Test your knowledge of reaction energetics:

Given the following *condensation* reaction:

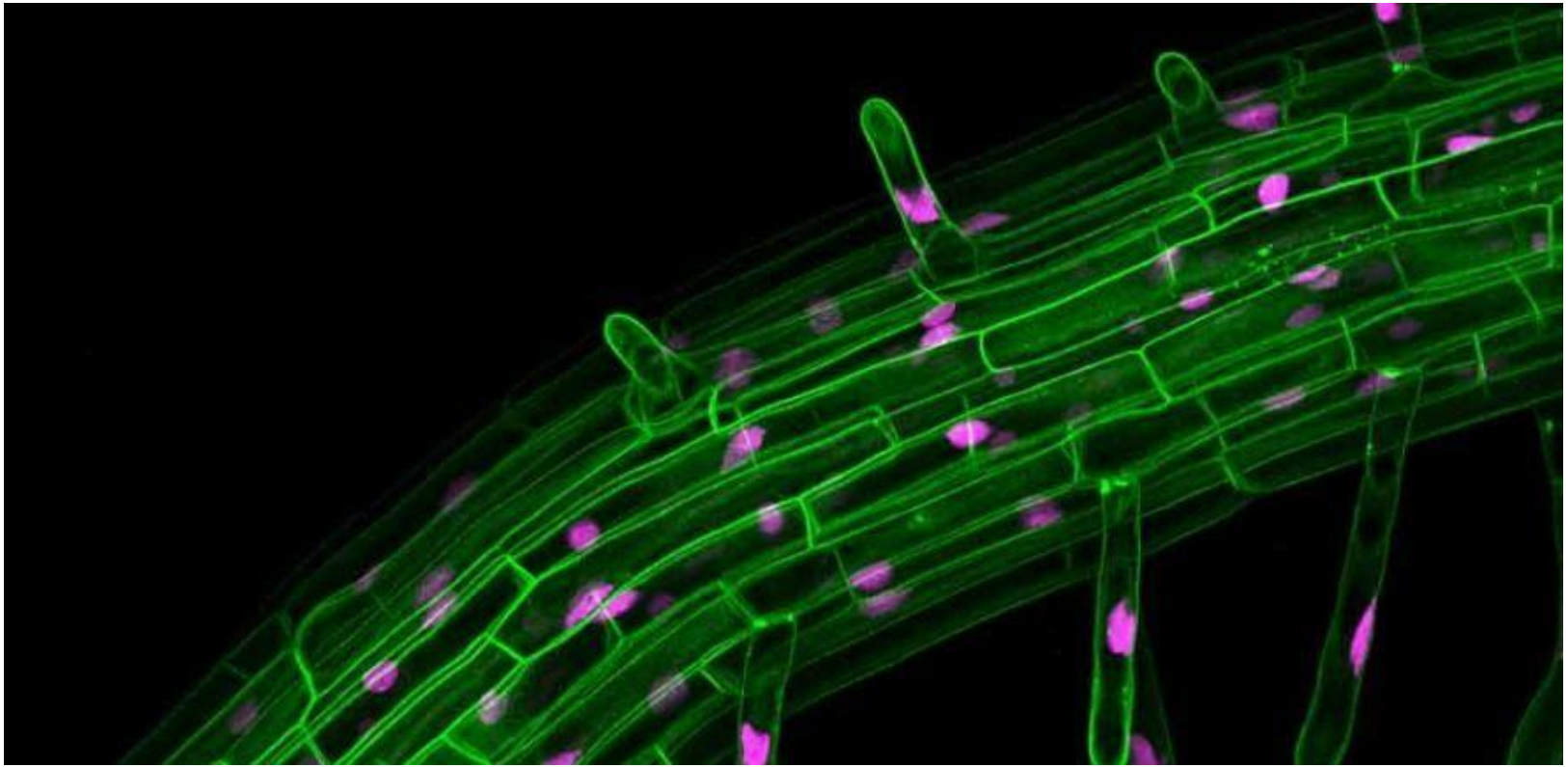
$$Glucose + Fructose \rightarrow Sucrose + H_2O;\ \Delta G = -23\ kJ/mol$$

Is the *hydrolysis* (*hydro*- water; *lysis*- to break) of sucrose a spontaneous reaction?

Draw a free energy diagram for this reaction showing the respective energy levels of reactants, products, and the transition state.

What would happen if we coupled the reaction to the hydrolysis of ATP ($\Delta G = -30.5\ kJ/mol$)?

Would coupling the reaction to ATP result in the stabilization of the transition state in the sucrose hydrolysis reaction?



Chapter 4 Part 1: Protein Structure, Function, and Control
Dr. Matthew Ellis

Learning Objectives for Chapter 4 Part 1:

Upon completing this module, **you should be able to**:

1) Understand the shape and structure of proteins (primary, secondary, tertiary, quaternary).

2) Describe the different regulatory control mechanisms of protein activity (inhibition, allostery, active vs. inactive forms).

3) Apply the above to the functionality of the molecular machine hemoglobin.

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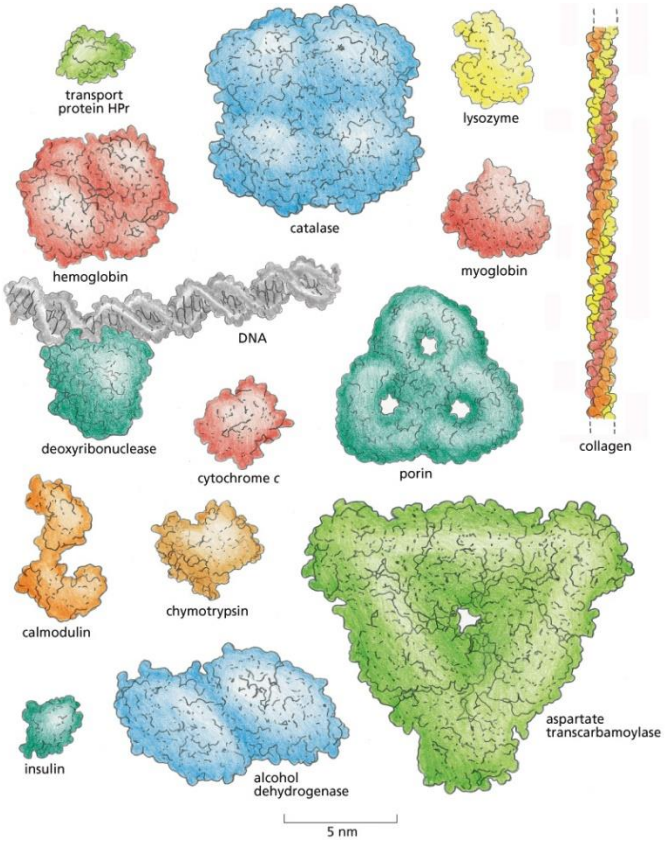
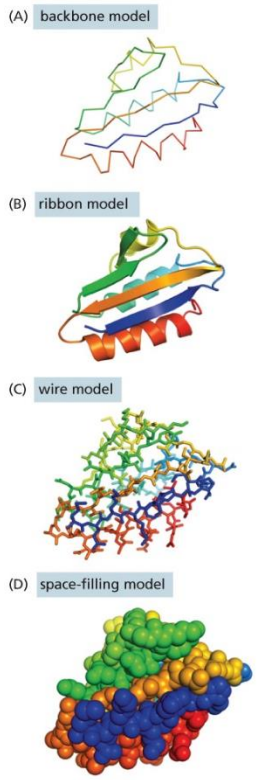
Proteins come in many shapes and are visualized in various formats

- Shape is largely determined by energy.
- Proteins will “fold” into a shape in which free energy retained is minimized – thus *energetically favorable* as it releases heat and increases entropy





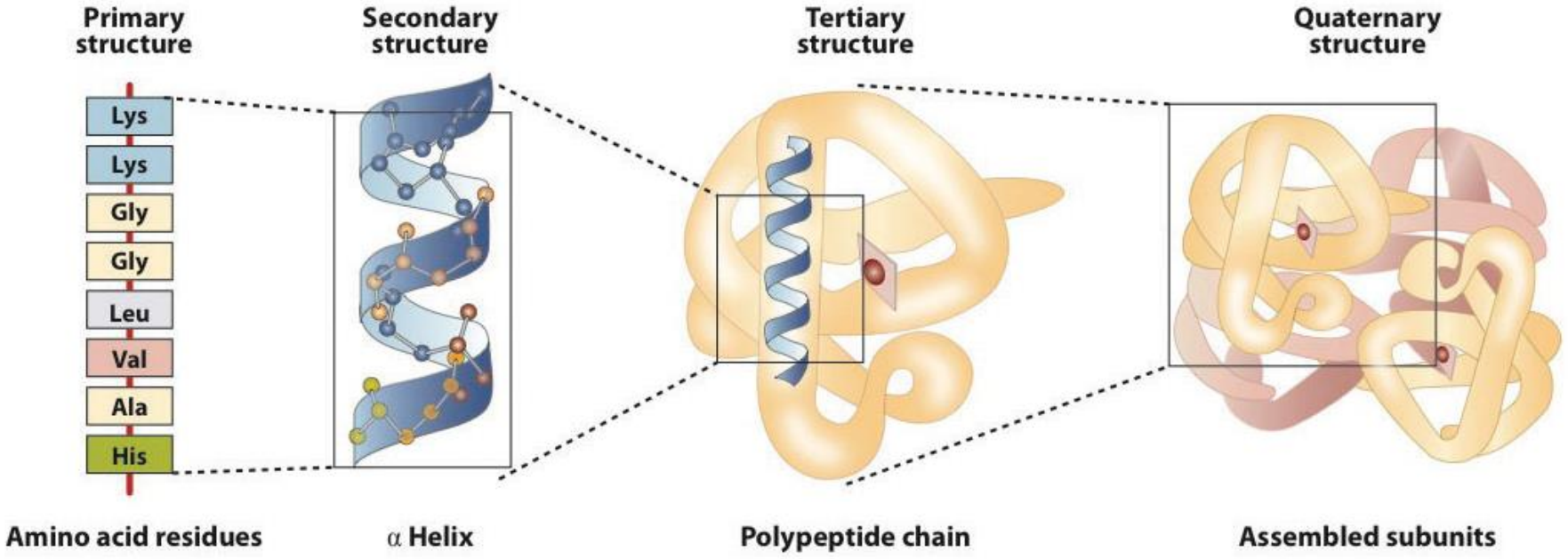
Levels of *protein structure*:

The sequence of amino acids in a protein

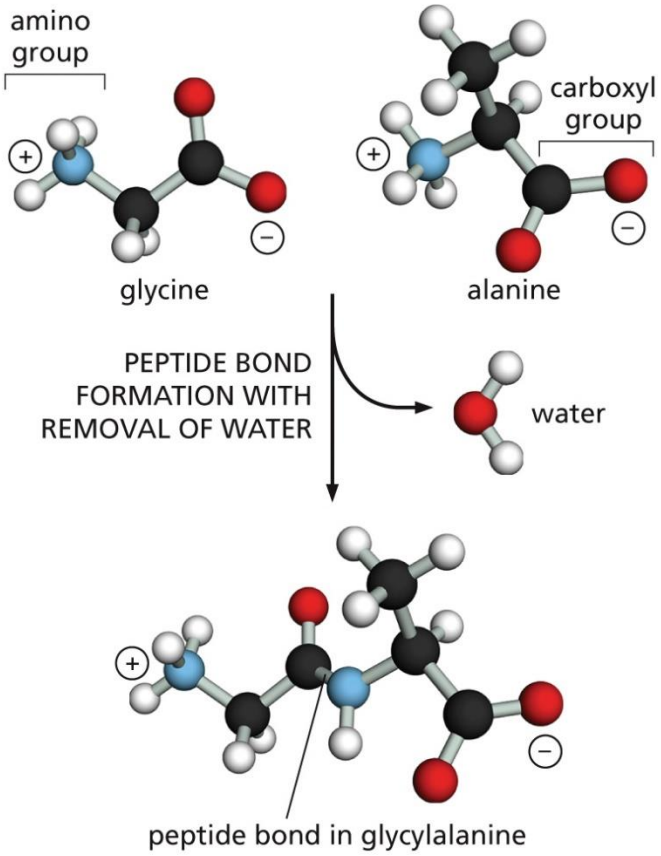
Intramolecular interactions along the *backbone* of the protein which form characteristic shapes (α-helices and β-sheets)

