Biochemistry Review

Topic 1: Central Dogma of Molecular Biology

Dogma: doctrine or code of beliefs accepted as authoritative

Dogma of Biology: DNA→RNA→Protein

- *DNA synthesis* occurs to replicate the DNA double helix (helical structure discovered by Watson and Crick in 1953)
- *RNA synthesis* (transcription) occurs to give rise to the mRNA transcript
- *Protein Synthesis* (translation) occurs to translate information via ribosomes from mRNA transcript into amino acids by "codons" on mRNA to form polypeptides
    - Mechanisms of epigenetics alter the protein functions, yet the DNA sequence is left unmodified

Personalized Medicine:

- A medical model that proposes the personalization of health care – medical decisions are being tailored specifically to the patient by use of genetic or other bodily information
- Defects in DNA are the source of many types of diseases – personalized medicine looks more at the genotype

Pharmacogenomics: Sequencing of genome to help predict which drugs someone would respond to the best – the cost of sequencing your genome is becoming less expensive and personalized medicine is becoming less expensive

Topic 2: Amino Acids

Amino Acids:

- Proteins are composed of linear polymers of amino acids called polypeptides
- L-Amino acids (stereoisomers) are the amino acids that make up proteins for human life
- Amino acids contain 4 components:
    - Amino Group (NH_2)
    - Carboxyl Group (COOH)
    - Hydrogen (H)
    - R-group (side chain)

Classes of Amino Acids:

- Non-polar
    - Non-polar side chains are hydrophobic
    - Proline (pro, P) is non-polar – it is an imino acid meaning its immediate synthetic precursor was an imino acid (i.e. contained an imine C=NH)
- ***All polar side chains are hydrophilic***
- Polar Charged: Basic
    - Contain basic groups – lysine (lys, K), arginine (arg, R), histidine (his, H)
- Polar Charged: Acidic
    - Contain carboxyl groups – glutamic acid (glu, E), aspartic acid (asp, D)

- Uncharged polar side chains
    - Contain hydroxyl groups: Serine (ser, S), threonine (thr, T), tyrosine(tyr, Y)

- Contain amide groups: glutamine (gln, Q), asparagine (asn, N)

AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
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

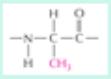
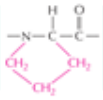
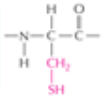
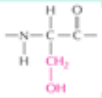
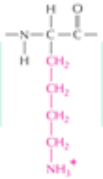
POLAR AMINO ACIDS (hydrophilic)

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 (hydrophobic)

Figure 4-3 Essential Cell Biology 3/e (© Garland Science 2010)

Memorize these amino acids…

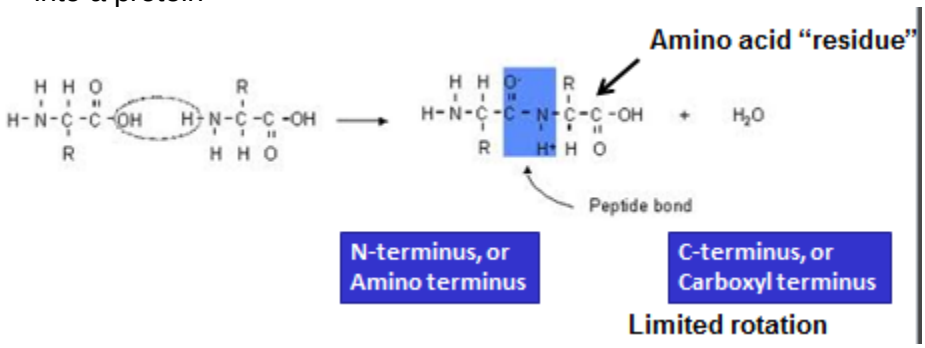
Name	Structure	3-Letter	1-Letter	Polarity	Side chain
Alanine		Ala	A	non-polar	non-polar
Glycine		Gly	G	non-polar	non-polar
Proline		Pro	P	non-polar	non-polar
Phenylalanine		Phe	F	non-polar	non-polar
Cysteine		Cys	C	non-polar	non-polar
Serine		Ser	S	polar	uncharged polar
Lysine		Lys	K	polar	positive
Aspartic Acid		Asp	D	polar	negative

- Specialized groups are found in amino acids for specific purposes relating to protein structure and function

