"Globular Proteins"

I. Overview

- Globular proteins are spherical (or "globelike") in overall shape.

- They are usually somewhat water-soluble, possessing many hydrophilic amino acids on their outer surface, facing the aqueous environment.

- More nonpolar amino acids face the interior of the protein, providing hydrophobic interactions to further stabilize the globular structure.

- This is in contrast to fibrous proteins, which:

    - Form long rodlike filaments
    - Are relatively inert or water-insoluble
    - Provide structural support in the extracellular environment

II. Globular Hemeproteins

- Hemeproteins are a group of specialized globular proteins that contain heme as a tightly bound prosthetic group

- The function of the heme group is dictated by the three-dimensional structure of the protein.

- In the mitochondrial electron transport chain:

    - The cytochrome protein structure allows for rapid and reversible oxidation-reduction electron transfer of the heme-coordinated iron
    - The iron reversibly transitions between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) states.

Role of Heme in Enzymes and Hemoglobin

- In the enzyme catalase, the heme group is structurally part of the enzyme's active site, which catalyzes the breakdown of hydrogen peroxide.

- The protein structure of hemoglobin can affect the alignment of the ferrous (Fe^{2+}) iron with respect to the plane of the heme prosthetic group.

- Changes in this alignment can affect the binding affinity and transport of oxygen by hemoglobin between the lungs and tissues.

A. Heme Structure

- Heme is a planar structure, comprised of:
    - A porphyrin ring
    - With ferrous iron (Fe^{2+}) coordinated in the porphyrin ring center.

- The iron is held in the center of the heme molecule by:
    - Bonds to four nitrogens of the porphyrin ring

- The heme Fe^{2+} can form two additional bonds, one on each side of the planar porphyrin ring.

- In hemoglobin:

    - One of these positions is coordinated to the side chain of a histidine residue of the globin molecule
    - The other position is available to bind O_2

B. Myoglobin Structure and Function

- Myoglobin, a hemeprotein present in heart and skeletal muscle, functions:

    - As an oxygen reservoir
    - And as an oxygen carrier that increases the rate of oxygen transport within the muscle cell

- Myoglobin consists of:

    - A single polypeptide chain
    - That is structurally similar to the individual polypeptide chains of the tetrameric hemoglobin molecule

- This homology makes myoglobin a useful model for interpreting some of the more complex properties of hemoglobin.

1. α-Helical Content

- Myoglobin is a compact molecule, with:
    - ~80% of its polypeptide chain folded into eight stretches of α-helix
- These α-helical regions are terminated either by:
    - The presence of proline, whose five-membered ring cannot be accommodated in an α-helix (see p. 16)
    - Or by β-bends and loops stabilized by:
        - Hydrogen bonds
        - Ionic bonds (Note: Ionic bonds are also termed electrostatic interactions or salt bridges)

2. Location of Polar and Nonpolar Amino Acid Residues

- The interior of the globular myoglobin molecule is composed almost entirely of nonpolar amino acids.
- Nonpolar amino acids are:
    - Packed closely together
    - Forming a structure stabilized by hydrophobic interactions between these clustered residues
- In contrast, polar amino acids are located almost exclusively on the surface, where:
    - They can form hydrogen bonds, both:
        - With each other
        - And with water

3. Binding of the Heme Group

- The heme prosthetic group of the myoglobin molecule:
    - Sits in a crevice lined with nonpolar amino acids
- Notable exceptions to the nonpolar lining are:
    - Two histidine residues, which are basic amino acids

- One of the histidine residues is:
    - The proximal histidine (F8), which binds directly to the Fe^{2+} of heme
- The second histidine is:
    - The distal histidine (E7), which:
        - Does not directly interact with the heme group
        - But helps stabilize the binding of O_2 to Fe^{2+}
- The protein (globin) portion of myoglobin:
    - Creates a special microenvironment for the heme that permits oxygenation
    - Oxygenation = Reversible binding of one oxygen molecule
- Oxidation (simultaneous loss of electrons by Fe^{2+} to ferric [Fe^{3+}] form):
    - Occurs only rarely

C. Hemoglobin Structure and Function

1. Location and Primary Function

- Hemoglobin is found exclusively in red blood cells (RBCs)
- Its main function is to:
    - Transport O_2 from the lungs to the capillaries of the tissues

2. Structure of Hemoglobin A (HbA)

- Hemoglobin A (HbA) is the major hemoglobin in adults
- Composed of four polypeptide chains:
    - Two α chains
    - Two β chains