Three-dimensional structure of the protein, including side chain (amino acid) interactions

Interactions between multiple polypeptides to form a multimeric protein



Amino acids are linked together by covalent peptide bonds



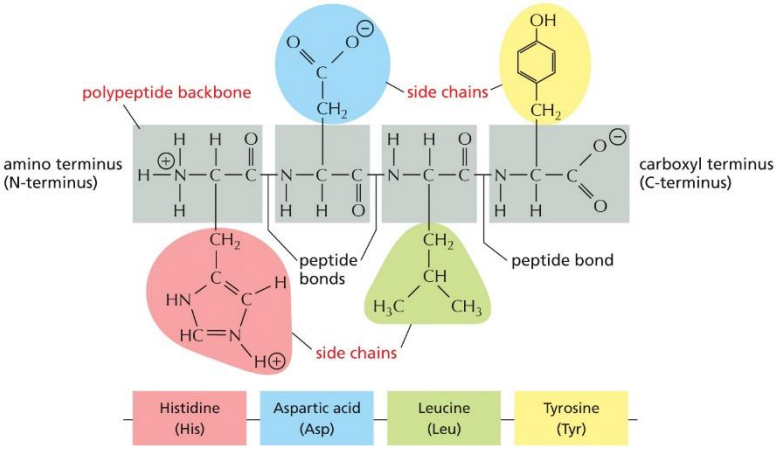
Primary Structure: Amino acid sequence from the N-terminus to the C-terminus

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negatively charged
Glutamic acid	Glu	E	negatively charged
Arginine	Arg	R	positively charged
Lysine	Lys	K	positively charged
Histidine	His	H	positively charged
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

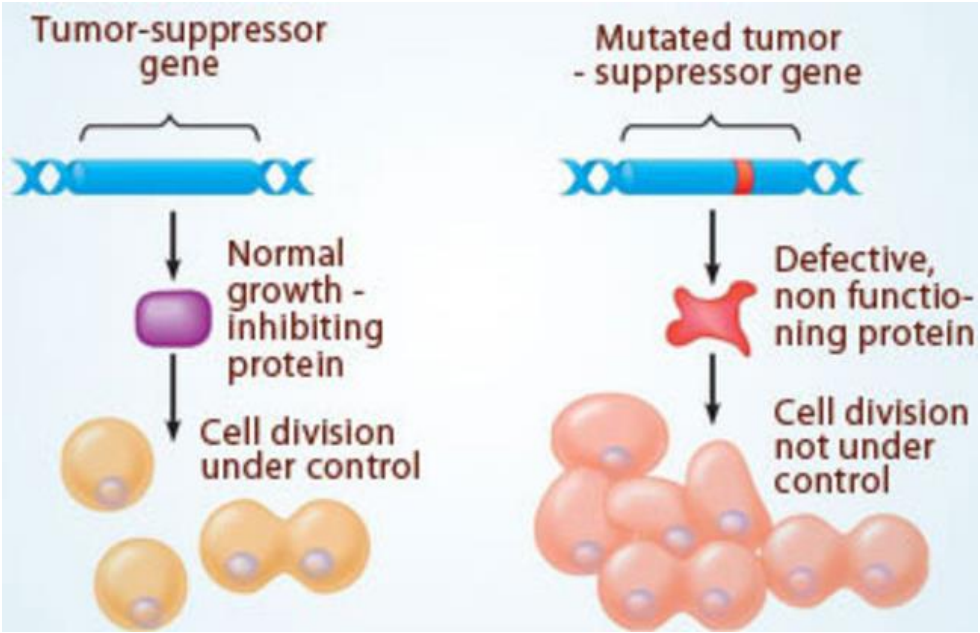
POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS



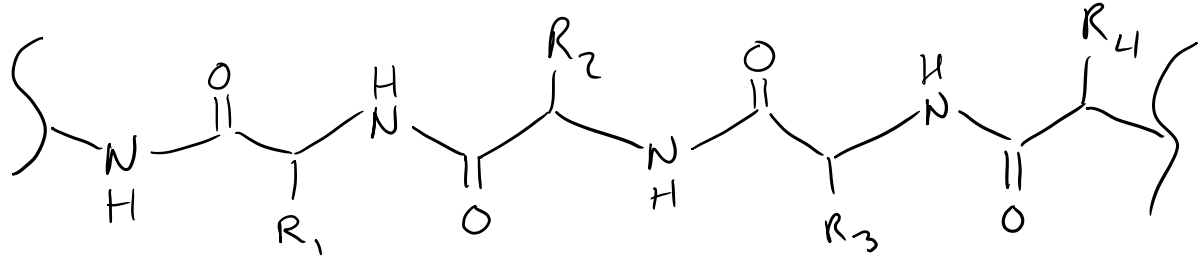
Irregularities in Protein Primary Sequence Lead to Disease



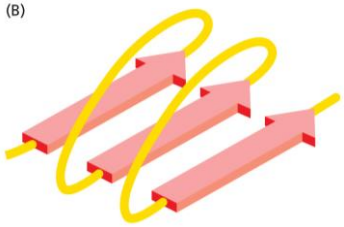
https://www.wonderwhizkids.com/cancer

***Secondary Structure*: protein backbone interactions**
The β-sheet

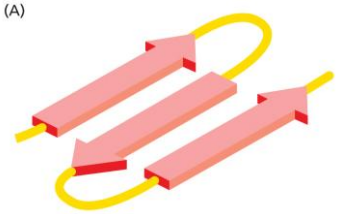
- Basic functional unit of a protein:



- β-sheets consist of two "rows" of these functional units aligned through *intramolecular hydrogen bonds*. They can be arranged in parallel or antiparallel configurations, as seen below:



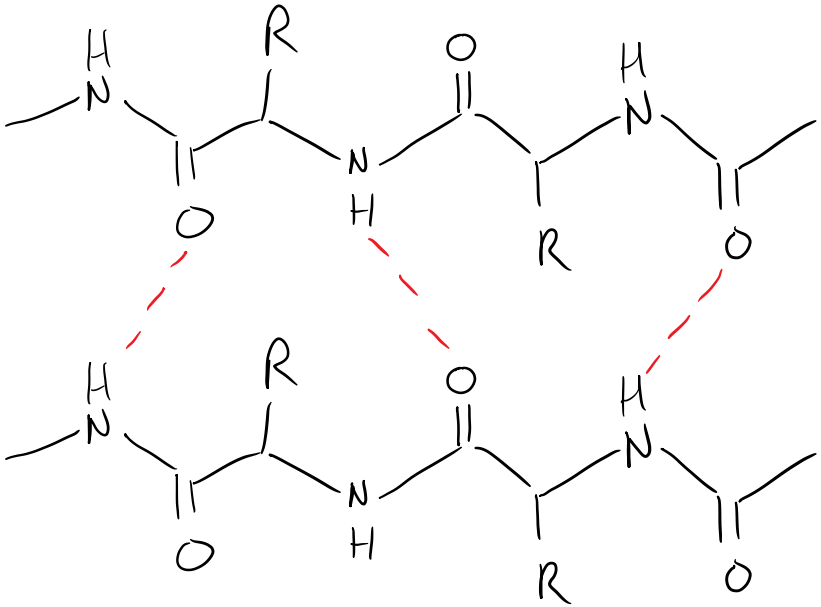
Parallel



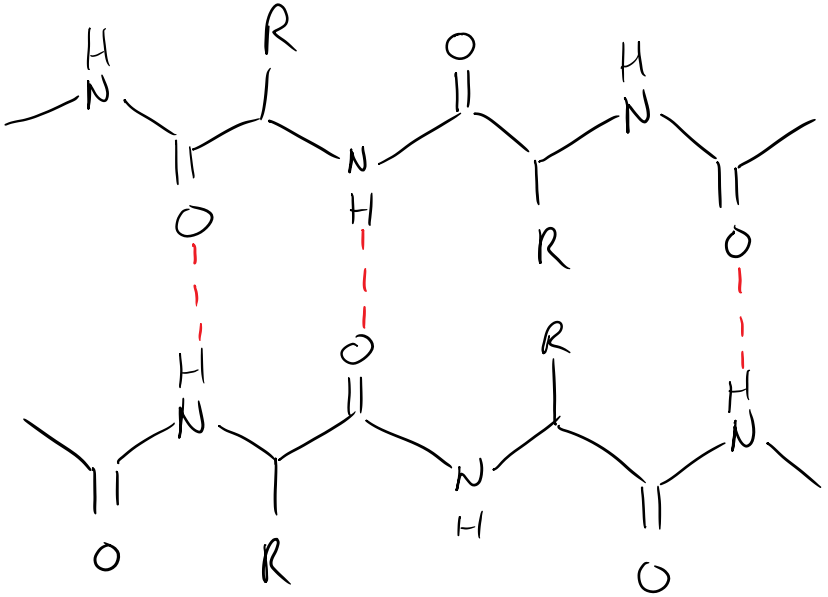
Anti-parallel

Hydrogen bonding in β-sheets

Parallel Configuration



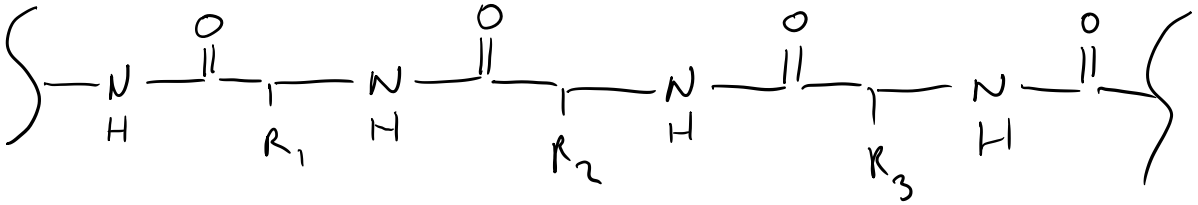
Antiparallel Configuration



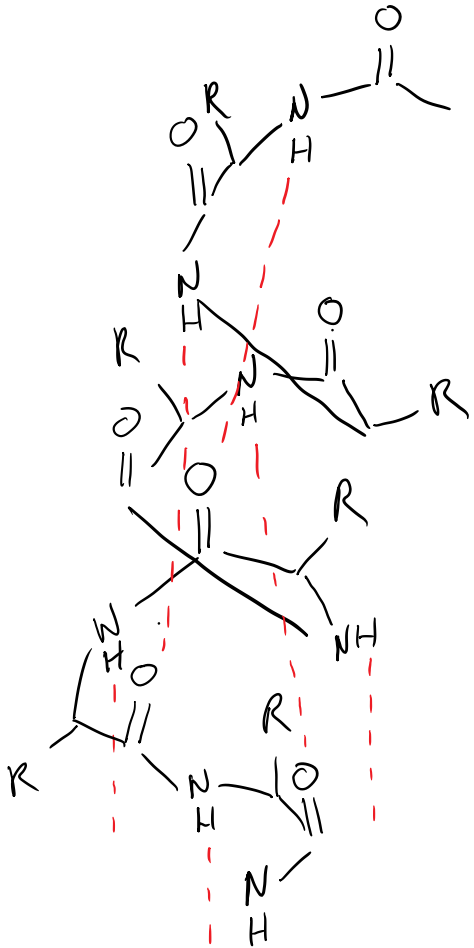
***Secondary Structure*: protein backbone interactions**
The α-helix

