Lecture of October 15, 2018 Chapter 4: Hemoglobin and Cooperativity; Proof of Grand Central Dogma

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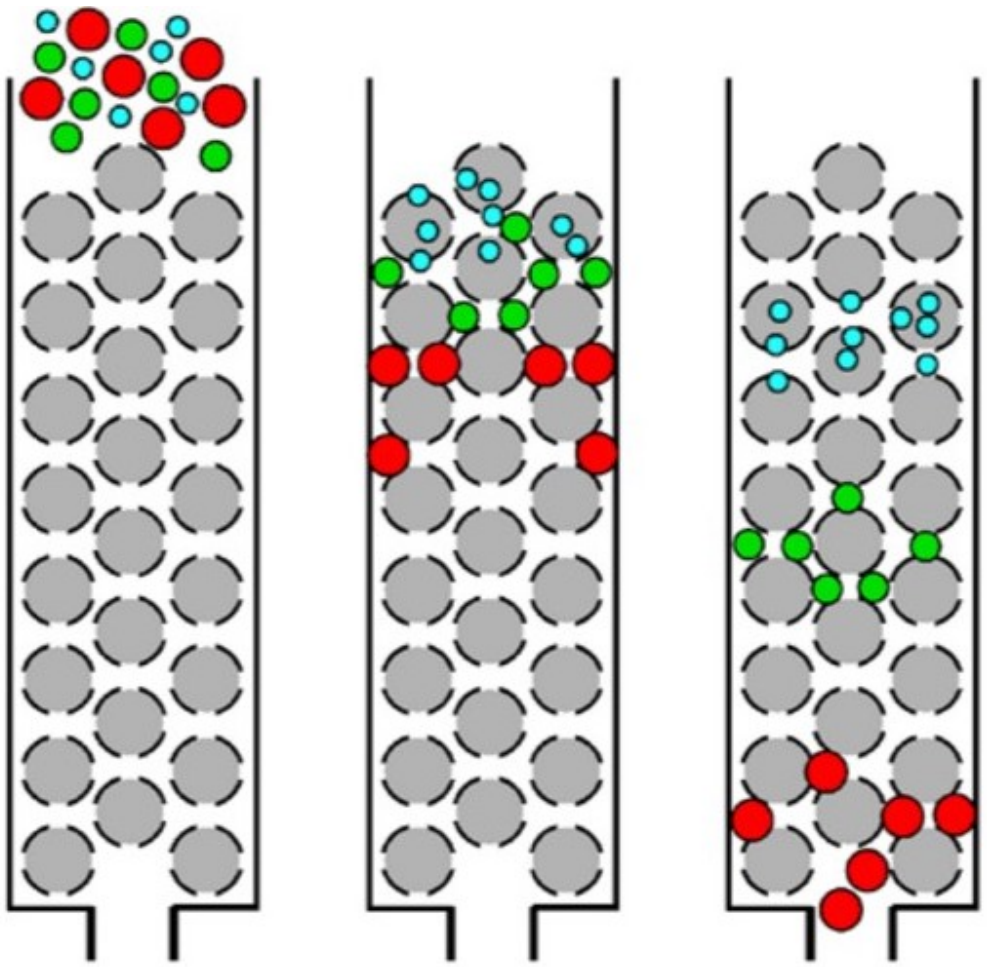
Section 4.1: Size Exclusion Chromatography; Figure 4.2A