- Chains are held together by noncovalent interactions

- Each chain (subunit) has:

    - Stretches of α-helical structure
    - A hydrophobic heme-binding pocket, similar to that in myoglobin

3. Complexity Compared to Myoglobin

- The tetrameric hemoglobin molecule is:

    - Structurally and functionally more complex than myoglobin

4. Additional Transport Functions

- Hemoglobin can transport:

    - Protons (H^+)
    - Carbon dioxide (CO_2)
    - From the tissues to the lungs

- Can carry four molecules of O_2 from the lungs to the cells of the body

5. Regulation of Oxygen-Binding

- The oxygen-binding properties of hemoglobin are:
  - Regulated by interaction with allosteric effectors

6. Physiological Relevance

- Obtaining O_2 solely by diffusion:
  - Greatly limits the size of organisms
- Circulatory systems overcome this limitation
- But transport molecules like hemoglobin are also required because:
  - O_2 is only slightly soluble in aqueous solutions such as blood

I. Quaternary Structure of Hemoglobin

A. Dimer Formation and Interactions

- The hemoglobin tetramer can be envisioned as composed of:
    - Two identical dimers: $\alpha\beta_1$ and $\alpha\beta_2$
- The two polypeptide chains within each dimer are:
    - Held tightly together primarily by hydrophobic interactions

B. Distribution of Hydrophobic Residues

- In this instance:
    - Hydrophobic amino acid residues are located:
        - Not only in the interior of the molecule
        - But also on a region of the surface of each subunit
- These interchain hydrophobic interactions:
    - Form strong associations between the α-subunit and β-subunit in each dimer

C. Interaction Between Dimers

- The two dimers are held together primarily by polar bonds
- These weaker interactions:
    - Allow the dimers to move with respect to one another
- As a result:
    - The two dimers occupy different relative positions in:
        - Deoxyhemoglobin versus oxyhemoglobin

a. T Form (Taut/Tense Form)

A. Structure of T Form

- The deoxy form of hemoglobin is called the "T" form, or taut (tense) form

- In this form:
    - The two αβ dimers interact through a network of ionic bonds and hydrogen bonds
    - These interactions constrain the movement of the polypeptide chains

B. Position of Iron and Oxygen Affinity

- In the T form:
    - The iron (Fe^{2+}) is pulled out of the heme planar structure

- The T conformation represents:
    - The low-oxygen-affinity form of hemoglobin

b. R Form (Relaxed Form)

A. Effect of O_2 Binding

- Binding of O_2 to hemoglobin:
    - Causes rupture of some polar bonds between the two αβ dimers
    - Allows movement of Fe^{2+} relative to the planar heme structure

B. Movement of Iron and Globin Chains

- Specifically:
    - O_2 binding to Fe^{2+} pulls the iron more directly into the plane of the heme ring
    - Because Fe^{2+} is linked to the proximal histidine (F8):
        - This movement shifts the globin chains
        - Alters the interface between the αβ dimers

C. R Conformation Properties

- This conformational change results in the:
  - "R" form, or relaxed form of hemoglobin

- The R form is:
  - The high-oxygen-affinity conformation

D. Oxygen Binding to Myoglobin and Hemoglobin

A. Binding Capacity

- Myoglobin:

  - Can bind only one O_2 molecule
  - Because it contains only one heme group

- Hemoglobin:

  - Can bind four O_2 molecules
  - One at each of its four heme groups

B. Degree of Saturation (Y)

- Y (Degree of Saturation):
    - Represents how many oxygen-binding sites are occupied
    - Can range from:
        - 0%: All sites are empty
        - 100%: All sites are full (fully saturated)

C. Clinical Note: Pulse Oximetry

- Pulse oximetry:
    - A noninvasive and indirect method for measuring oxygen saturation of arterial blood
    - Based on differences in light absorption:
        - Between oxyhemoglobin and deoxyhemoglobin

I. Oxygen-Dissociation Curve

A. Definition

- A graph of degree of saturation (Y) at different partial pressures of oxygen (pO_2 or PO_2)
- Called the oxygen-dissociation curve

B. Comparison: Myoglobin vs. Hemoglobin

- Myoglobin has a higher O_2 affinity at all pO_2 values than hemoglobin
- P50 (partial pressure needed for 50% saturation):
    - Myoglobin: ~1 mm Hg
    - Hemoglobin: ~26 mm Hg
- Interpretation:
    - Higher oxygen affinity → Lower P50 (binds O_2 more tightly)

a. Myoglobin

A. Curve Shape

- The oxygen-dissociation curve is hyperbolic

B. Reason for Hyperbolic Shape

- Due to reversible binding of a single O_2 molecule
- Simple equilibrium:
    - $Mb + O_2 \rightleftarrows MbO_2$