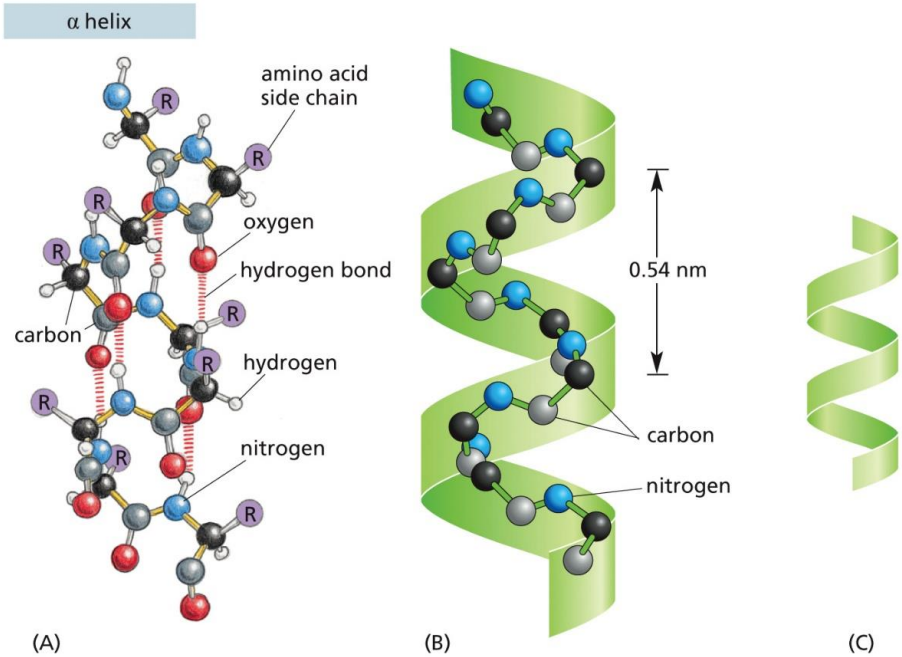
- α-helices similarly take advantage of hydrogen bonding interactions, however now each –NH– group will interact with the double bonded oxygen 3-4 residues (R-group containing functional unit) away

- Again, the basic functional unit of a protein backbone may orient itself to form an α-helix:



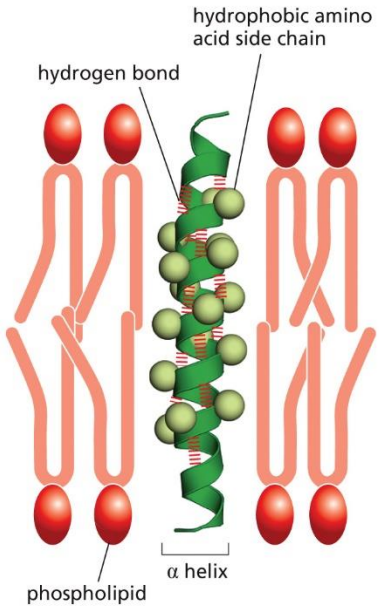
α-helices



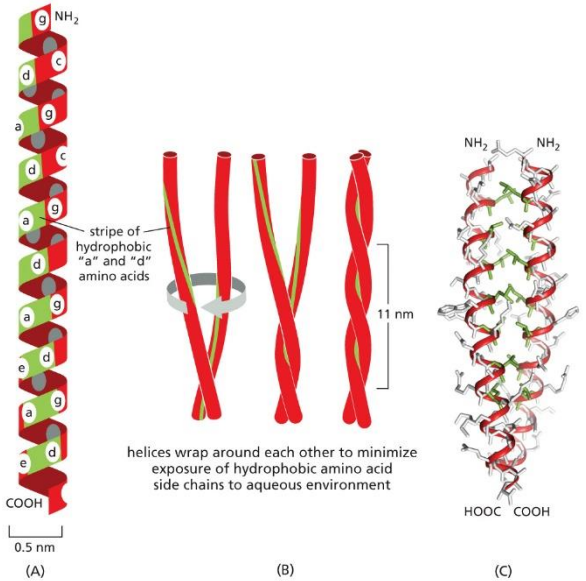

α helix
amino acid
side chain
oxygen
hydrogen bond
carbon
hydrogen
nitrogen
0.54 nm
carbon
nitrogen
(A)
(B)
(C)

Properties of α–helices

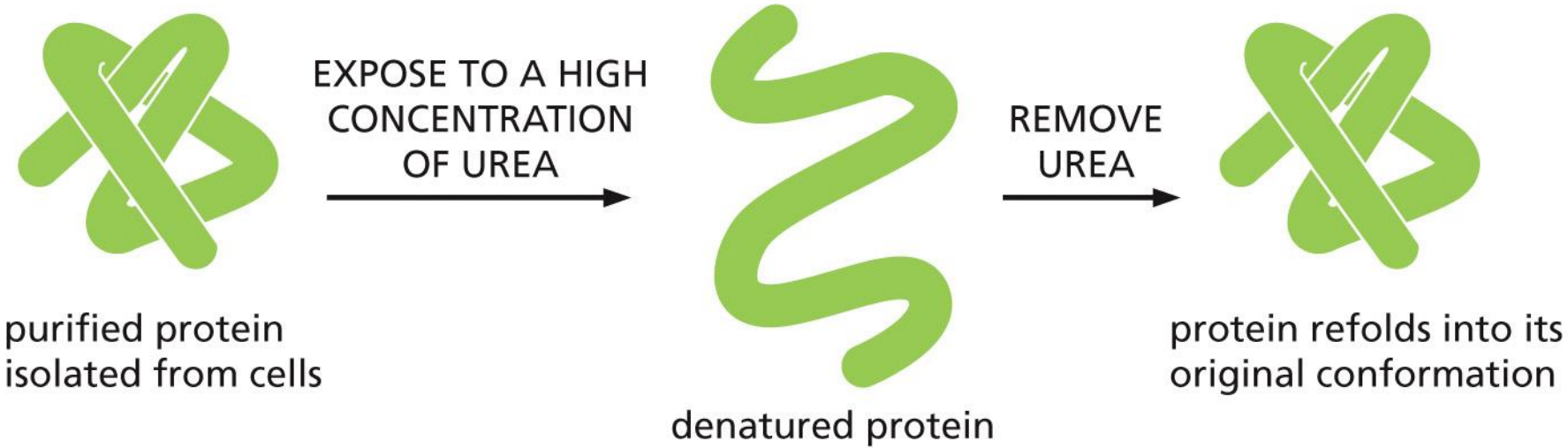
α helices can insert into membranes, useful for proteins with cell membrane functionality



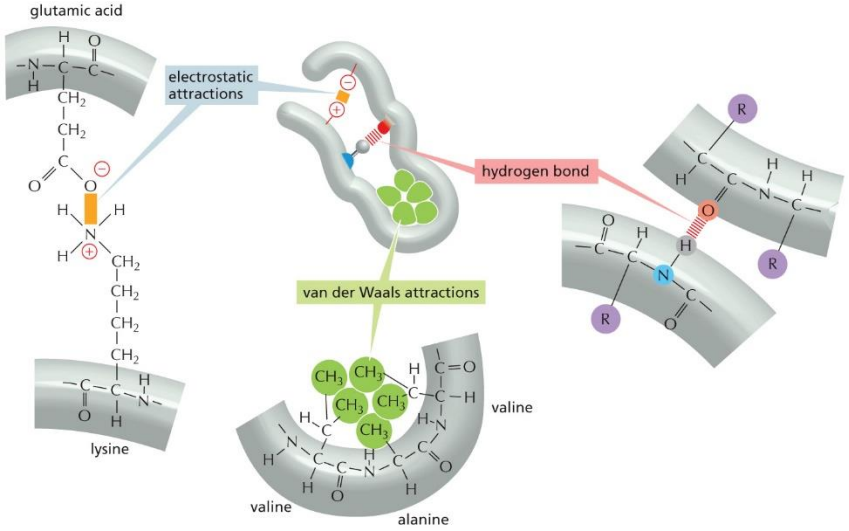
α helices can combine to form stiff coiled coils, providing enhanced strength and functionality to the protein

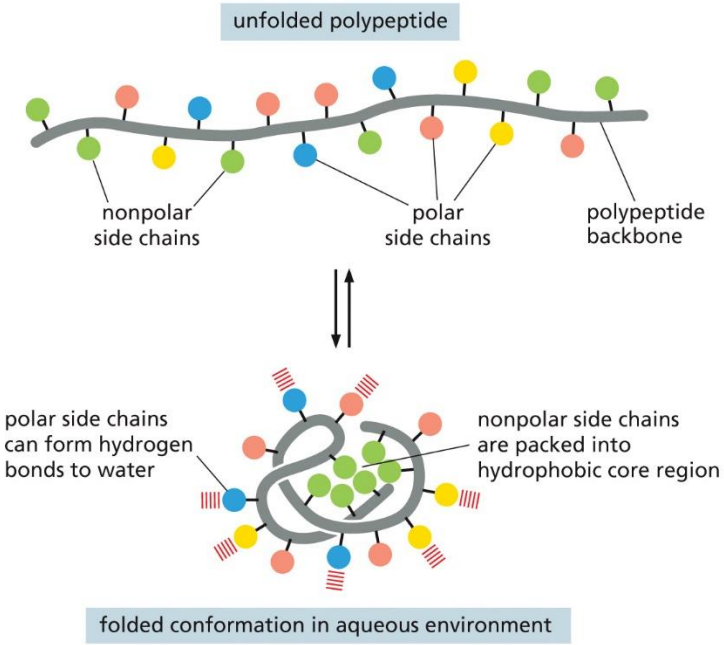


***Tertiary Structure*: Amino acid side chain interactions further specify shape**

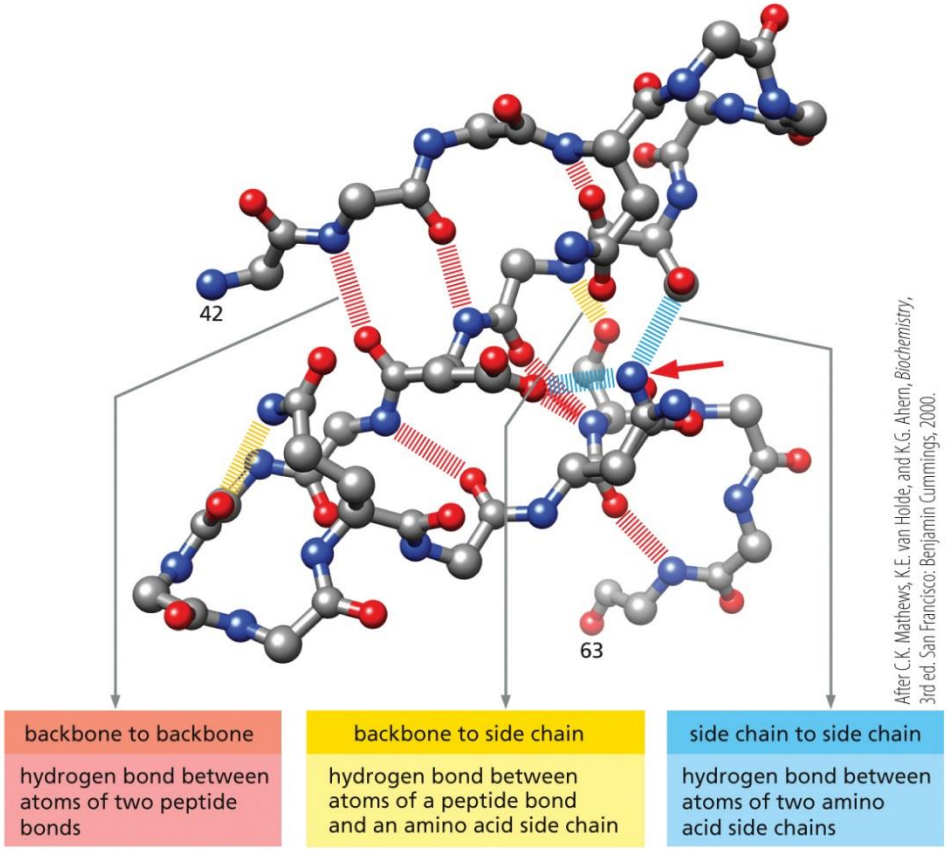


Tertiary Structure: Amino acid side chain interactions further specify shape

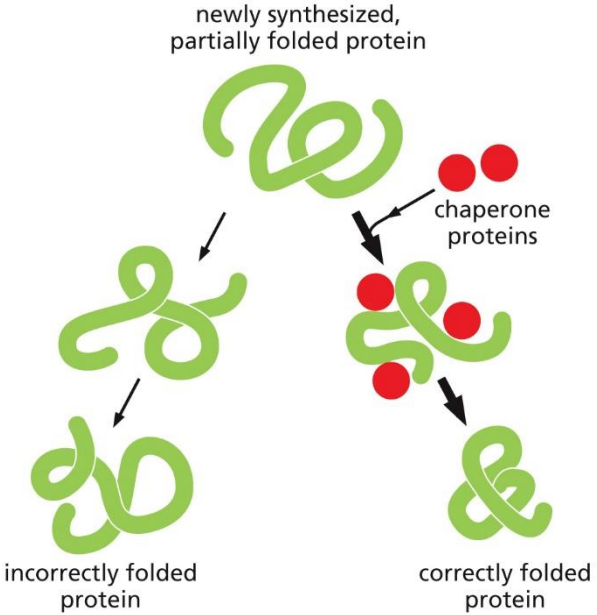


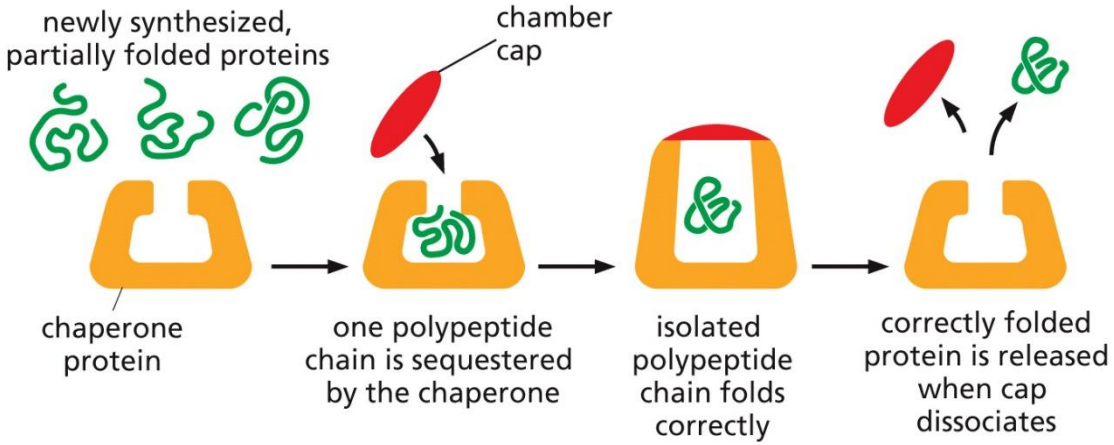


Hydrogen bonds play an extra important role in stabilizing proteins upon folding



Specialized proteins called chaperone proteins aid in proper folding of proteins.