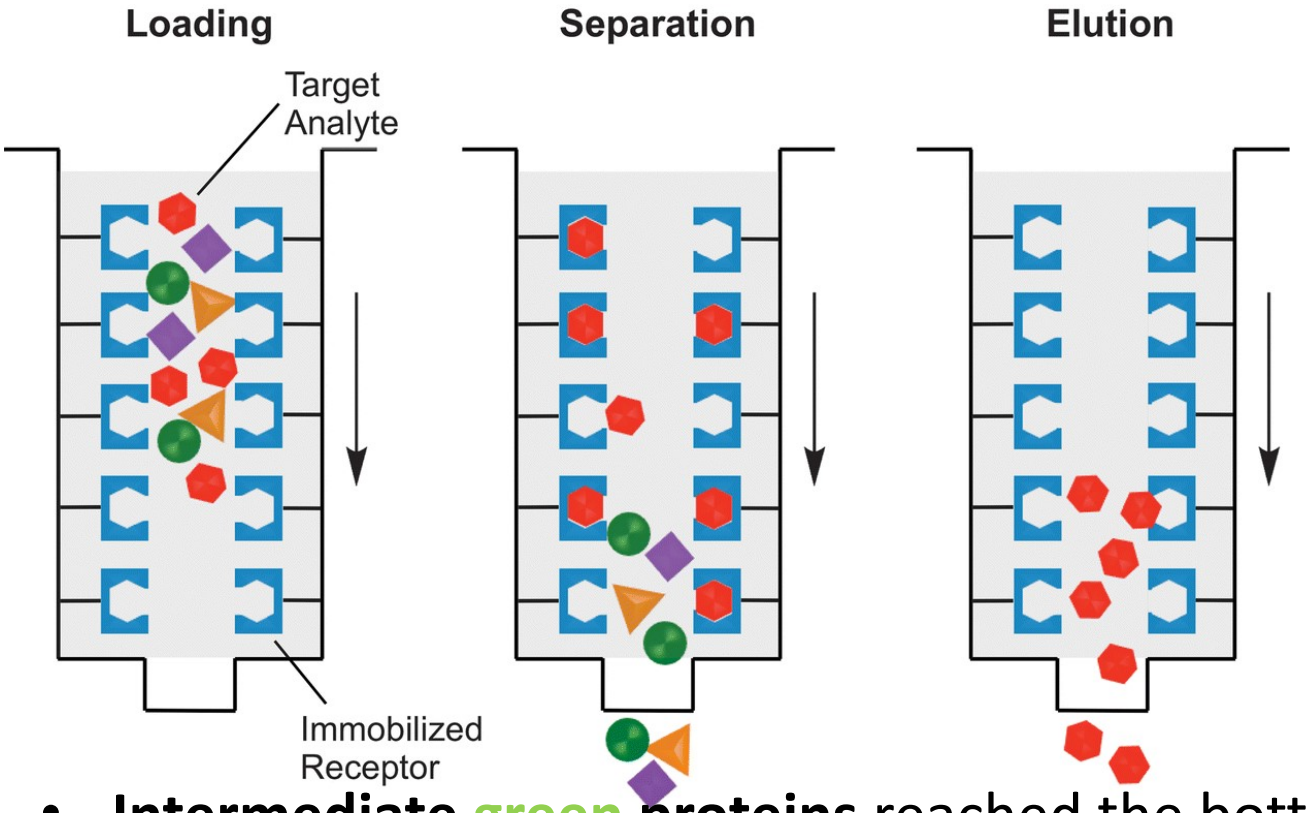
SLIDE 2



Purpose is to **separate red, green, blue proteins** by **size:**

- **Column Matrix** filled with cross-linked polymers with **small holes**.
- **Small blue proteins** are embedded in the small holes, while larger **red proteins** flow unimpeded to the bottom.
- **Intermediate green proteins** reached the bottom after **red proteins**

Section 4.1: Affinity Chromatography



SLIDE 3

Purpose is to **separate red, green, blue proteins** by **Chemical Affinity:**

- **Column Matrix** liganded with chemical units with affinity for **red proteins**

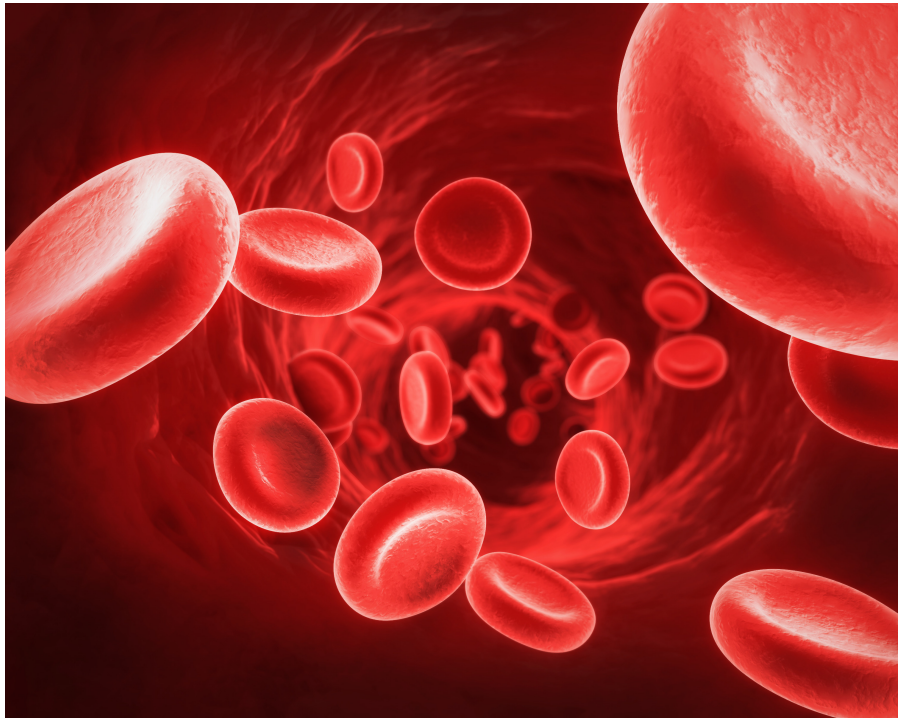
- **Intermediate green proteins** reached the bottom Only **red proteins** bind to **ligands**, while **green, blue proteins** flow through
- **red proteins** are eluted (washed out) by addition of solvents with solutes with **affinity** for the **ligands**