Topic 3: Protein Structure

Formation of a Peptide Bond

- A peptide bond is an amide linkage which bonds two amino acids together by process of a condensation reaction
- The N-terminus (amino terminus) bonds to the C-terminus (carboxyl terminus)
- Residue: the part of the polypeptide which does not participate in the peptide bond – called this because water is lost in the reaction
- The nature of the bond limits its rotation, which limits the way that a polypeptide can fold into a protein



Polypeptides

- Polypeptides are always written from "N" to "C" terminus
- Peptides are small numbers of joined residues (e.g. dipeptide, tripeptide)
- Backbone is the repeating part of a polypeptide (-N-C-C-N-C-C-N-C-C-)
- Side chains project from the backbone
- E.g. Valine-Histidine-Leucine-Proline-Threonine-Glutamic Acid-Glutamic acid is known as Val-His-Leu-Pro-Thr-Glu-Glu or VHLPTEE – no matter which way you flip it, the peptide is always read N-to-C

Effect of Side Chain on Structure of Polypeptide and Protein

- There are three major properties of a side chain that effect the structure of a protein:
    1. Physical Size
        - The orientation of side chains in space alter how the protein folds (i.e. larger side chains might not fit where a smaller one can)
    2. Hydrophobic or Hydrophilic (charged or uncharged)
        - The charge or polarity of a side chain can be used to predict the charge of an amino acid
        - Three parts of an amino acid can carry a charge: amino group, carboxylic acid and the side chain (only on some amino acids)
    3. Ability form hydrogen bonds
        - Hydrogen bonds can be formed between either another part of the polypeptide or another peptide from itself

Calculating Total Charge of an Amino Acid

- Begin with the charge of each group, then add the charges together
    - Acidic side-chains: if a hydrogen is stripped off, the group carries a 1^- charge
    - Basic side chains: if you add a hydrogen, the group carries a 1^+ charge
- To calculate the charge of an amino acid, you need to know two things:
    1. The tendency of groups to attract a proton (pKa)
    2. The number of protons available in the solution (pH)

- pKa (constant) tells you at which pH (variable)the proton will be attached/detached to the amino acid

pH Scale

- The pH scale is a logarithmic scale based on the [H+]
- $pH=-\log_{10}[H^+]$

Dissociation

- Acidic and basic amino acids ionize (become charged) when placed in water
- K_a is the dissociation constant – a quantitative measure of the strength of an acid
- $K_a = \frac{[H+][RCOO-]}{[RCOOH]}$
- $pKa = -\log_{10}Ka$

Example Calculation
Problem: A 0.2 M solution of a generic weak acid (HA) has a pH of 2. Determine the pKa.

1) Write the dissociation equation for the acid: HA <===> H+ + A^-
2) Write the equilibrium expression: Ka = ([H+] [A^-]) / [HA]

a) We will use the pH to calculate the [H+]. We know pH = -log [H+], therefore [H+] = 10^-pH
[H+] = 10^{-2} =0.01M
b) From the dissociation equation, we know there is a 1:1 molar ratio between [H+] and [A^-]. Therefore:
[A^-] = 0.01M
c) the final value, [HA] is given in the problem. In the example being discussed, 0.20 M is the value we want.
c1) Ka = [(0.01)(0.01)]/2
c2) Ka = 0.0005
3) pKa= -logKa = -(-3.3)=3.3