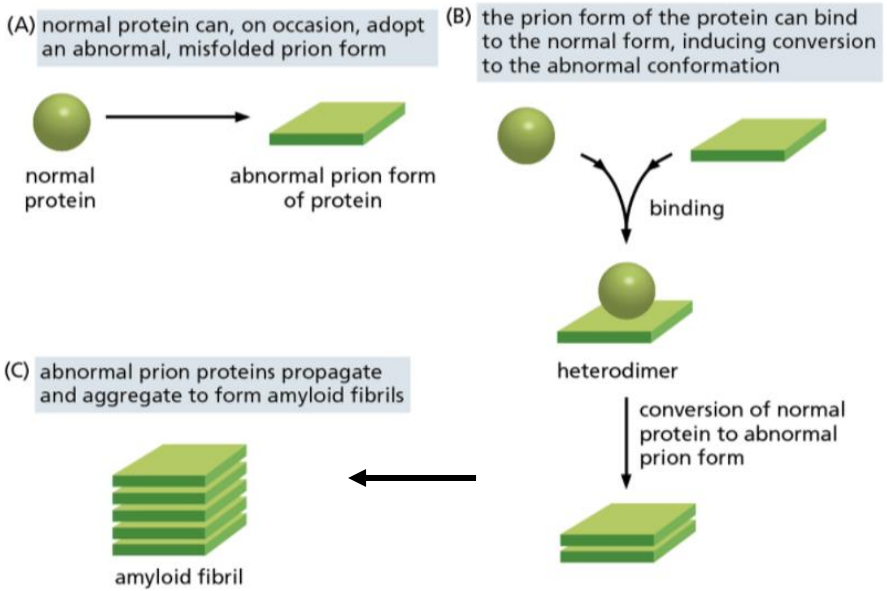




Misfolded proteins can contribute to disease

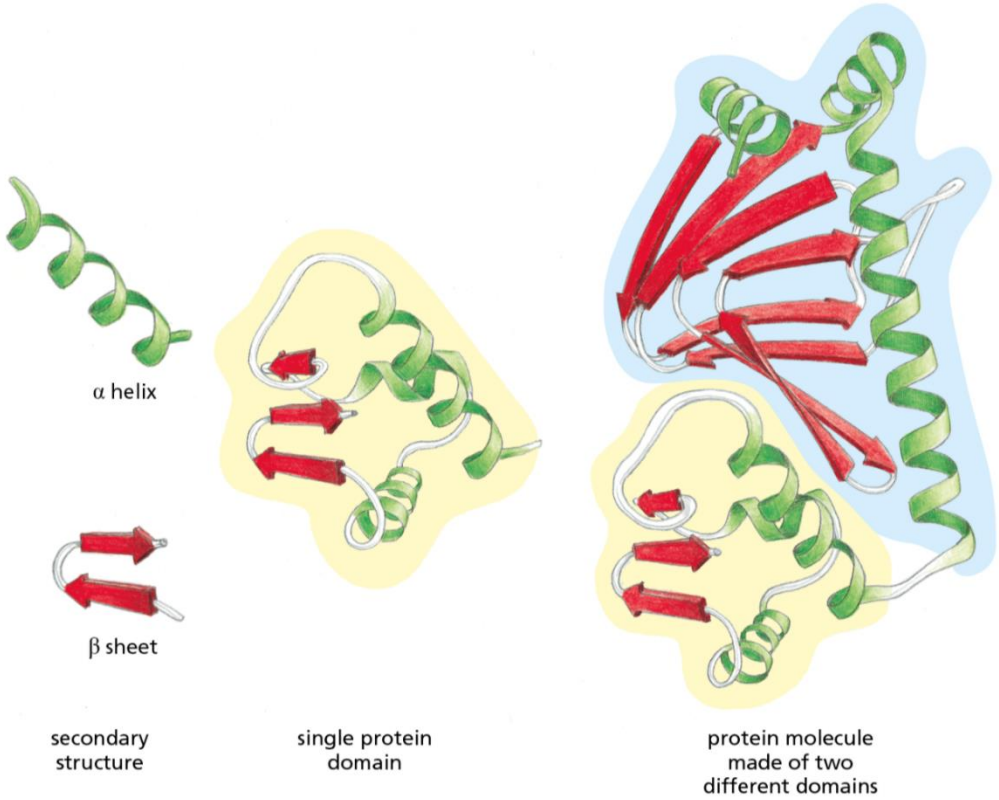
Creutzfeldt-Jakob ("Mad Cow") Disease

"infectious" prions



Prions: misfolded proteins that have the ability to transmit their misfolded shape onto normal variants of the same protein

Many proteins are composed of separate ***functional domains***

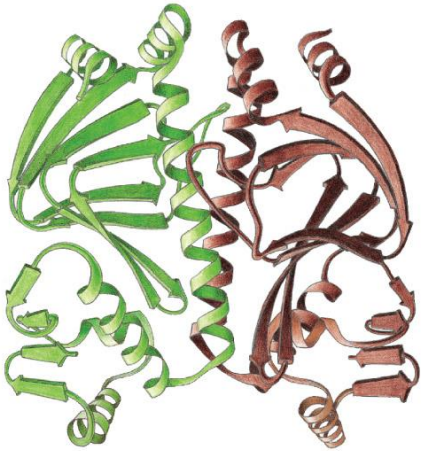


- Domains are any segment of a polypeptide chain that can fold and function independently
- Different domains are associated with different functions

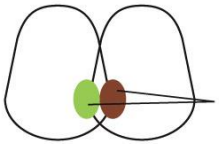
Example of Bacterial CAP protein:

Blue region: cyclic-AMP binding domain
Yellow region: DNA binding domain

Quaternary Structure: Protein molecules often contain more than one polypeptide chain (subunit)

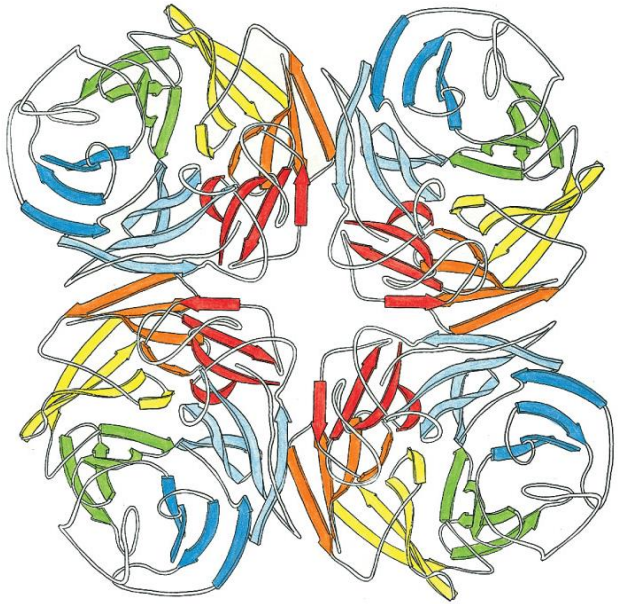


dimer of the CAP protein

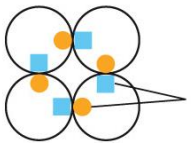


dimer formed by interaction between a single, identical binding site on each monomer

(A)



tetramer of neuraminidase protein



tetramer formed by interactions between two nonidentical binding sites on each monomer

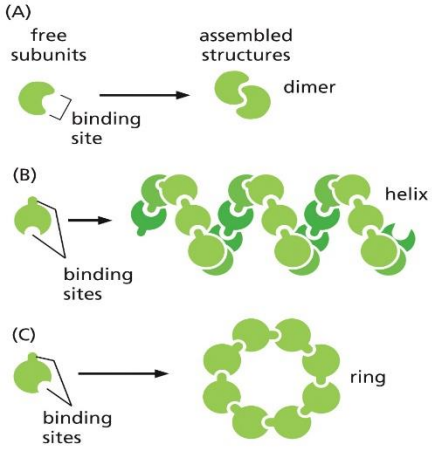
(B)

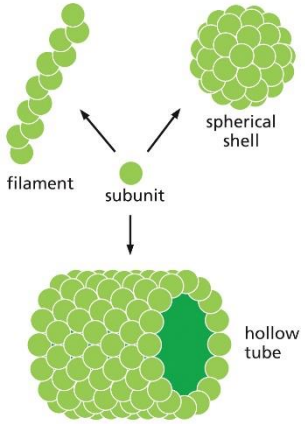
**In general these polypeptide subunits are held together by many non-covalent bonds (binding site)*

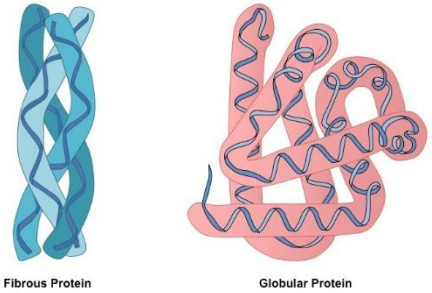
Identical protein structures can assemble into complex structures: *macromolecular machines*

- Proteins can generally form into filaments, tubes, or spheres depending on arrangement of binding sites

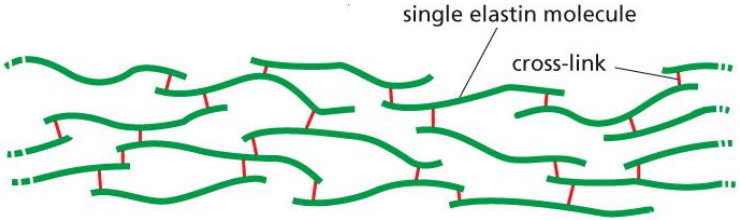
- Proteins generally form shapes that are associated with its distinct function in a cell:
  - **fibrous** are elongated and span long distances
  - **globular** are round and compact

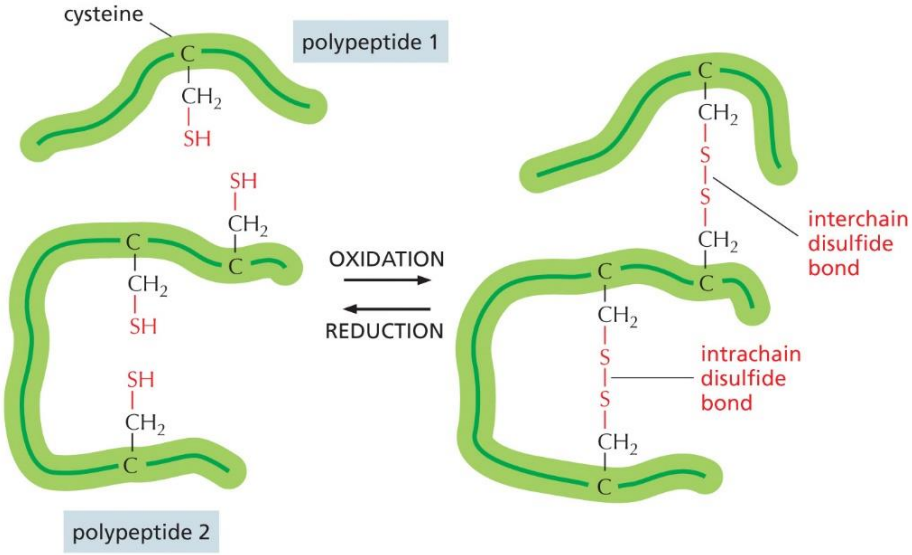






Some proteins are stabilized by *covalent crosslinks* (extracellular proteins, antibodies)