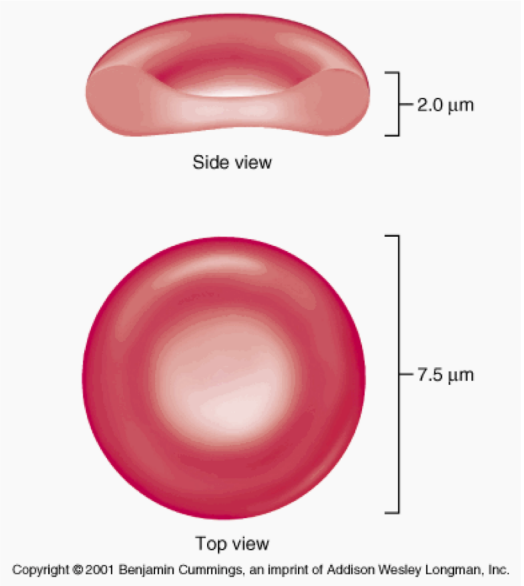
SLIDE 2

See **Figure 4.2 B**

Section 4.2: Hemoglobin (Hb) and Cooperativity

SLIDE 4

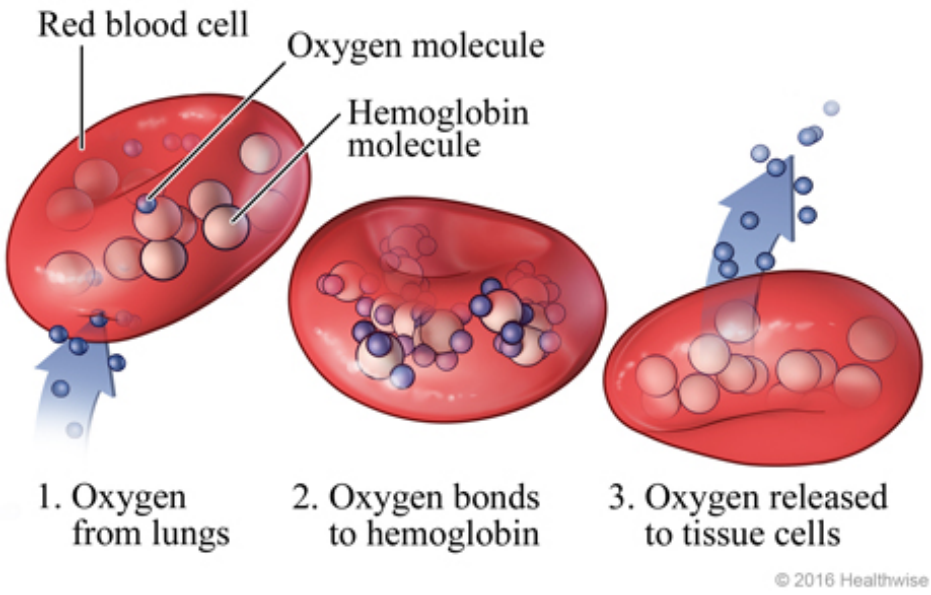


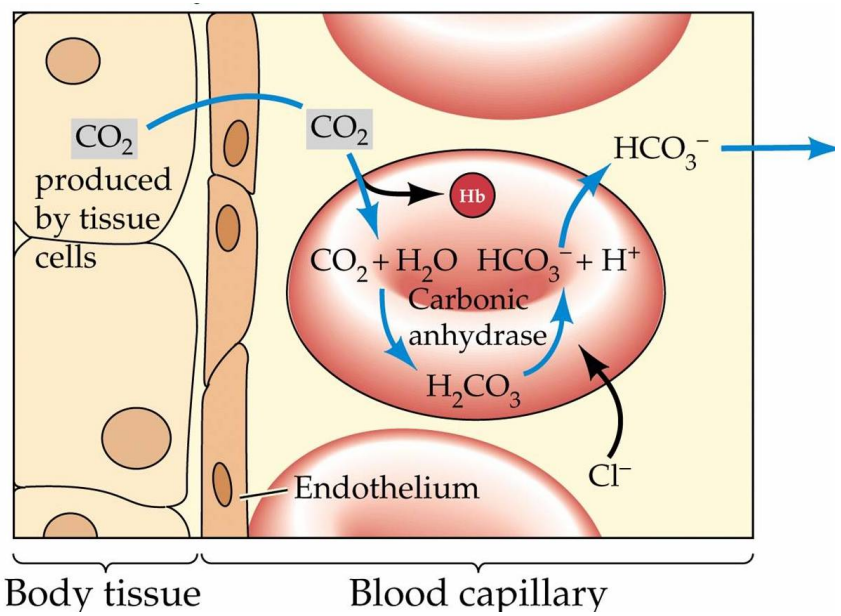


- Every day a human produced about 2×10^6 Red Blood Cell (RBC), every day