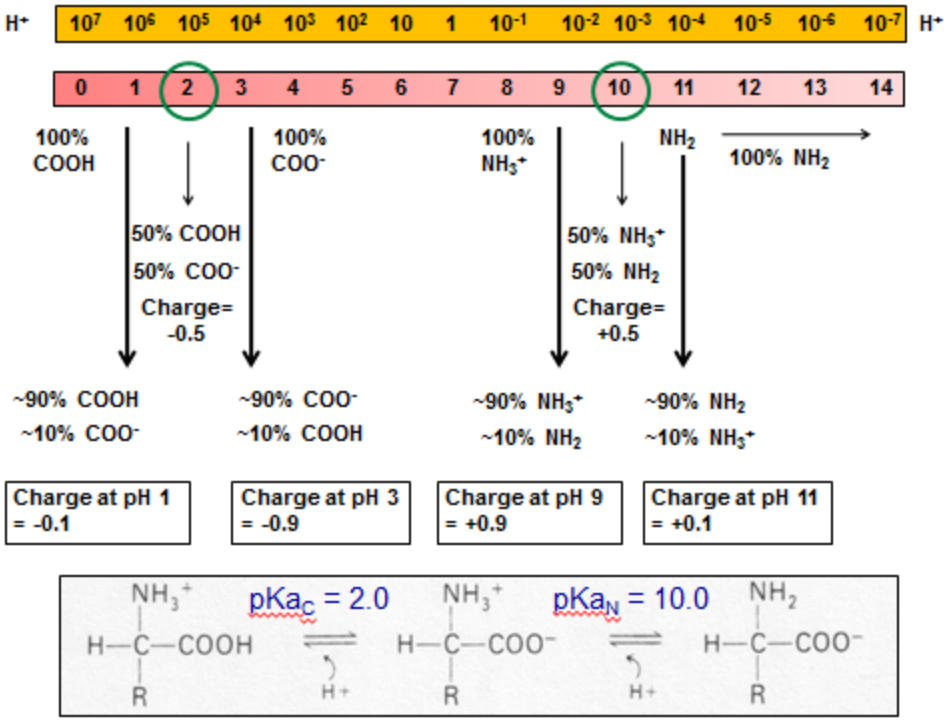
Rules for Calculating Amino Acid Net Charge

- At differing pH's you may have different forms of an amino acid
- When pH<pKa the group will be protonated
- When pH>pKa protons will be stripped off
- When pH=pKa, 50% of the groups will be ionized (neutral)
- **It is important to remember the pH/pKa scales are logarithmic**

Example Calculation:
At pH=1, Find the charge of the carboxyl group (pKa=2)

- **There are 10x more H+ at pH 1 than at pH 2, the ratio of COOH:COO- will be 10:1**
- In terms of %, this means that there will be 10/11 or ~90% COOH, and 1/11 or ~10% COO-

Charge of carboxyl group at pH 1 =
= 0.9 (0) + 0.1(-1) = 0 + (-0.1)
= -0.1

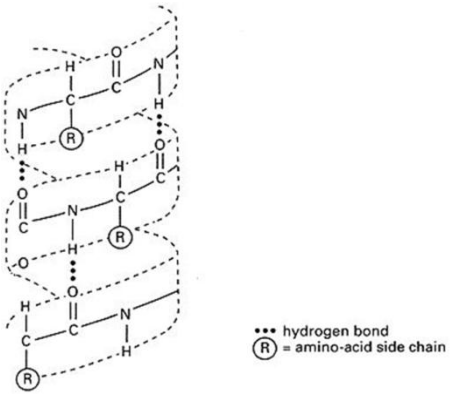


Primary Structure

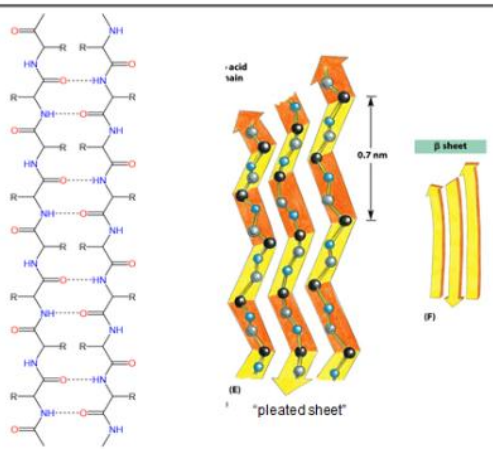
- Essentially just the sequence of amino acids (i.e. the polypeptide)
- The minimum size of a protein is defined as about 50 residues; smaller chains are referred to as peptides
- Primary structure is the sequence of residues which make up the protein

Secondary Structure

- Secondary structure is the local folding of the polypeptide backbone and is stabilized by hydrogen bonds between N-H and C=O groups
- The majority of proteins in nature exhibit secondary structure
- A polypeptide chain may have different regions which take on different secondary structures
- There are two major types of secondary structure
    1. A-helix
        - Stabilized by hydrogen bonding between an amino group (NH) and the carbonyl group (CO)—these two groups are oriented parallel to the axis
        - A-helices are arranged in a coil spring arrangement
        - There is no interior space in an A-helix – side chains project outwards
        - Dimensions: 3.6 residues/turn, 0.54nm/turn
        - Proline often inhibit the formation of A-helices due to their ring like structure