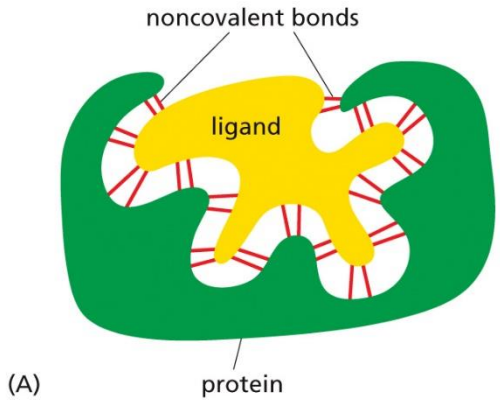


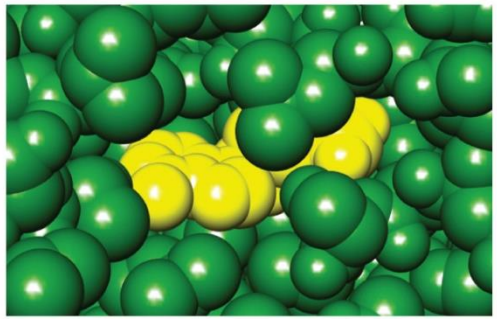


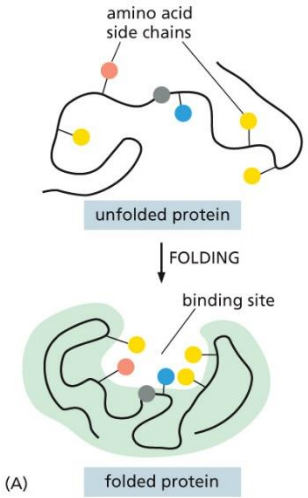
- *Disulfide bonds* (sulfur-sulfur bonds between cysteine amino acid residues) can link two amino acids from the same protein together or join multiple chains in a large protein complex
  - Helps to maintain and stabilize tertiary and quaternary structures

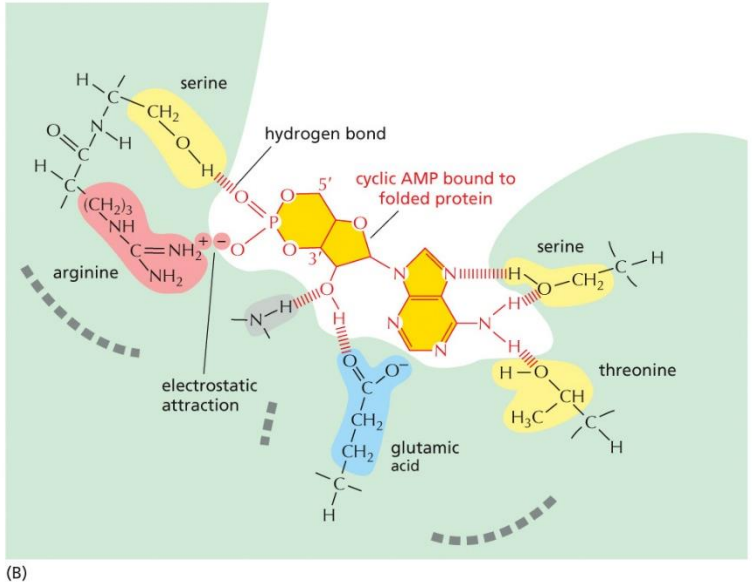
- Disulfide bonds are broken by reducing agents (mercaptoethanol or Dithiothreitol (DTT) commonly used in labs)
  - More on this next lecture!

All proteins bind to other molecules (ligands) through *binding sites*









- Binding site is a cavity within surface formed by specific arrangement of amino acid side chains and noncovalent interactions brought together during folding
  - Region that binds ligands will be different for different ligands

SQUARECAP Q#1-2

Learning Objectives for Chapter 4 Part 1:

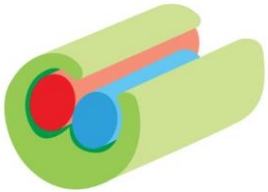
Upon completing this module, **you should be able to**:

1) Understand the shape and structure of proteins (primary, secondary, tertiary, quaternary).

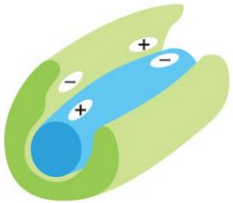
2) Describe the different regulatory control mechanisms of protein activity (inhibition, allostery, active vs. inactive forms).

3) Apply the above to the functionality of the molecular machine hemoglobin.

Recall: enzymes are highly specialized catalysts that greatly speed up the rate of chemical reactions



(A) enzyme binds to two substrate molecules and orients them precisely to encourage a reaction to occur between them



(B) binding of substrate to enzyme rearranges electrons in the substrate, creating partial negative and positive charges that favor a reaction

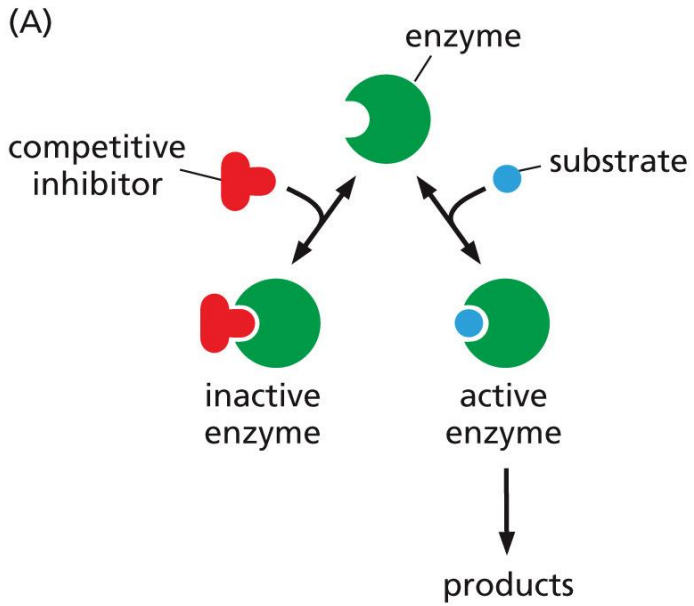
(C) enzyme strains the bound substrate molecule, forcing it toward a transition state that favors a reaction

TABLE 4–1 SOME COMMON FUNCTIONAL CLASSES OF ENZYMES

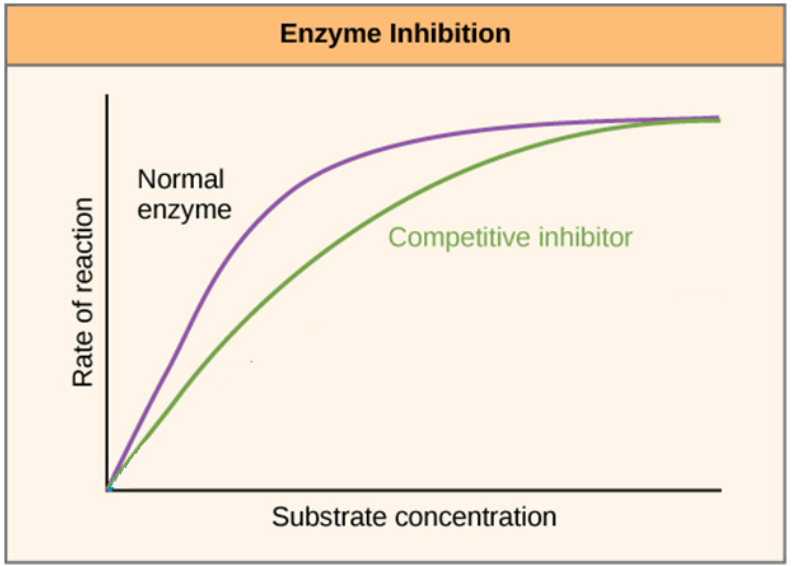
Enzyme Class	Biochemical Function
Hydrolase	General term for enzymes that catalyze a hydrolytic cleavage reaction
Nuclease	Breaks down nucleic acids by hydrolyzing bonds between nucleotides
Protease	Breaks down proteins by hydrolyzing peptide bonds between amino acids
Ligase	Joins two molecules together; DNA ligase joins two DNA strands together end-to-end
Isomerase	Catalyzes the rearrangement of bonds within a single molecule
Polymerase	Catalyzes polymerization reactions such as the synthesis of DNA and RNA
Kinase	Catalyzes the addition of phosphate groups to molecules. Protein kinases are an important group of kinases that attach phosphate groups to proteins
Phosphatase	Catalyzes the hydrolytic removal of a phosphate group from a molecule
Oxido-reductase	General name for enzymes that catalyze reactions in which one molecule is oxidized while the other is reduced. Enzymes of this type are often called oxidases, reductases, or dehydrogenases
ATPase	Hydrolyzes ATP. Many proteins have an energy-harnessing ATPase activity as part of their function, including motor proteins such as myosin (discussed in Chapter 17) and membrane transport proteins such as the Na^+ pump (discussed in Chapter 12)

Enzyme names typically end in "-ase," with the exception of some enzymes, such as pepsin, trypsin, thrombin, lysozyme, and so on, which were discovered and named before the convention became generally accepted, at the end of the nineteenth century. The name of an enzyme usually indicates the nature of the reaction catalyzed. For example, citrate synthase catalyzes the synthesis of citrate by a reaction between acetyl CoA and oxaloacetate.

Drugs can inhibit enzyme activity: ***competitive inhibition***



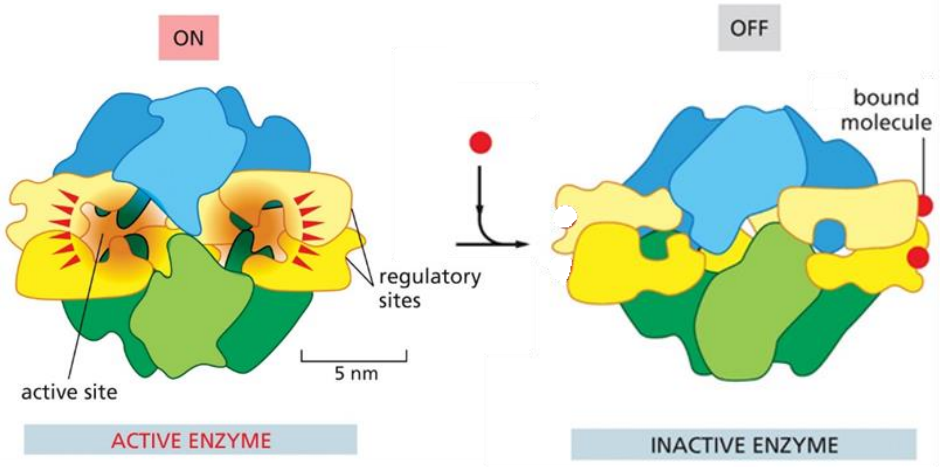
Rate of enzymatic product formation

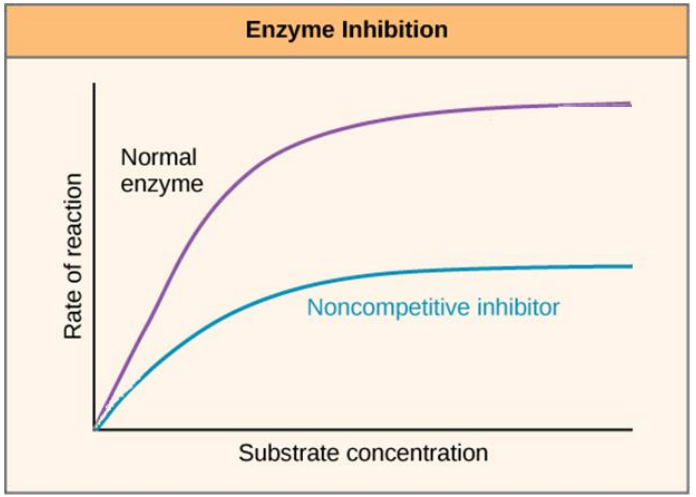


- A competitive inhibitor binds to the same enzyme active site as the substrate, this:
  - Slows down the reaction rate
  - Requires more substrate that normally necessary to oversaturate the system

https://www.khanacademy.org/science/ap-biology/cellular-energetics/environmental-impacts-on-enzyme-function/a/enzyme-regulation