C. Effect of O_2 Levels

- Equilibrium shifts:
    - To the right when O_2 is added
    - To the left when O_2 is removed

D. Functional Role of Myoglobin

- Binds O_2 released by hemoglobin at low pO_2 (as in muscle)

- Releases O_2 within the muscle cell in response to oxygen demand

b. Hemoglobin

A. Curve Shape

- The oxygen-dissociation curve is sigmoidal

B. Reason for Sigmoidal Shape

- Due to cooperative binding:
    - Binding of O_2 at one subunit increases the O_2 affinity of the remaining subunits

C. Cooperative Binding Mechanism

- First O_2 binds with difficulty
- Subsequent O_2 molecules bind with increasingly higher affinity
- Reflected by:
    - Steep upward curve between 20-30 mm Hg pO_2

E. Allosteric Effectors of Hemoglobin

A. Definition

- Hemoglobin's ability to reversibly bind O_2 is influenced by:
    - pO_2 (partial pressure of oxygen)
    - pH of the environment
    - pCO_2 (partial pressure of carbon dioxide)
    - 2,3-bisphosphoglycerate (2,3-BPG)

B. Mechanism

- These are called allosteric ("other site") effectors:
    - They bind at sites other than the O_2-binding site
    - Cause structural changes that affect O_2 binding to heme iron at other sites within the hemoglobin molecule

- Myoglobin (monomeric) is not influenced by allosteric effectors

I. Oxygen (O_2) as an Allosteric Effector

A. Cooperativity and Structural Change

- The sigmoidal O_2-dissociation curve reflects structural changes initiated at one subunit and transmitted to others
- Net effect of cooperativity:
  - Hemoglobin's affinity for the 4th O_2 molecule is ~300 times greater than for the 1st O_2 molecule
- O_2 itself acts as an allosteric effector:
  - Stabilizes the R (relaxed) form of hemoglobin

a. Loading and Unloading of Oxygen

- In lungs (high pO_2):
  - Hemoglobin becomes almost fully saturated ("loaded") with O_2

- In peripheral tissues (low pO_2):
    - Hemoglobin releases ("unloads") O_2 for use in oxidative metabolism
- Cooperative binding allows efficient delivery of O_2 in response to small changes in pO_2

b. Significance of the Sigmoidal Oxygen-Dissociation Curve

- The steep slope of hemoglobin's curve in the physiological pO_2 range enables:
    - Efficient O_2 uptake in lungs
    - Effective O_2 release in tissues
- In contrast, myoglobin (with a hyperbolic curve):
    - Has high O_2 affinity throughout the range
    - Would not release significant O_2 in tissues
    - Therefore, not suitable for O_2 delivery function like hemoglobin

2. Bohr Effect

A. Definition

- Bohr effect = The phenomenon where O_2 release from hemoglobin is enhanced under certain conditions:
    - ↓ pH (↑ $[H^+]$)
    - ↑ pCO_2 (partial pressure of CO_2)
- Both conditions cause:
    - ↓ O_2 affinity of hemoglobin
    - Rightward shift of the oxygen-dissociation curve
    - Stabilization of the T (tense/deoxy) form

B. Opposite Conditions

- ↑ pH or ↓ CO_2 concentration causes:
    - ↑ O_2 affinity
    - Leftward shift in the oxygen-dissociation curve
    - Stabilization of the R (relaxed/oxy) form

a. Source of the Protons That Lower pH

- In metabolically active tissues, concentrations of H^+ and CO_2 are higher than in alveolar capillaries

- In these tissues, the enzyme carbonic anhydrase (zinc-containing) catalyzes:

    - $CO_2 + H_2O \rightleftarrows H_2CO_3$ (carbonic acid)
    - H_2CO_3 then spontaneously ionizes into:
        - HCO_3^- (bicarbonate, major blood buffer)
        - H^+ (proton responsible for ↓ pH):
            - $H_2CO_3 \rightleftarrows HCO_3^- + H^+$

A. Role of pH Gradient

- H^+ ions produced by:
    - $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+$
    - Contribute to lowering pH in peripheral tissues

- pH gradient between lungs and tissues:

    - Lungs: Higher pH
    - Tissues: Lower pH

- This pH difference:
    - Favors unloading of O_2 in tissues
    - Favors loading of O_2 in the lungs
- Therefore:
    - O_2 affinity of hemoglobin is finely regulated by small shifts in pH
    - Makes hemoglobin a highly efficient O_2 transporter

b. Mechanism of the Bohr Effect

- Deoxyhemoglobin has greater affinity for H^+ than oxyhemoglobin
- Due to:
    - Ionizable functional groups (e.g., histidine side chains) that:
        - Have higher pKa in deoxyhemoglobin than in oxyhemoglobin

- When $[H^+]$ increases ($\downarrow$ pH):
    - These groups become protonated (charged)
    - Form ionic bonds (salt bridges)
    - These bonds stabilize deoxyhemoglobin (T form)
    - Resulting in decreased oxygen affinity
- Note: Hemoglobin also functions as an important blood buffer

Schematic Representation

- Bohr effect reaction:
    - $HbO_2 + H^+ \rightleftarrows HbH + O_2$
        - Oxyhemoglobin $\rightarrow$ Deoxyhemoglobin
- Interpretation:
    - $\uparrow H^+$ (or $\downarrow pO_2$) $\rightarrow$ shifts equilibrium to the right (favors O_2 release)
    - $\uparrow pO_2$ (or $\downarrow H^+$) $\rightarrow$ shifts equilibrium to the left (favors O_2 binding)

3. 2,3-BPG Effect on Oxygen Affinity

A. General Overview

- 2,3-Bisphosphoglycerate (2,3-BPG) is a major regulator of O_2 binding to hemoglobin
- It is the most abundant organic phosphate in RBCs
    - Its concentration is approximately equal to hemoglobin
- Source: Synthesized from an intermediate of the glycolytic pathway

B. 2,3-BPG Binding to Deoxyhemoglobin

- Decreases O_2 affinity by binding only to deoxyhemoglobin
- Does not bind to oxyhemoglobin

- Binding of 2,3-BPG:
    - Stabilizes the T (tense) form of hemoglobin
- Schematic reaction:
    - $HbO_2 + 2,3\text{-BPG} \rightleftarrows Hb\text{-}2,3\text{-BPG} + O_2$
        - Binding of 2,3-BPG promotes O_2 release

C. 2,3-BPG-Binding Site

- One molecule of 2,3-BPG binds to a pocket in deoxyhemoglobin
    - Formed by the two β-globin chains
- The pocket:
    - Contains positively charged amino acids
        - These form ionic bonds with negatively charged phosphate groups of 2,3-BPG

- Important note:
    - Amino acid replacement at this site can result in hemoglobin variants with abnormally high O_2 affinity
        - Can lead to compensatory erythrocytosis (↑ RBC production)

- Oxygenation of hemoglobin:
    - Narrows the central pocket
    - Causes release of 2,3-BPG

D. Shift in Oxygen-Dissociation Curve

- Hemoglobin without 2,3-BPG:
    - Has very high O_2 affinity

- Hemoglobin with 2,3-BPG:
    - Has reduced O_2 affinity
    - Shifts the oxygen-dissociation curve to the right

- This shift:
    - Enables hemoglobin to release O_2 more efficiently at tissue-level pO_2

D. 2,3-BPG Levels in Chronic Hypoxia or Anemia

- Increased 2,3-BPG occurs in chronic hypoxia and anemia
    - Seen in conditions such as:
        - Chronic Obstructive Pulmonary Disease (COPD) (e.g., emphysema)
        - High altitudes (↓ pO_2)
        - Chronic anemia (↓ RBC count)
- Mechanism:
    - Low oxygen availability → ↑ 2,3-BPG in RBCs
- Effect:
    - ↑ 2,3-BPG → ↓ O_2 affinity of hemoglobin
    - Promotes greater O_2 unloading in peripheral tissues
    - Helps meet tissue oxygen demands despite reduced O_2 delivery

E. 2,3-BPG in Transfused Blood

- Stored blood in blood banks:
    - Gradually depletes its 2,3-BPG content
- Consequences:
    - Hemoglobin in stored blood has abnormally high O_2 affinity
    - Leads to poor O_2 unloading → acts as an O_2 "trap"
- Recovery:
    - Transfused RBCs can restore 2,3-BPG within 6–24 hours
- Clinical concern:
    - Severely ill patients may not tolerate delayed O_2 delivery

- Solution:
    - Stored blood is treated with "rejuvenation solution"
        - Rapidly restores 2,3-BPG
        - Also restores ATP lost during storage