- RBC is needed to transport O_2 to the body, and removes CO_2 from the body

Section 4.2: Hemoglobin (Hb) and Cooperativity

SLIDE 5



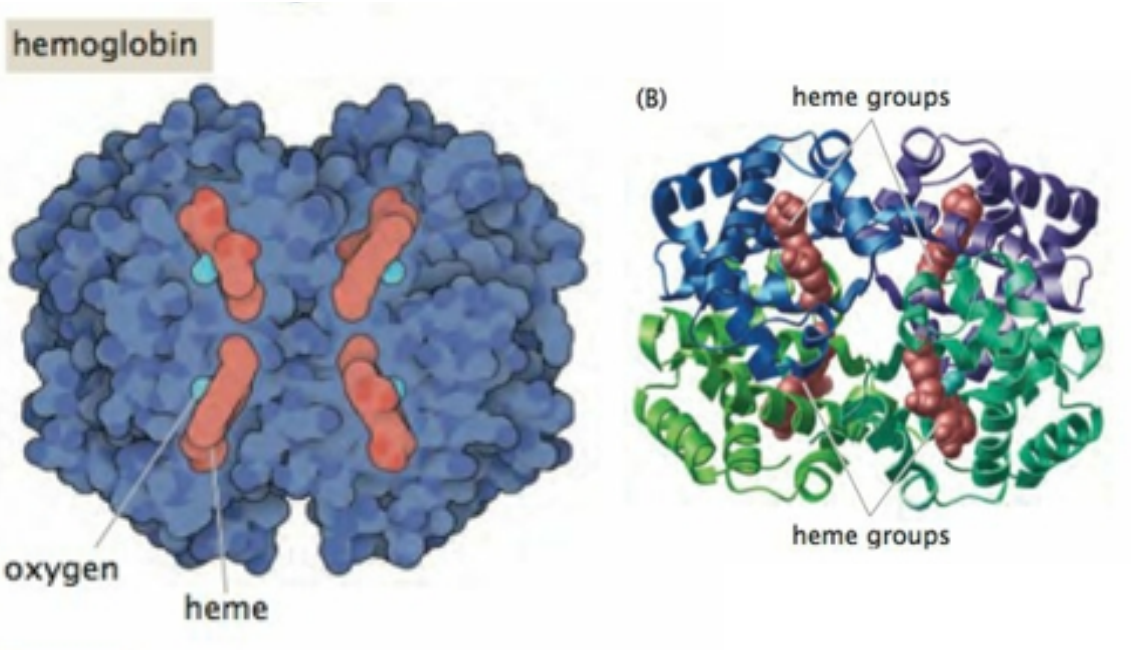


- Hemoglobins (Hb) are proteins in RBC that bind to O_2 from the lungs, and transport and released them in the tissues