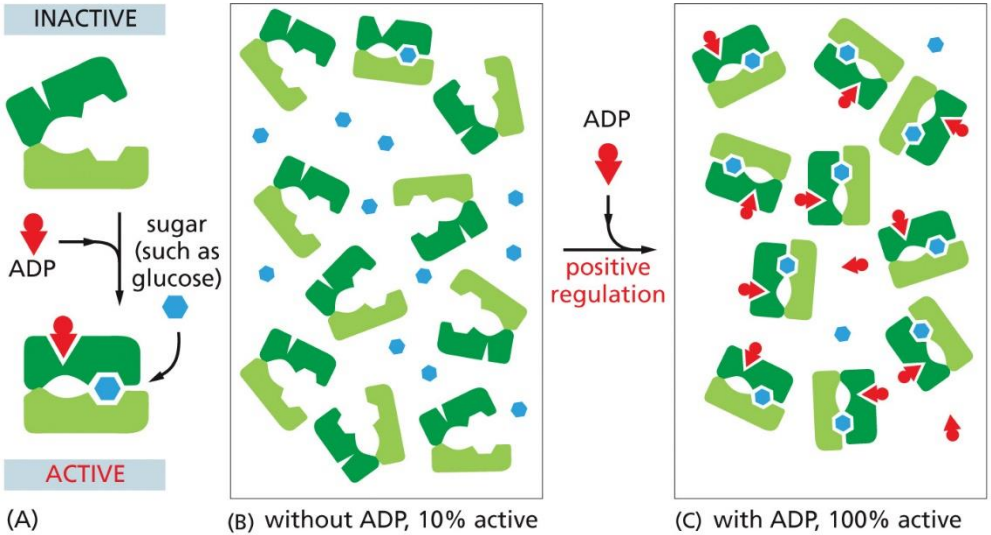
Allosteric **sites: alternative sites away from the active site can also regulate enzyme activity**



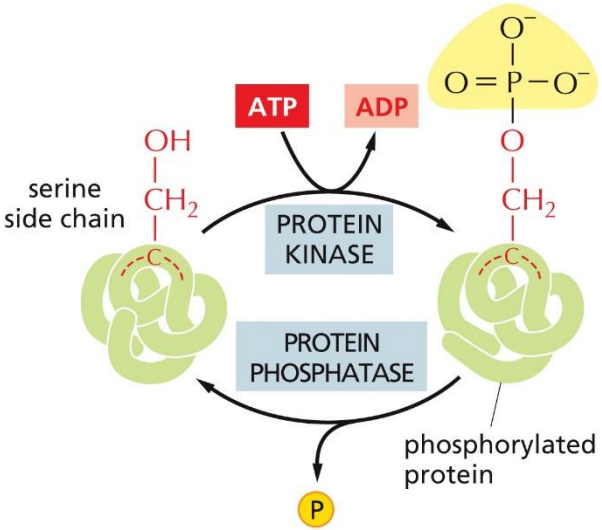


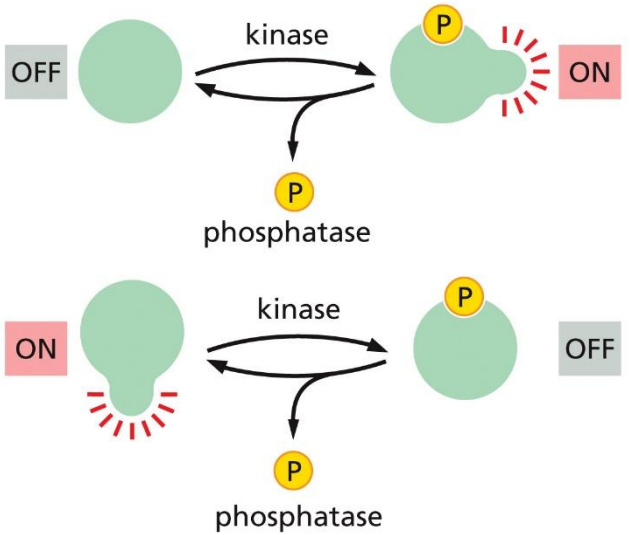
- A *non-competitive inhibitor* binds to an allosteric site, preventing product formation
  - Leads to a lower maximum reaction rate as increasing substrate concentration cannot overcome inactivity of enzymes

Allosteric sites can also augment enzymatic activity through *cooperativity*



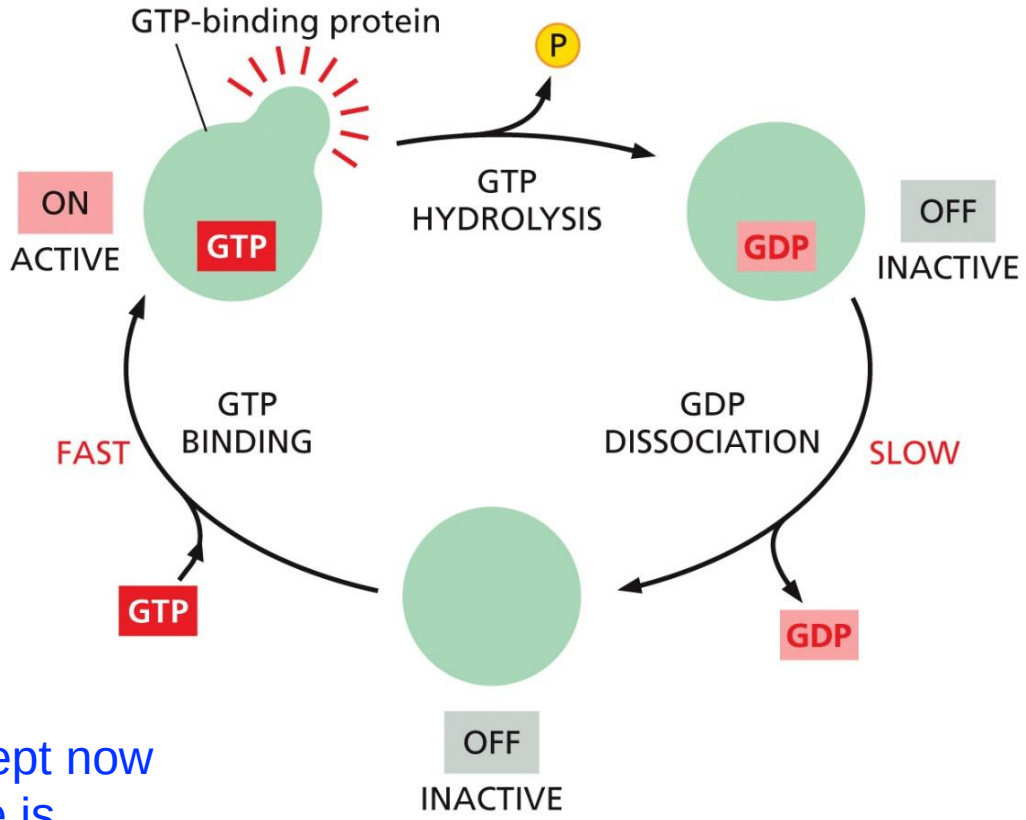
Phosphorylation **(addition of a phosphate group) is a common molecular "switch" regulating protein activity**





Phosphorylation can increase or decrease activity of target protein

GTP-binding proteins form another class of molecular switches



GTP is like ATP, except now the nitrogenous base is guanine, rather than adenine

SQUARECAP Q#3-4

Learning Objectives for Chapter 4 Part 1:

Upon completing this module, **you should be able to**:

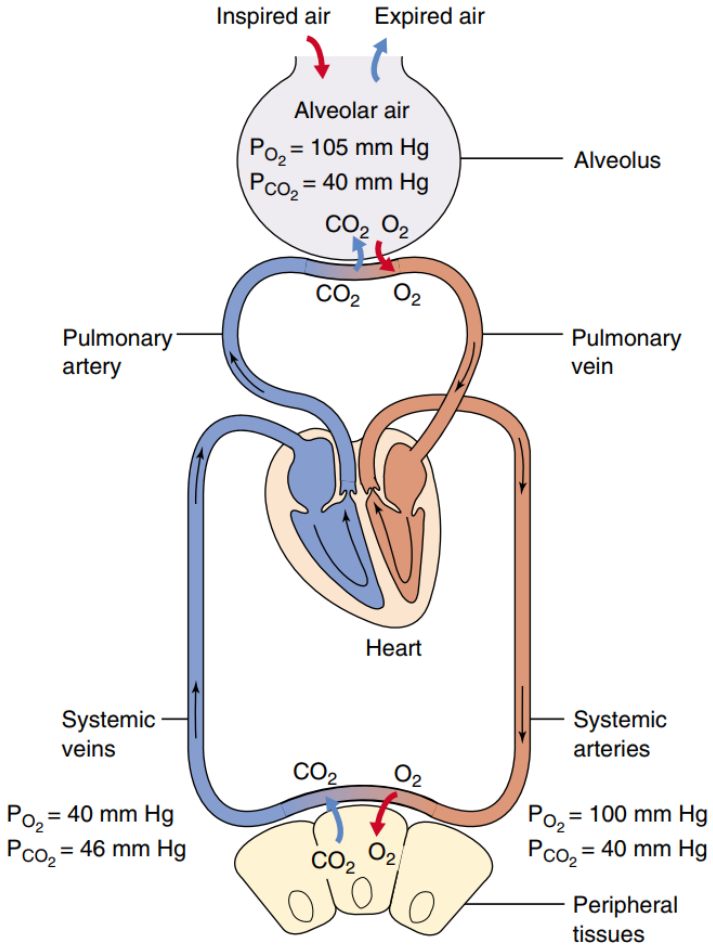
1) Understand the shape and structure of proteins (primary, secondary, tertiary, quaternary).

2) Describe the different regulatory control mechanisms of protein activity (inhibition, allostery, active vs. inactive forms).

3) Apply the above to the functionality of the molecular machine hemoglobin.

Hemoglobin

The role of hemoglobin is to deliver oxygen inhaled through the lungs to the tissues to serve as a substrate for cellular metabolism while simultaneously trafficking carbon dioxide accumulated in the tissues as a biproduct of metabolism back to the lungs to be exhaled.