F. Clinical Application 3.1: 2,3-BPG Offloads Oxygen to the Tissues

Scenario: Two individuals compared

1. Sea-level Individual (5 mmol/L 2,3-BPG)

- At sea level:
    - Hemoglobin O_2 saturation in lungs: ~100%
    - In tissues: ~60% saturation → 40% O_2 delivered
- At high altitude:
    - Lung saturation: ~90%
    - Tissue saturation: ~60%
    - O_2 delivery drops to ~30%

2. High-altitude Acclimatized Individual (8 mmol/L 2,3-BPG)

- Increased 2,3-BPG shifts the O_2-binding curve rightward
- Lung saturation: ~80%
- Tissue saturation: ~40%
- O_2 delivery remains ~40%, similar to sea level

Conclusion:

- Increased 2,3-BPG compensates for reduced lung O_2 uptake by improving O_2 unloading in tissues
- Allows consistent oxygen delivery despite environmental hypoxia

4. CO_2 Binding to Hemoglobin

- Major transport form of CO_2:
    - As bicarbonate ion (HCO_3^-) via hydration (catalyzed by carbonic anhydrase)
- Alternate transport form:
    - Carbamate formation with hemoglobin:
        - Reaction:
        - $Hb-NH_2 + CO_2 \rightleftarrows Hb-NH-COO^- + H^+$
        - CO_2 binds to terminal amino groups on globin chains (not the heme iron)
        - Forms carbaminohemoglobin
- Functional consequence:
    - Stabilizes T (tense/deoxy) form of hemoglobin
    - Decreases O_2 affinity → Right shift of O_2-dissociation curve
    - Facilitates O_2 unloading in tissues
- In lungs:
    - CO_2 dissociates from hemoglobin
    - Released in the exhaled breath

5. CO (Carbon Monoxide) Binding to Hemoglobin

- Forms carboxyhemoglobin:
    - CO binds to the heme iron in hemoglobin
    - Binding is tight but reversible
    - Shifts hemoglobin to R (relaxed) form
- Effect on O_2 binding:
    - Remaining heme sites bind O_2 with abnormally high affinity
    - O_2-dissociation curve shifts left
    - Sigmoid curve becomes hyperbolic
    - Impaired O_2 release to tissues → causes tissue hypoxia
- Affinity facts:
    - Hemoglobin's affinity for CO is ~220× greater than for O_2
    - Even low environmental CO levels → toxic carboxyhemoglobin concentrations

- Sources of CO:
    - Environmental pollution
    - Tobacco smoke (↑ CO levels in smokers)
- CO toxicity mechanisms:
    - Tissue hypoxia
    - Direct cellular toxicity
        - CO also inhibits Complex IV (cytochrome c oxidase) of the electron transport chain
- Treatment:
    - 100% oxygen at high pressure (hyperbaric oxygen therapy)
    - Promotes rapid dissociation of CO from hemoglobin

Additional Note: Nitric Oxide (NO) and Hemoglobin

- NO binding:
    - Hemoglobin can carry nitric oxide
- Role of NO:
    - Potent vasodilator
- Hemoglobin modulates NO:
    - Can salvage or release NO
    - Influences vascular diameter and blood pressure regulation

F. Minor Hemoglobins

- Human Hemoglobin A (HbA) is only one of several related oxygen-carrying proteins
- All hemoglobins are tetramers:
    - Composed of 2 α-globin (or α-like) + 2 β-globin (or β-like) chains

- Other hemoglobins include:
    - HbF (Fetal Hemoglobin) — dominant in fetal life
    - HbA_2 — low levels in adults
    - HbA_1c — glycated form of HbA

1. Fetal Hemoglobin (HbF)

Structure

- Tetramer: $\alpha_2\gamma_2$
    - Two α chains: same as in HbA
    - Two γ chains: members of the β-globin gene family

a. HbF Synthesis During Development

- 1st month after conception:
    - Embryonic hemoglobins synthesized by yolk sac
    - Example: Hb Gower 1 = $\zeta_2\varepsilon_2$
        - 2 zeta (ζ) chains (α-like)
        - 2 epsilon (ε) chains (β-like)

- 5th week of gestation:
    - Site of globin synthesis shifts:
        - From yolk sac → liver → bone marrow
    - Primary product = HbF
- Late fetal life:
    - HbF is the major hemoglobin (~60% of total hemoglobin in RBCs)
- 8th month of gestation:
    - HbA synthesis begins in bone marrow
    - HbA gradually replaces HbF postnatally

b. 2,3-BPG Binding to HbF

- HbF has higher O_2 affinity than HbA
    - Due to weaker binding of 2,3-BPG

- Reason:
    - γ chains of HbF lack some positively charged residues present in β chains
    - These positive residues are important for 2,3-BPG binding
- Physiologic consequence:
    - Less 2,3-BPG binding → higher O_2 affinity
    - Facilitates O_2 transfer from maternal blood → fetal RBCs across the placenta
- If 2,3-BPG is removed:
    - HbF and HbA show similar O_2 affinity