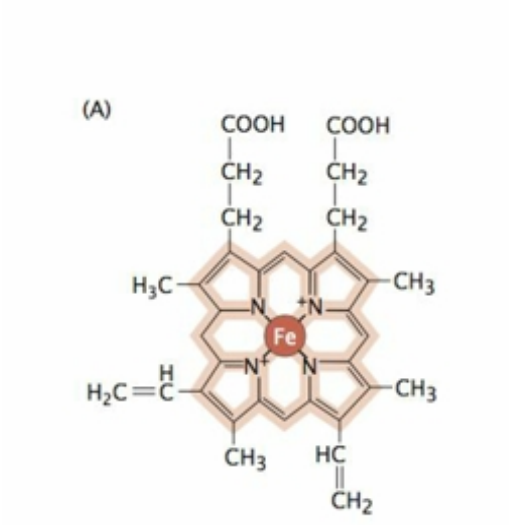
- Hemoglobins (Hb) also bind to CO_2 from the tissues, and transport them to the lungs to be exhale out of the body

Section 4.2: Hemoglobin (Hb) and Cooperativity

SLIDE 6



- A Hemoglobin (Hb) in licorice representation, on the left and in ribbon representation on the right.
- **4 Heme groups (in RED)** is bound to the Hb



- A **Heme** an iron(Fe)containing compound of the porphyrin class that forms the nonprotein part of hemoglobin.

Section 4.2: Hemoglobin (Hb) and Cooperativity

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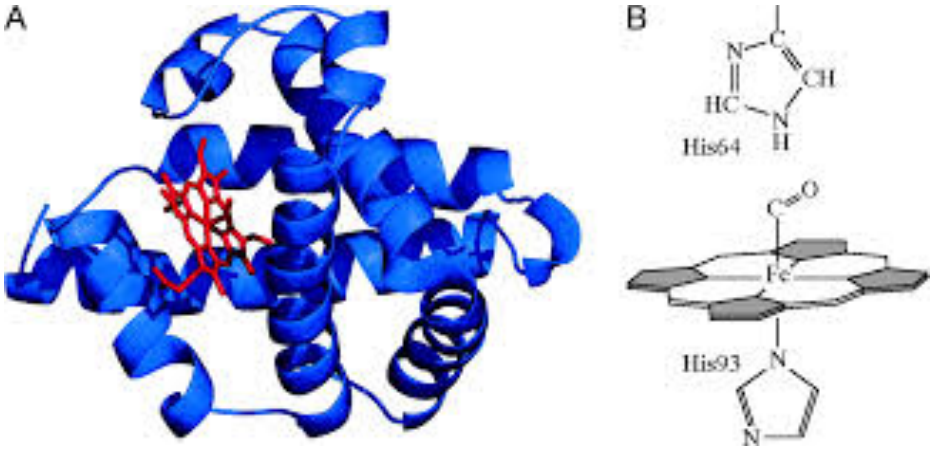

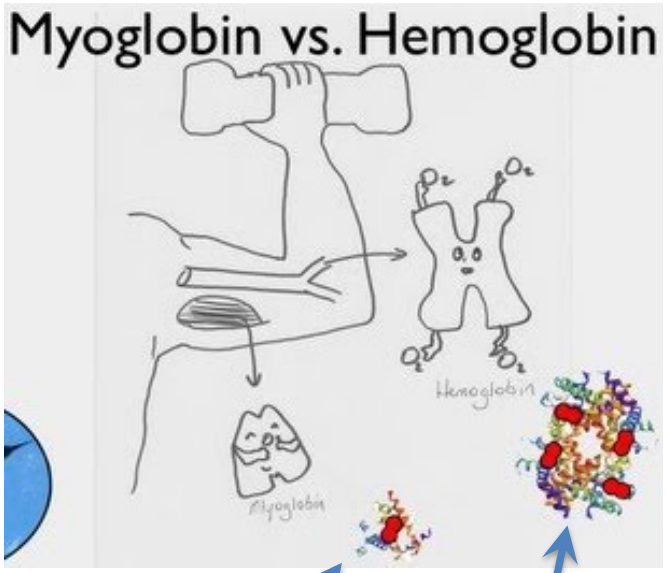

- **(A)** Myoglobin (MHC) in ribbon representation with **one Heme groups (in RED)**
- **(B) Heme structure**

- Oxygen (O_2) from the lung is transported by **Hemoglobin** (Hb), and released to **Myoglobin** in the tissue