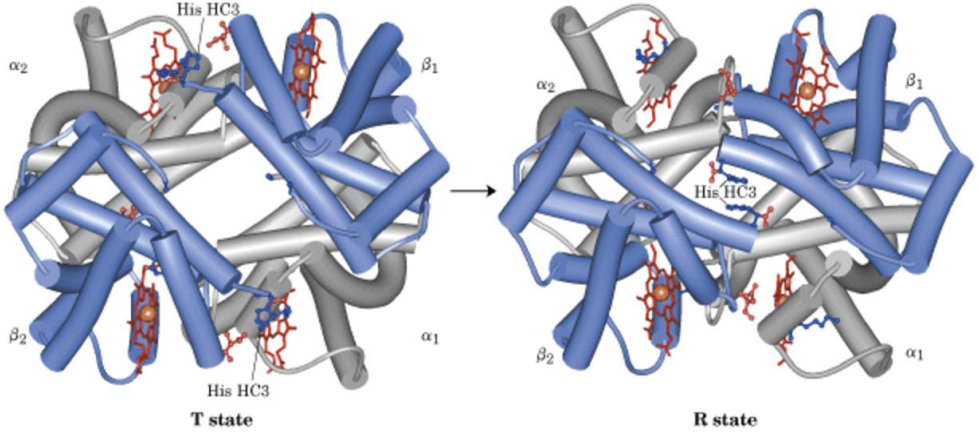
http://www.mhhe.com/biosci/genbio/raven6b/graphics/raven06b/other/raven06_53.pdf

Hemoglobin Structure

- Tetrameric protein complex made up of 2 α and 2 β subunits, each of which contains an oxygen binding site (4 binding sites total)

- Exists in conformational equilibrium between two states:

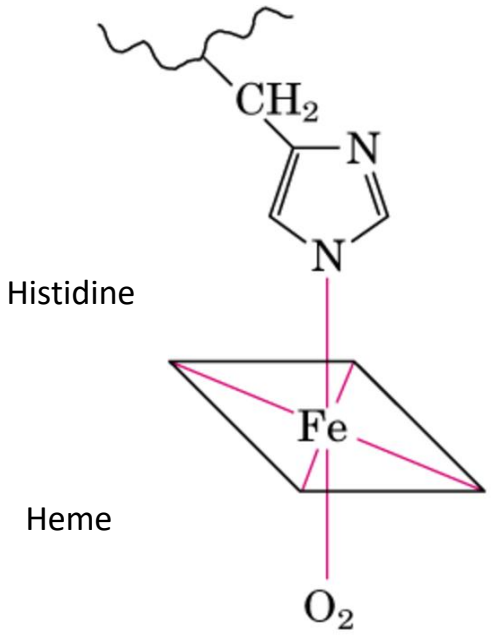
Tense (T) **Relaxed (R)**

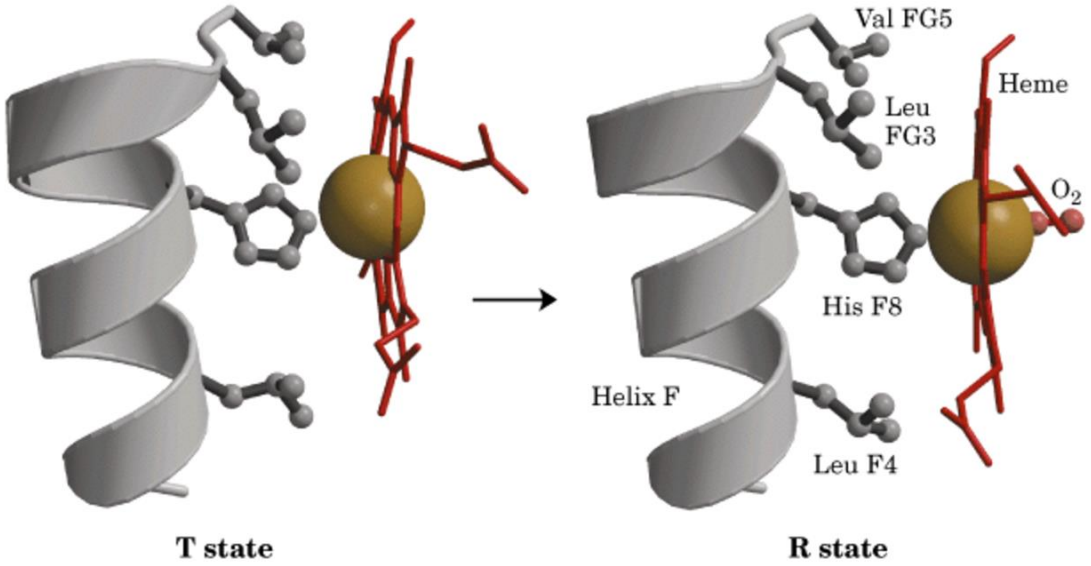


Low affinity for O_2 **High affinity for O_2**

http://cbc.arizona.edu/classes/bioc462/462a/NOTES/hemoglobin/hemoglobin_function.htm#o5

O_2 Binding Destabilizes the T State of Hemoglobin

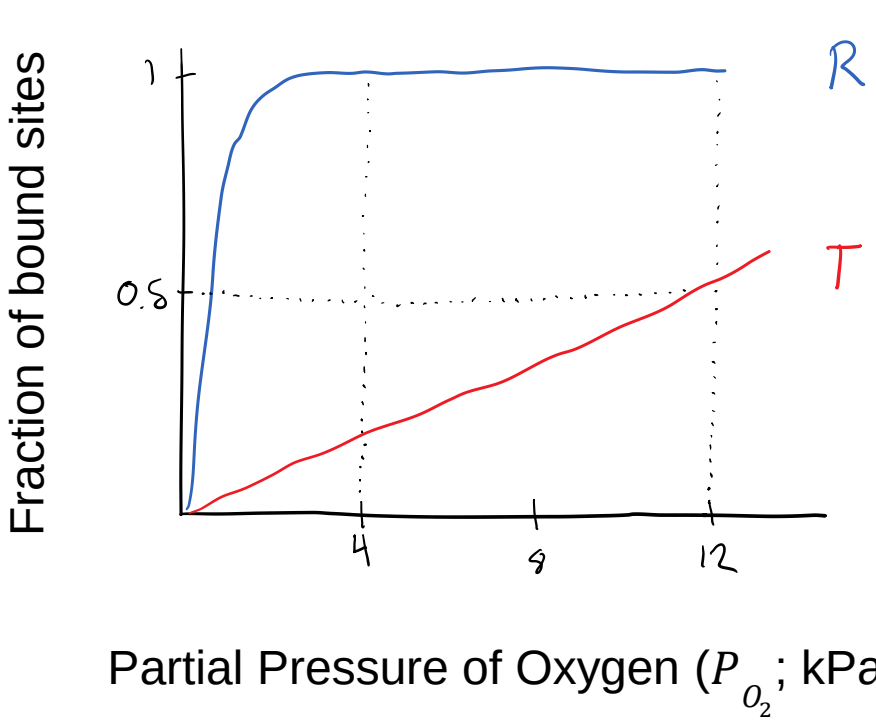




- This is known as **positive cooperativity**, as **O_2 binding increases the binding of further O_2** as the R state has a higher affinity for oxyen

http://cbc.arizona.edu/classes/bioc462/462a/NOTES/hemoglobin/hemoglobin_function.htm#o5

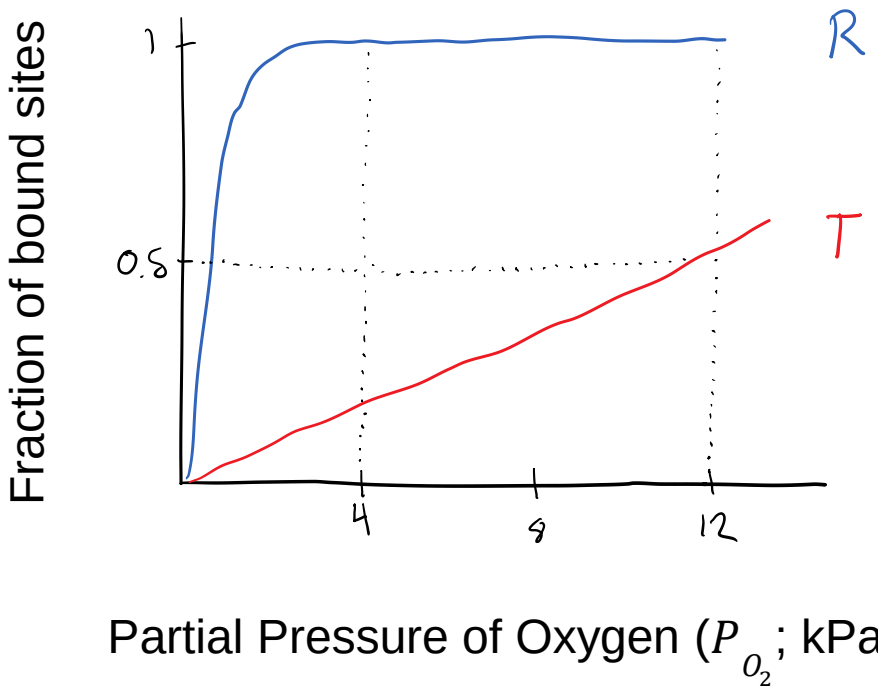
Binding Curves for Hemoglobin States



- What would happen if all hemoglobin only existed in the T state?
- What would happen if all hemoglobin only existed in the R state?
- Why does the T state exist at all if it has sub-optimal binding affinity for oxygen?
- What would be the ideal situation for which state for hemoglobin to exist in?

P_{O_2} = 12 kPa in human lungs; P_{O_2} = 4 kPa in human peripheral tissues

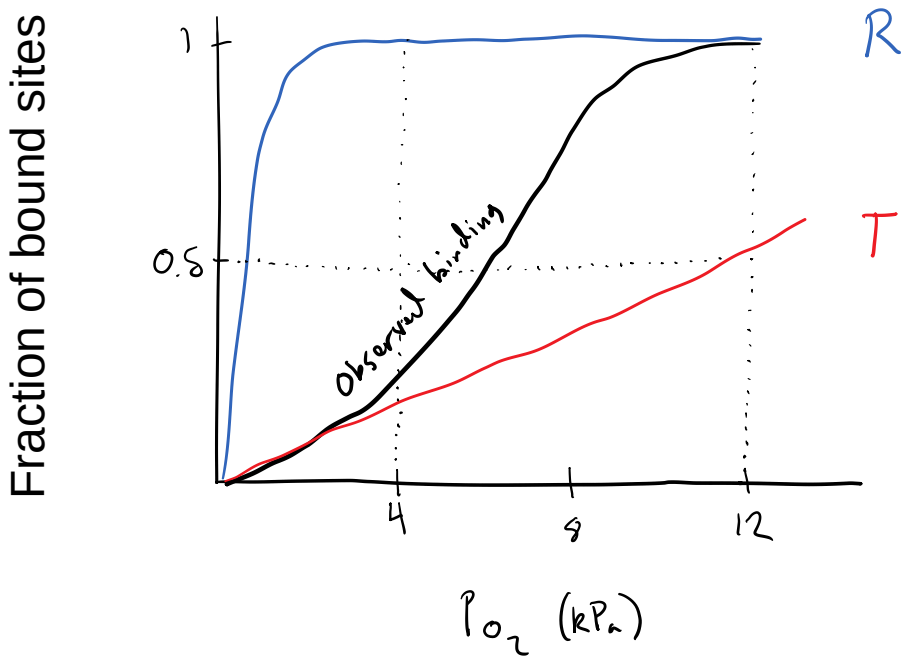
Binding Curves for Hemoglobin States



- What would happen if all hemoglobin only existed in the T state?
  - You would get a lot less oxygen picked up in the lungs due to low affinity (poorly functioning hemoglobin)
- What would happen if all hemoglobin only existed in the R state?
  - You would not allow oxygen to be dissociated to the tissues
- Why does the T state exist at all if it has sub-optimal binding affinity for oxygen?
  - To offload oxygen effectively in the tissues, as it has lower affinity than the R state and thus more capable of delivering the payload to the desire site
- What would be the ideal situation for which state for hemoglobin to exist in?
  - The ideal situation would be R in the lungs and T in the tissues, for maximal pick up and drop off oxygen

P_{O_2} = 12 kPa in human lungs; P_{O_2} = 4 kPa in human peripheral tissues

Observed Hemoglobin Binding Curve



P_{O_2} = 12 kPa in human lungs

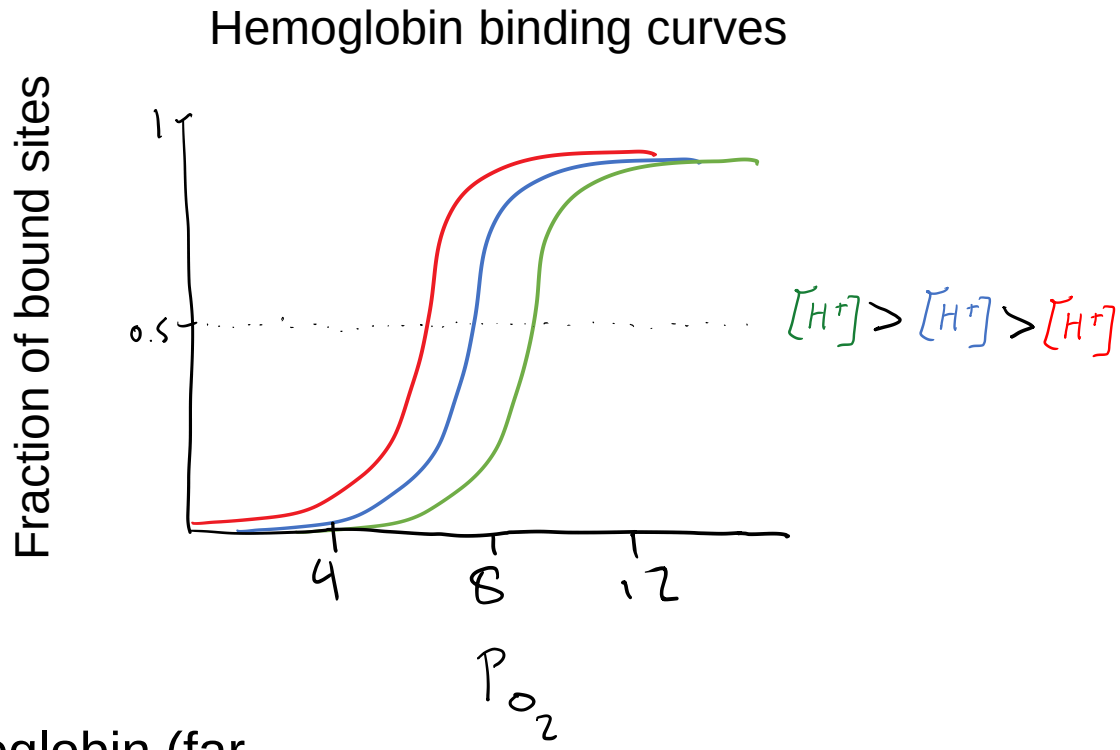
P_{O_2} = 4 kPa in human peripheral tissues

- The experimentally determined binding curve for hemoglobin is a hybrid of the T and R states, to allow oxygen pick-up in the lungs and oxygen drop off in the tissues

Oxygen Release

- H^+ (proton) binding to hemoglobin stabilizes the T conformation (lower binding affinity for oxygen)

- As the peripheral tissues of the body are at a *lower pH than the lungs*, increased incidence of proton binding causes O_2 to be released



- Why is the green curve hemoglobin (far right) more likely to dissociate its oxygen?