2. Hemoglobin A_2 (HbA_2)

- Minor component of normal adult hemoglobin
- Appears shortly before birth
- In adults: constitutes ~2% of total hemoglobin

Structure

- Tetramer: $\alpha_2\delta_2$
    - 2 α-globin chains
    - 2 δ-globin chains

3. Hemoglobin A_1c (HbA_1c)

Definition

- Formed via nonenzymatic glycation of hemoglobin A (HbA)
- Sugar molecules, primarily glucose, are added to HbA

Glycation Process

- Nonenzymatic and dependent on plasma glucose concentration
- Glycation occurs at the N-terminal valines of the β-globin chains

Structure

- Glucose residues covalently attached to HbA
- Produces HbA_1c, the most abundant glycated form

Clinical Significance

- Increased HbA_1c levels found in patients with diabetes mellitus
    - Due to prolonged exposure of RBCs (120-day lifespan) to elevated glucose levels
- Used as a marker for average blood glucose levels

III. Globin Gene Organization

- Understanding gene organization is essential to grasp hemoglobin-related genetic disorders.
- Globin genes direct synthesis of different globin chains via gene families located on separate chromosomes.
- Gene expression begins in RBC precursors with transcription of the globin gene.

Gene Expression Process

- DNA → Transcription → pre-mRNA
- Two introns are spliced out
- Three exons are joined to form mature mRNA
- Mature mRNA undergoes translation to produce globin chains

A. α-Gene Family

- Located on Chromosome 16
- Called the α-gene cluster

Contains:

- Two functional α-globin genes
- ζ (zeta) gene
  - Expressed early in embryonic development
  - Produces α-globin-like chains (part of embryonic hemoglobin)

Additional Notes:

- Also includes globin-like pseudogenes
    - Structurally similar but nonfunctional
    - Do not produce any globin chains

B. β-Gene Family

- Located on Chromosome 11
- Called the β-gene cluster

Contains:

- One β-globin gene (functional in adult HbA)
- Four additional β-globin-like genes:
    a. ε (epsilon):
        - Expressed early in embryonic development
    b. Two γ (gamma) genes:
        - Gγ and Aγ
        - Expressed in fetal hemoglobin (HbF)
    c. δ (delta) gene:
        - Produces the δ-globin chain
        - Found in HbA_2 (minor adult hemoglobin)

IV. Hemoglobinopathies

- Group of genetic disorders involving:
    - Structurally abnormal hemoglobin
    - Reduced synthesis of normal hemoglobin
    - Or both abnormalities (rare)

Types of Hemoglobinopathies:

- Qualitative hemoglobinopathies:
    - Due to structural defects in globin chains
    - Examples:
        - Sickle cell anemia (HbS)
        - Hemoglobin C disease (HbC)
        - Hemoglobin SC disease (HbSC = HbS + HbC)

- Quantitative hemoglobinopathies:
    - Due to reduced production of globin chains
    - Example:
        - Thalassemias

A. Sickle Cell Anemia (Hemoglobin S Disease)

- Caused by a point mutation in the β-globin gene
  - Substitution of valine for glutamic acid at position 6
  - Produces β^S-globin chain, forming abnormal HbS ($\alpha_2\beta^S_2$)

Genetic Pattern:

- Autosomal recessive disorder
- Affected individuals inherit two mutant alleles (one from each parent)

Morphological Impact:

- HbS polymerizes under low oxygen → RBC sickling
- Sickled RBCs:
  - Are crescent/sickle-shaped
  - Less flexible → block blood flow
  - Have reduced lifespan (<20 days) vs. 120 days in normal RBCs
  - Leads to chronic hemolytic anemia and hyperbilirubinemia

Onset:

- Symptoms begin once HbF declines post-infancy and HbS dominates

Clinical Features:

- Painful crises (vaso-occlusive episodes)
- Chronic anemia
- Hyperbilirubinemia
- Increased risk of infections (especially in infancy)
- Acute chest syndrome
- Stroke
- Splenic and renal dysfunction
- Bone changes due to marrow hyperplasia
- Reduced life expectancy: median age ~ mid-40s

Sickle Cell Trait (Heterozygous Individuals)