2. B-sheet
    - The polypeptide chain folds back on itself so that the polypeptide strain lays side by side forming a rigid (corrugated) stucture
    - Hydrogen bonds are formed between amino and carbonyl groups of the adjacent polypeptide strand which stabalizes the structure
    - The primary structure can fold back on itself in either a parallel (N-terminus are lined up at end of sheet) or antiparallel (N and C termani are lined up at end of sheet) arrangement



    - Primary structure determines how the secondary sequence is formed
    - **Beta sheets are can be formed by a single polypeptide chain folding back on itself or two or more separate polypeptide chains hydrogen bonding to each other**

Tertiary Structure

- Tertiary structure describes how regions of secondary structure fold together (i.e. the 3-dimensional arrangement of a polypeptide chain
- Tertiary structure is the result of interactions stabilized by bonds between side chains, or between side chains and the polypeptide backbone
- Types of Interactions that affect protein structure:
    - Electrostatic reactions (ionic bonds), hydrogen bonds and van der Waals forces between residues can give the tertiary protein shape – far weaker than covalent bonds (takes multiple reactions to stabilize protein structure)
    - Hydrophobic side chains tend to cluster towards the inside of the protein, hydrophilic side chains tend to be on the outer-layer of the protein as they prefer to be exposed to the aqueous environment
    - Covalent di-sulfide bonds can form between cysteine residues in an oxidizing environment – these only exist in non-cytoplasmic proteins as there are enzymes present in the cytoplasm to remove disulfide proteins
        - These bonds can form between two cysteine residues on either the same polypeptide (intra-chain) or different polypeptides (inter-chain)
- Domains exist in tertiary structure and are a critical concept for understanding larger proteins
    - Domains are distinct regions of a protein which is an independently folded part of a protein that folds into a stable structure
    - Often domains are connected by a loosely folded region and my create clefts between them—structural domains often have their own function

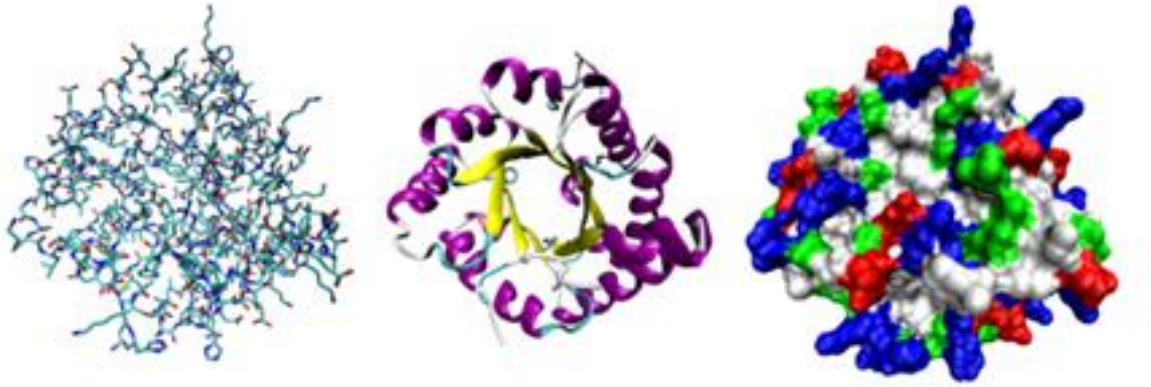
Quaternary Structure

- Proteins that consist of more than one polypeptide chain is known as a quaternary protein
- A singular polypeptide of a quaternary protein is known as a subunit of the protein

- The same forces (hydrophobic forces, hydrogen bonds, van der Waals, ionic bonds and disulfide bonds) that hold together a tertiary structure also are used to hold together subunits in a stable complex
    - An example of a 4° protein is p53 – it is a tumor suppressor protein which consists of 4 different polypeptides (Tetramer)
- A common quaternary structure which forms is the “coiled-coil”
    - This structure is stabilized by a hydrophobic stripe of residues along two A-helices which coil around each other and bury the hydrophobic side chains in order to minimize exposure to aqueous environment and form a stable structure

Visualizing Protein Structure

- Biochemists often use different type of figures to indicate protein structure



- Wire (Left): shows side chains and their proximity and predicts how specific amino acids are involved in function
- Ribbon (Middle): aids visualization of secondary structure
- Space-Filling (Right): shows the theoretical space of which each atom on the outside surface will take up – also helps to show how protein would react with water or other proteins