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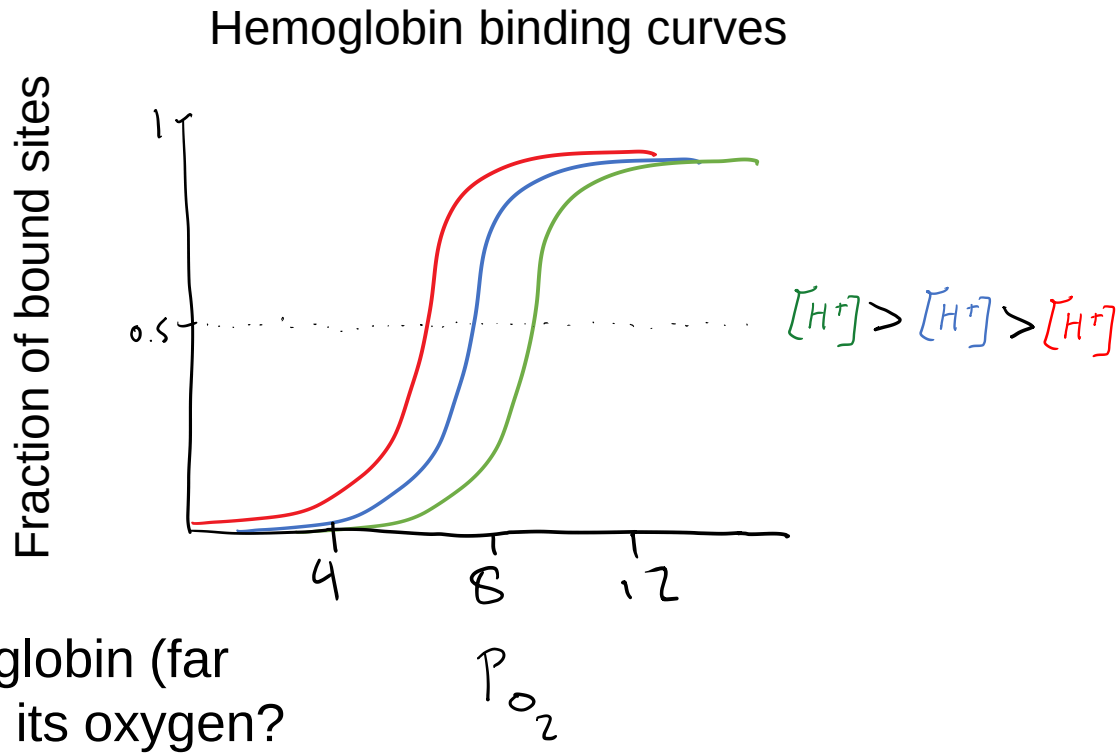
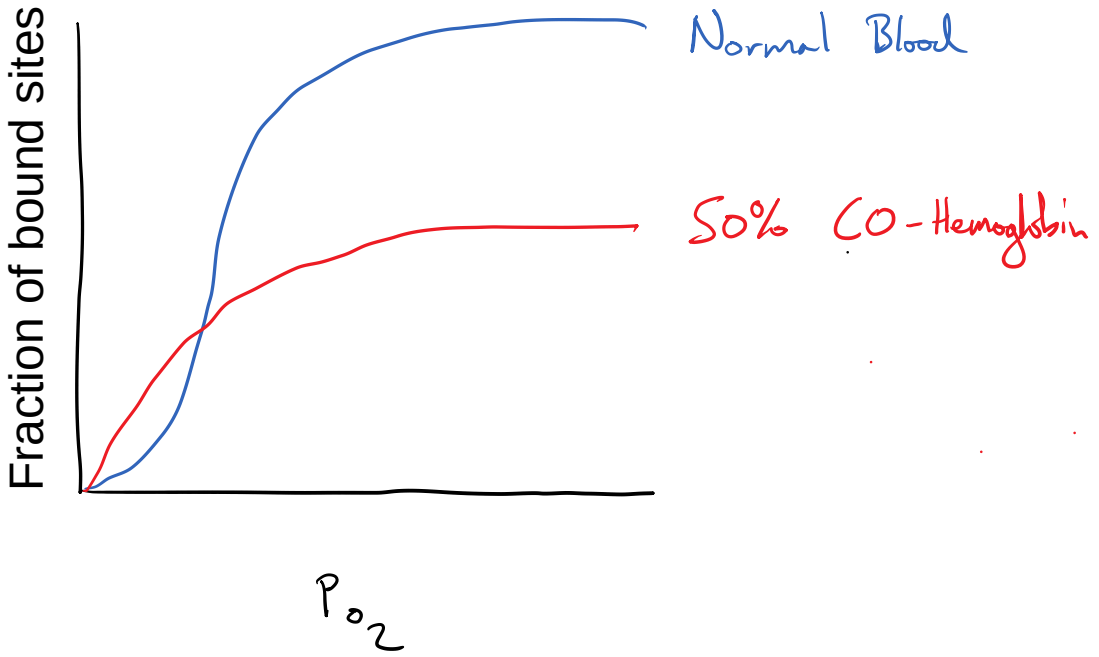


- Why is the green curve hemoglobin (far right) more likely to dissociate its oxygen?
  - The oxygen dissociation curve has shifted to the right, meaning it requires more oxygen to bind to the same fraction of hemoglobin binding sites so at 4 kPa P_O2 and low pH (high H+) in the tissues hemoglobin will be more likely to dissociate oxygen from itself

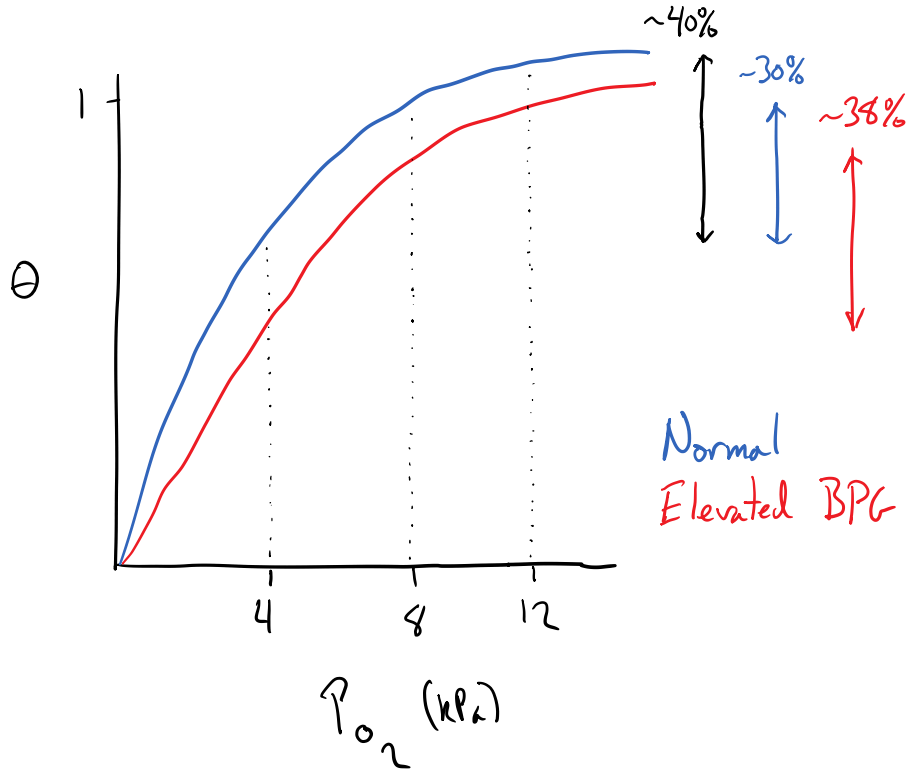
Hemoglobin & Smoking

- Carbon monoxide (CO) is released into the lungs as a result of smoking cigarettes
- CO binds to the *same position* as O_2 in hemoglobin with an almost *250-fold higher affinity*
- This binding event is *irreversible and allosterically increases* O_2 *binding to hemoglobin*
  - Meaning the bound oxygen that does bind is unlikely to be released into the tissues



Hemoglobin and High-Altitude Adaptation

- 2,3-Bisphosphoglycerate (BPG) is a normal circulating molecule in human red blood cells (where hemoglobin is found)
- BPG binding to hemoglobin favors the T state, lowering the affinity for oxygen
- BPG levels are elevated under conditions of tissue hypoxia (lower than normal oxygen levels) so as to allow for *enhanced oxygen release* when it is needed most



Fetal Hemoglobin

- Fetal hemoglobin is composed of 2 α and 2 γ subunits as contrasted to the 2 α and 2 β subunit of adult hemoglobin.
- These alternate subunits provide an *altered binding affinity to BPG* (recall BPG lowers the binding affinity for oxygen).
  - Would you expect fetal hemoglobin to have a higher or a lower binding affinity *for BPG* compared to adult hemoglobin?
  - Would you expect pregnant people to have an elevated or lowered level of BPG?

Fetal Hemoglobin

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- These alternate subunits provide an *altered binding affinity to BPG* (recall BPG lowers the binding affinity for oxygen).
  - Would you expect fetal hemoglobin to have a higher or a lower binding affinity *for BPG* compared to adult hemoglobin?
    - Fetal hemoglobin lacks a histidine residue critical in the binding of BPG, and so the binding affinity is lower than that of adult hemoglobin. This makes sense because it consequently allows fetal hemoglobin a higher binding affinity to oxygen than adult hemoglobin, so the fetus can obtain needed oxygen from the mother.
  - Would you expect pregnant people to have an elevated or lowered level of BPG?
    - Similarly, pregnant people have a 30% elevation in their BPG levels, further lowering the affinity for oxygen, thus increasing the likelihood it will be released, and allowing it to be taken up by the fetus.

Hemoglobin Summary

- Hemoglobin is a complex molecular machine which operates between two conformations (T and R) to allow for the optimal level of binding affinity to oxygen at the appropriate time
  - Positive cooperativity of oxygen binding and release augments these effects
- Allosteric interactions play an important role in facilitating the conformational changes between the T and R states
  - H^+, CO_2, BPG all stabilize the T state to facilitate oxygen release to the tissues
- You should never smoke cigarettes, because carbon monoxide is a poison which permanently reduces the ability of hemoglobin to transport oxygen

Learning Objectives for Chapter 4 Part 1:

Upon completing this module, **you should be able to**:

1) Understand the shape and structure of proteins (primary, secondary, tertiary, quaternary).

2) Describe the different regulatory control mechanisms of protein activity (allostery, active vs. inactive forms).

3) Apply the above to the functionality of the molecular machine hemoglobin.

Feedback/Reflection

https://forms.gle/vbdZXee89Dh9diY78