- Have one normal β-globin allele and one sickle allele
- Blood contains both HbA and HbS
- Usually asymptomatic
    - Rare sickling under extreme dehydration or exertion
- Represent 1 in 12 African Americans
- Have a normal lifespan
- Condition termed sickle cell trait, not sickle cell disease

1. Amino Acid Substitution in HbS β Chains

- Composition of HbS molecule in sickle cell anemia:
    - 2 normal α-globin chains
    - 2 mutant β^S-globin chains
- Molecular change:
    - Glutamate (Glu) at position 6 in β-globin replaced by valine (Val)
    - Glutamate: negatively charged, polar
    - Valine: neutral, nonpolar
- Effect on charge and electrophoretic mobility:
    - HbS has less negative charge than HbA
    - During alkaline pH electrophoresis, HbS migrates more slowly toward the anode than HbA
    - Electrophoresis of lysed RBC hemoglobin is a routine diagnostic test for:
        - Sickle cell anemia (disease)
        - Sickle cell trait
    - DNA analysis can also diagnose sickle cell anemia

2. Sickling and Tissue Anoxia

- Cause of sickling:
    - Valine substitution creates a hydrophobic protrusion on β^S-globin chain
    - This interacts with a complementary hydrophobic pocket on another HbS molecule
- Polymerization under low O_2 tension:
    - Deoxygenated HbS polymerizes inside RBCs
    - Forms insoluble fibrous polymers
    - Leads to stiffening and distortion of RBCs → rigid, sickle-shaped cells
- Consequences of sickled RBCs:
    - Block small capillaries, impairing blood flow
    - Causes localized tissue anoxia → leads to:
        - Severe pain
        - Ischemic cell death (infarction)
    - Anoxia further increases deoxygenated HbS, worsening sickling

- RBC and capillary size mismatch:
    - RBC diameter: ~7.5 μm
    - Capillary diameter: ~3–4 μm
    - Sickled RBCs:
        - Have less deformability
        - Increased adhesion to endothelium
        - Result in microvascular occlusion

3. Variables That Increase Sickling

- Sickling is worsened by factors that increase deoxygenated HbS, including:
    - ↓ pO_2 (low oxygen tension)
    - ↑ pCO_2 (high carbon dioxide)
    - ↓ pH (acidosis)
    - Dehydration
    - ↑ 2,3-BPG concentration in RBCs

4. Treatment

- Supportive management:
    - Adequate hydration
    - Analgesics (for pain management)
    - Aggressive antibiotics if infection present
- Transfusion therapy:
    - Used in high-risk patients to prevent fatal vessel occlusion
    - Reduces stroke risk
    - Risks of transfusion include:
        - Iron overload (hemosiderosis)
        - Blood-borne infections
        - Immunologic reactions
- Pharmacologic therapy:
    - Hydroxyurea (hydroxycarbamide):
        - An antitumor drug
        - Increases HbF (fetal hemoglobin) levels
        - HbF reduces sickling by diluting HbS

- Hydroxyurea therapy effects:
    - ↓ Frequency of painful vaso-occlusive crises
    - ↓ Overall mortality in sickle cell anemia patients
- Curative option:
    - Stem cell transplantation is a potential cure
- Newborn screening:
    - Sickle cell anemia included in newborn screening panels
    - Enables early initiation of prophylactic antibiotics
    - Helps prevent early complications and mortality

5. Selective Advantage of the Heterozygous State

- High prevalence of β^S mutation in Black Africans despite harmful homozygous effects suggests selective benefit for heterozygotes

- Protection against malaria:
    - Heterozygotes (HbAS) less susceptible to severe malaria caused by Plasmodium falciparum
    - P. falciparum requires RBCs for part of its lifecycle
    - Shortened RBC lifespan in heterozygotes may prevent parasite maturation
    - Provides evolutionary advantage in malaria-endemic regions

- Geographic correlation:
    - Sickle cell gene distribution in Africa closely matches malaria endemic zones

B. Hemoglobin C Disease

- Nature of HbC mutation:
    - Single amino acid substitution at position 6 of β-globin chain
    - Lysine replaces glutamate (vs. valine in HbS)

- Electrophoresis pattern:
    - HbC is less negatively charged
    - Migrates more slowly toward anode than HbA or HbS

- Clinical features of HbC disease (HbCC):
    - Rare homozygous patients
    - Have mild chronic hemolytic anemia
    - Do not experience infarctive crises
    - No specific therapy generally needed