Protein Folding

- A folded biologically-active protein is considered to be in its “native” state
- Folding pathways are determined by the sequence of amino acids and their properties (polar, non-polar, charged, uncharged)
- Molecular chaperones are proteins which aid the folding of other proteins by masking hydrophobic regions or protecting membrane-bound proteins which must stay unfolded until they reach their distination
- Proteins can be unfolded or denatured by a treatment with solvents that disrupt weak bonds which are vital to stabilizing protein structure
    - Organic solvents can disrupt hydrophobic interactions
    - High concentrations of urea of guanidine interfere with hydrogen bonding
    - Extreme pH or high temperatures can alter charge and break bonds
- Denatured proteins have a random, flexible conformation that usually lack biological function
- If the denaturing condition is removed, some proteins will re-fold and regain activity—however this is not always the case

Protein Families

- Proteins can be classified in a number of ways, according to structure, function, location and properties
- All proteins arise from evolutionary process – they are classified by their relativity, proteins which arise from the same ancestor are called “homologous proteins” and have the same conserved residues
- Studying the conserved residues of two homologous proteins gives clues to the structure and function of the proteins

Topic 4: Protein Function

Types of Proteins

Name	Function	Example
Enzyme	catalyzes covalent bond breakage or formation	protein kinase
Structural	provides mechanical support to cells & tissues	keratin
Transport	carries small molecules or ions	hemoglobin
Motor	generates movement in cells and tissues	myosin
Storage	stores small molecules or ions	ferritin
Signal	carries signals from cell to cell	insulin
Receptor	detects signals and transmits them to the cell	rhodopsin
Gene Regulator	binds to DNA to switch genes on or off	p53

Protein Flexibility

- Protein flexibility is critical for many different functions
- Proteins that are not rigid can undergo modest changes in structure called conformational changes which are driving forces behind the action of proteins

Protein Topography

- The surfaces of proteins have a large variety of shape and electronic topography due to the various properties of side chains and tertiary structures that can bring a variety of residues together in space

Ligand-binding Protein

- Many proteins have evolved to bind to particular molecules with great specificity using multiple weak bonds forming a complex
- A small area of the protein is complementary to the structure of the binding molecule (called a ligand)
- In the case of enzymes, the active site is complementary to the substrate (or ligand)
- Ligand binding is characterized by a dissociation constant: K_d whose value is based on the molecules affinity to each other

$$P + L \rightleftharpoons PL \qquad Kd = \frac{[P]\ [L]}{[PL]}$$

*High Affinity=Tight Binding=Small K_d

Protein Complexes

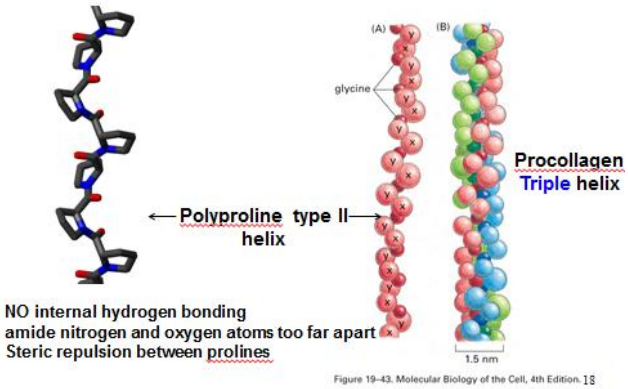
- Protein complexes contain many proteins linked together to form a supramolecular structure – performs a specific job within the cell
- Ribosomal Complex
  - Performs protein synthesis – A specific complex of more than 50 proteins and RNAs
  - Ribsome is an extremely complicated complex
- Proteasome
  - Degrades 99% of proteins in humans – very important for destroying damaged proteins in body which can be detrimental
  - Proteasomes are shaped like a hollowed barrel – proteins come in one end and amino acids come out the other
  - Proteasome uses ATP to unfold proteins

Case Study: Collagen

- Collagen is the most common protein in animals (25-30%) – an extracellular protein that forms very strong fibrils which strengthen: bone, skin and basement membranes of arteries, veins, and many other tissues
- Various diseases arise from mutations in collagen genes
- All 20 amino acids are present in collagen – however every 3rd amino acid residue is glycine
- Collagen also has a very high (20%) proline content -- many prolines become hydroxylated which are factors important to final structure of the protein (lysine also becomes hydroxylated) – prolines prevent the formation of an alpha helical structure
- Different forms of the collagen (numbered 1-14) protein have different lengths