Section 4.2: Hemoglobin (Hb) and Cooperativity

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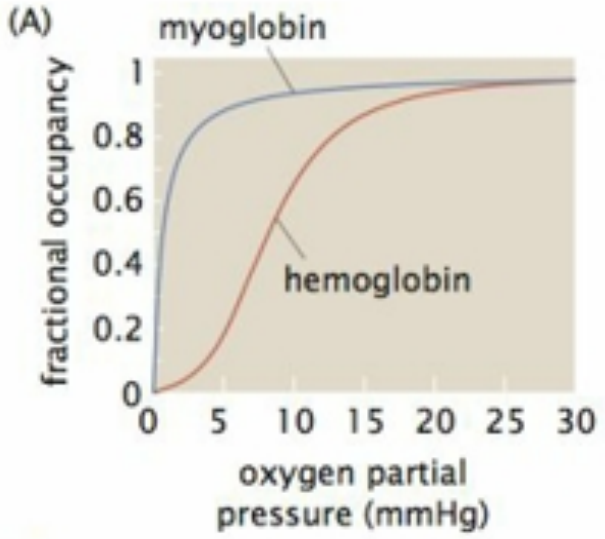

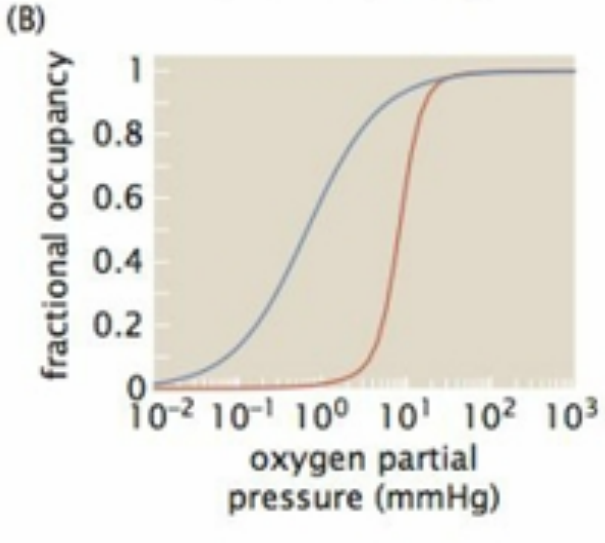

- **Binding of O_2** to **Hemoglobin (Hb)** and **Myoglobin** is cooperative
- For **Hb**, which can bind up to **4 O_2** simultaneously, the curve on the left means that **Hb** will either bind **zero** or **4** O_2, but **never** 1, 2 or 3 oxygen.
- The physical explanation is that the binding of **one** O_2 increase the oxygen affinity of Hb so much that it will readily bind to more O_2
- This **all** or **nothing transition** is termed **cooperativity**.

Section 4.2: Hemoglobin (Hb) and Cooperativity

Cooperativity is a key **concept** in **Biological Functions:**

- Hemoglobin-O_2 binding
- Protein Folding: protein are either **folded** or **not folded**.
- Genetic Control: example is in section 3.2.2 (oscillators and clocks) where cyclin proteins accumulates steadily in a cell, until it exceeds a critical amount, which initiates cell division.

Section 4.2: Hemoglobin (Hb) and Cooperativity

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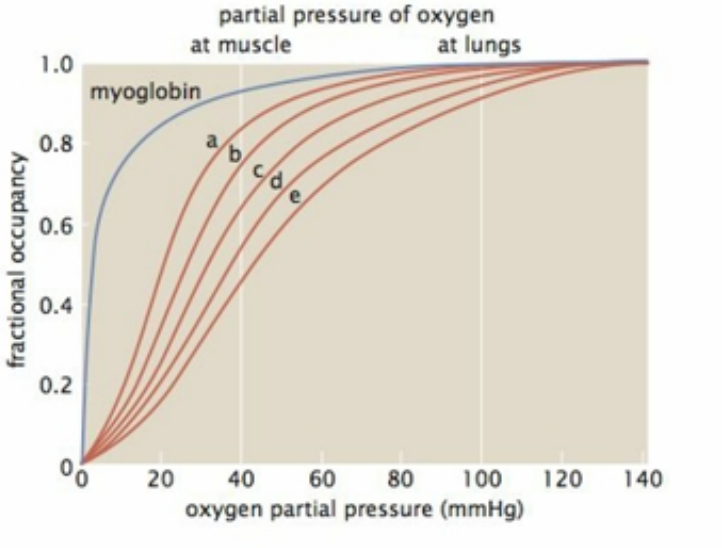
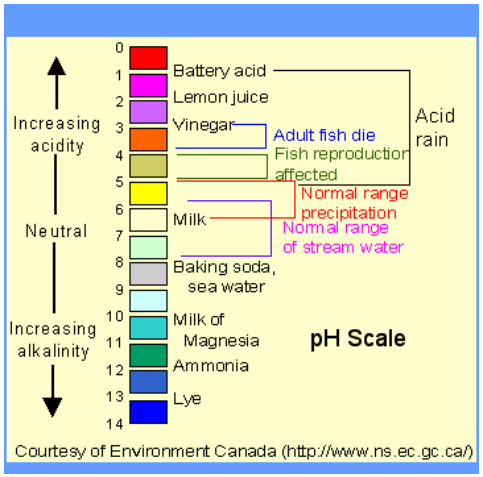