C. Hemoglobin SC Disease

- Definition:
    - A form of RBC sickling disease
    - Some β-globin chains carry the HbS mutation (valine substitution)
    - Others carry the HbC mutation (lysine substitution)

- Genetic nature:
    - Patients are compound heterozygotes
        - Both β-globin genes are abnormal but different
- Clinical features:
    - Hemoglobin levels are higher than in sickle cell anemia
        - May be low-normal
    - Painful crises:
        - Less frequent and less severe than in sickle cell anemia
    - Clinical variability is significant among individuals

D. Methemoglobinemias

- Definition:
    - Oxidation of heme iron from Fe^{2+} (ferrous) to Fe^{3+} (ferric) state
    - Methemoglobin (Fe^{3+}) cannot bind O_2

- Causes:
    - Acquired:
        - Due to drugs (e.g., nitrates)
        - Endogenous reactive oxygen species
    - Congenital:
        - NADH-cytochrome b_5 reductase deficiency (aka NADH-methemoglobin reductase)
            - Enzyme that reduces Fe^{3+} back to Fe^{2+}
        - HbM production due to rare mutations in α- or β-globin chains
            - HbM is resistant to enzymatic reduction
- Newborns:
    - RBCs have ~50% lower capacity to reduce methemoglobin compared to adults

- Clinical presentation:
    - Chocolate cyanosis:
        - Blue skin & mucous membranes
        - Brown-colored blood
    - Symptoms depend on tissue hypoxia:
        - Anxiety
        - Headache
        - Dyspnea
        - Severe cases: coma or death
- Treatment:
    - Methylene blue
        - Acts as an electron acceptor
        - Oxidized as Fe^{3+} is reduced back to Fe^{2+}

E. Thalassemias

- Definition:
    - Hereditary hemolytic anemias caused by imbalanced synthesis of globin chains
    - Most common single-gene disorders in humans
- Normal physiology:
    - Coordinated synthesis of α- and β-globin chains
        - Forms HbA ($\alpha_2\beta_2$)
- Pathology:
    - Defective synthesis of either α- or β-globin chain
    - Leads to reduced hemoglobin concentration
- Genetic causes:
    - Can include:
        - Whole gene deletions
        - Point mutations
        - Nucleotide deletions or substitutions

- Classification:
    - α^0- or β^0-thalassemia: No chain production
    - α^+- or β^+-thalassemia: Reduced chain production

1. β-Thalassemias

- Defect:
    - Reduced or absent β-globin synthesis
    - Usually due to point mutations affecting mRNA production
    - α-globin synthesis is normal

- Consequences:
    - Excess α-globin chains:
        - Cannot form stable tetramers
        - Precipitate in erythroid precursors → premature cell death
    - Increased levels of:
        - HbA_2 ($\alpha_2\delta_2$)
        - HbF ($\alpha_2\gamma_2$)

- Genetics:
    - Two β-globin genes per individual (1 per chromosome 11)
    - Classification based on number of affected genes:
        - β-Thalassemia trait (minor):
            - One defective β-globin gene
            - Mild anemia, usually no treatment needed
        - β-Thalassemia major (Cooley anemia):
            - Both β-globin genes defective
            - No β-chain production

- Clinical features of β-Thalassemia major:
    - Healthy at birth (β-globin not expressed prenatally)
    - Symptoms appear after a few months:
        - Severe anemia
        - Ineffective erythropoiesis
        - Skeletal deformities (due to extramedullary hematopoiesis)

- Treatment:
    - Regular blood transfusions (lifesaving)
        - Risk: Iron overload
    - Iron chelation therapy improves outcome
    - Only cure: Hematopoietic stem cell transplantation

2. α-Thalassemias

- Definition:
    - Disorders with decreased or absent α-globin chain synthesis
    - Most commonly caused by deletional mutations

- Genetics:
    - Each person has 4 α-globin genes (2 on each chromosome 16)
    - Severity depends on the number of defective alleles:

Levels of α-Globin Deficiency:

1.1 defective gene:

- "Silent" carrier
- No clinical symptoms

1.2 defective genes:

- α-Thalassemia trait
- Usually mild anemia or asymptomatic

1.3 defective genes:

- Hemoglobin H disease (HbH, β_4)
- Moderate to severe hemolytic anemia

1.4 defective genes:

- Hemoglobin Bart's disease (Hb Bart, γ_4)
- Leads to:
  - Hydrops fetalis
  - Fetal death
- Reason: α-globin chains are essential for HbF ($\alpha_2\gamma_2$) formation

- Note:
    - Both α- and β-thalassemias offer heterozygote advantage against malaria