Synthesis of Collagen

1) The construction of the collagen fibre begins with the synthesis of an individual polypeptide
2) Once formed, the chains are hydroxylated in the ER by two different enzymes
   - Proline ----- (prolyl hydroxylase) → hydroxyproline
   - Lysine ------ (lysyl hydroxylase) → hydroxylysine
3) Once hydroxylated, three collagen strands "polyproline type II helices" associate at the C-terminal and then wind around each other to form an extremely tight rope-like structure – the hydroxyl groups on hydroxyproline form hydrogen bonds which hold the procollagen triple helix together
   - The small side chain of glycine every third residue allows the chains to come close together
   - Hydrolysine residues are necessary as sites of sugar addition that increases solubility, and they also serve in a cross-linking reaction that creates covalent bonds between collagen chains



4) Procollagen is secreted out of the cytosol and the propeptides are trimmed off by specific proteases to leave only the triple-stranded rope part
5) These trimmed molecules (tropocollagen) then assemble into polymers called "fibrils"

- These molecules often appear stripped because there are spaces between procollagen triple helices

6) Covalent crosslinks (by lysine)between neighbouring fibrils are formed to give the extremely strong fibre

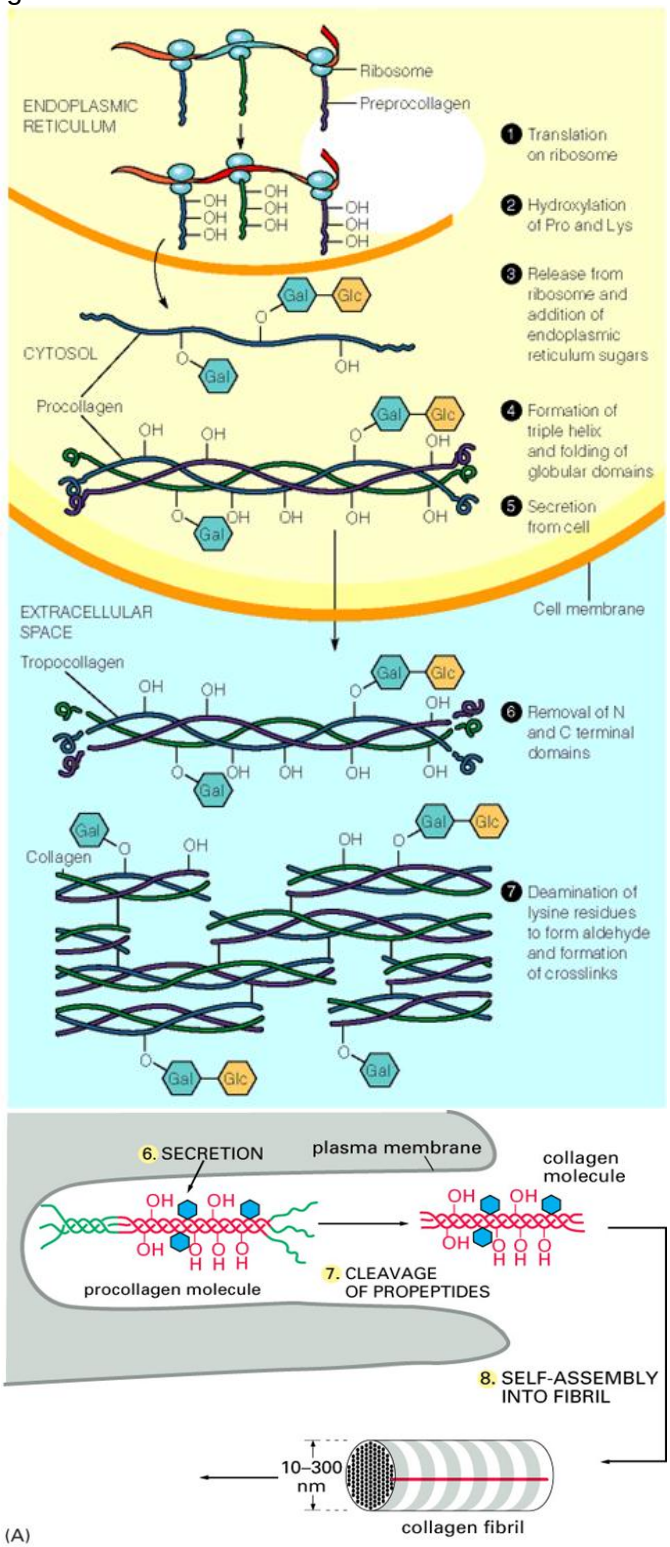


Figure 19–47 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

Collagen Properties

- Glycine every 3rd residue is essential – any other amino acid pushes the chain apart
- Gives a thin (1.5nm), long (300nm), very strong molecule
- Quite hydrophobic and insoluble due to many exposed hydrophobic side chains
- Must first be synthesized as a precursor which is soluble called “propeptides” but then extensions on N- and C- are removed once they pass through the cell membrane and is now called “tropopeptides”