Figure 4.5: Binding curves for oxygen uptake by hemoglobin as a function of pH revealing the Bohr effect. The hemoglobin binding curves are shown for five values of the pH: (a) 7.5, (b) 7.4, (c) 7.2, (d) 7.0, and (e) 6.8. The vertical lines indicate the partial pressures experienced in muscle and in the lungs. (Adapted from R. E. Dickerson and I. Geis, Hemoglobin: Structure, Function, Evolution and Pathology. Benjamin/Cummings, 1983.)



Bohr Effect: Christian Bohr (1904)

- **Binding of O_2** to **Hemoglobin (Hb)** binding affinity to O_2 decreases with acidity (low pH) and increasing concentration of CO_2

Section 4.2: Hemoglobin by the Numbers

- Average of 5L of blood in a human
- Mass density of blood$\rho = 1060\,\frac{kg}{m^3}$
- $\sim 5\times10^{6}$ RBC per μL of blood
- 15 g of hemoglobin (Hb) per deciliter of blood
- 25×10^{12} RBC and 750 g of hemoglobin in an average adult
- If one hemoglobin is about 64000 Dalton, giving 7×10^{21}Hb in average adult
- About 3×10^{8} Hb in on eRBC

Section 4.3: Bacteriophages and Molecular Biology, Proof of Central Dogma

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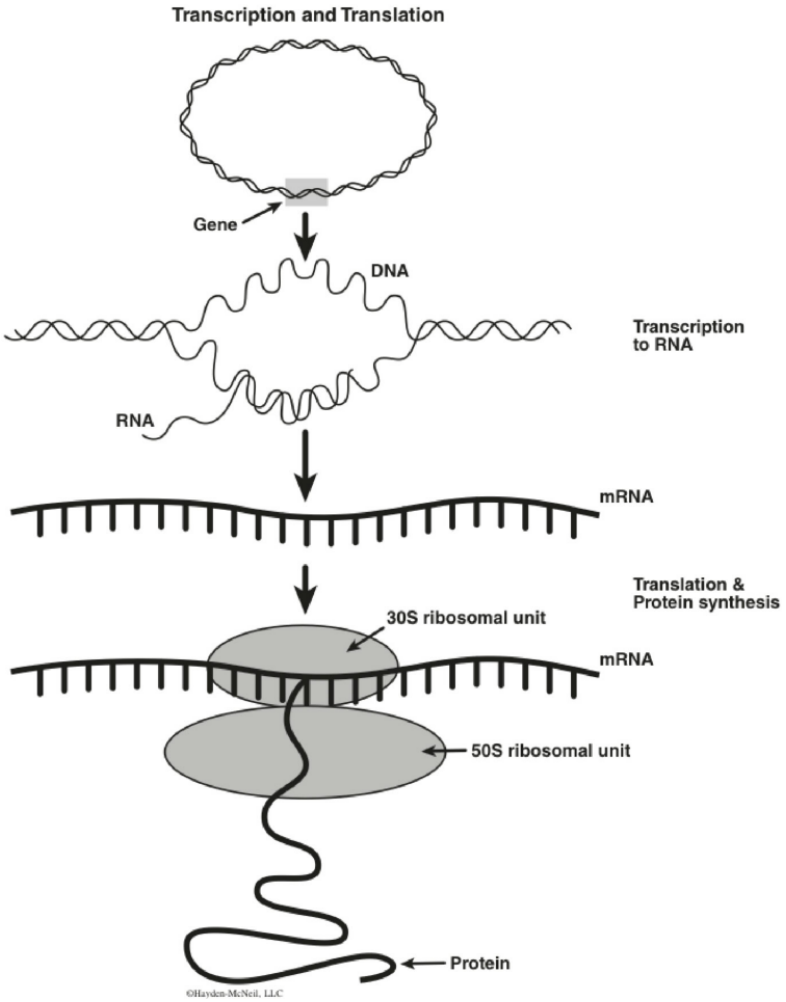

Transcription and Translation

- **Genotypes is coded in DNA** and proteins bring about **Phenotypes**
- Where is the proof that DNAs and not proteins are the code of life?

Section 4.3: Bacteriophages and Molecular Biology, Proof of Central Dogma

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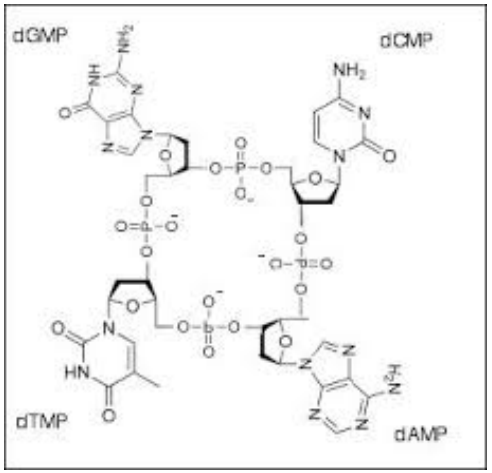
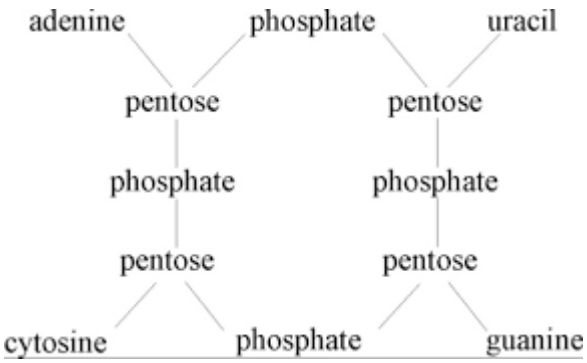


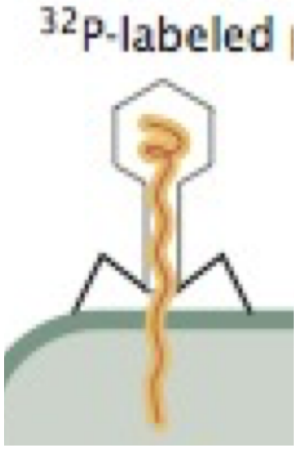


Tetranucleotide Hypothesis

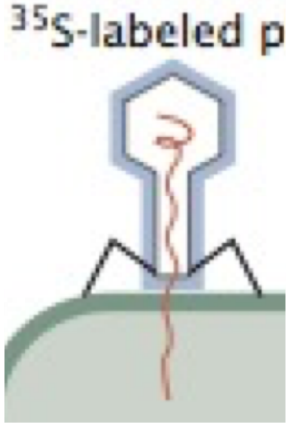
- Proposed that DNA was made up of equal amount of A, G, C, T bases
- Code of life is written on the **proteins** part of **chromosomes**.

Section 4.3: Bacteriophages and Molecular Biology, Proof of Central Dogma

Proof that **code** of **life (genotype) encoded** in **DNA/RNA: Hershey-Chase experiment**



Phage Viruses multiply by inserting RNAs (their genetic materials) into a Bacteria, and using the bacteria's machineries to produce RNAs and proteins needed to replicate.



- Viruses are grown in media with **radioactive** phosphorous ^{32}P
- Only **RNAs** contain **Phosphorous**

$$^{32}_{17}P \longrightarrow {}^{32}_{16}S + {}^{0}_{-1}e + \nu + 1.71\ \text{MeV}$$

- Viruses are grown in media with **radioactive sulfur** ^{35}S
- Only **proteins** contain Sulfur

$$^{35}_{16}S \rightarrow {}^{0}_{-1}e + {}^{35}_{17}Cl + \bar{\nu}$$

Section 4.3: Bacteriophages and Molecular Biology, Proof of Central Dogma

Proof that **code** of **life (genotype) encoded** in **DNA/RNA: Hershey Chase experiment**