Collagen-Related Diseases

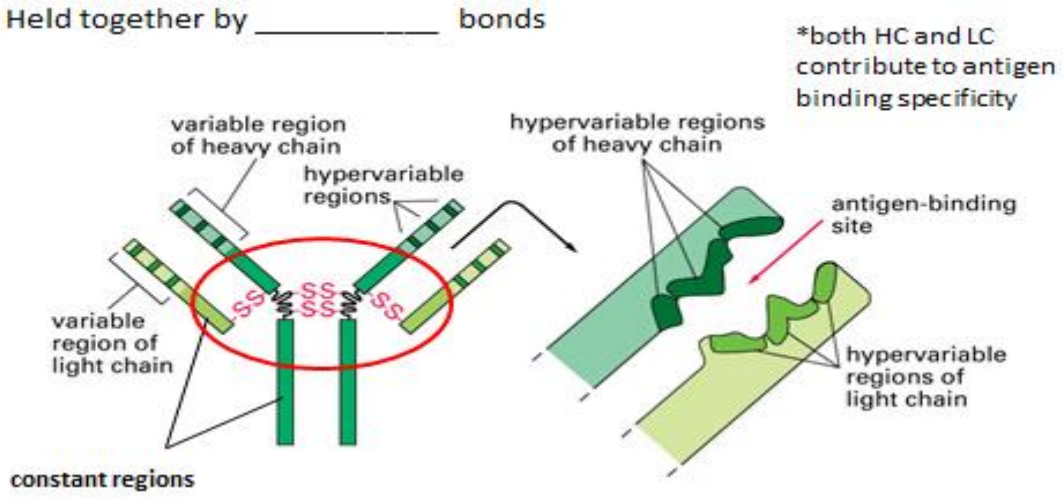
- Scurvy: The enzyme prolyl hydroxylase is an iron containing enzyme which requires the presence of ascorbic acid – the disease scurvy results from insufficient amount of ascorbic acid and prolines not being hydroxylated
- Osteogenesis Imperfecta (Bad Bone): often a glycine mutation which prevents proper assembly of the triple helix – leads to a lack of collagen resulting in malformed or abesnt bone
- Ehlerys-Danlos Syndrome (EDS): group of inheritied connective tissue disorders which is caused by a defect in the synthesis of collagen

Case Study: Antibodies

- Antibodies are proteins produced by the Immune system that bind tightly and specifically to foreign substances (bacteria and parasites) introduced into the body – their amazing specificity has led to powerful tools in medicine and studying of proteins
- Immunoglobulin are soluble proteins that recognize and tag antigens for destruction— there are many types: A,D,E,G and M
- IgG is the most common type of immunoglobulin found in blood and is most generally useful for biotechnology

IgG Tetramers

- There are billions of different IgGs that can be made by an individual – each being specific to a certain antigen
- IgGs are composed of 4 polypeptide chains (2 identical pairs): 2 identical heavy chains and 2 identical light chains which are held together by disulfide bonds
- Differing IgG’s have similar sequences, except in two regions:
    - Variable Regions: allows antibodies to bind to many similar antigens
    - Hypervariable Regions: allows antibodies to bind highly specifically to an antigen
- Outside of variable and hypervariable there are regions known as constant regions which are the same for all IgGs



IgG Domains

- Light chain is made up of 2 Ig domains which are β-sheets (2 sets in total) in both the variable and constant domains
- Heavy chain is made up of 4 Ig domains
- The disulfide bonds within those β-sheets are what hold them tightly together
- The looping at the end of the β-sheets in the variable region form the "antigen binding site"
- Different IgG molecules will have different amino acid sequences in these loops, and the different amino acid sequences will produce different sequences
- Multiple weak bonds are involved in antigen binding, thus requiring strong surface complementarity

Functional Segments of Immunoglobulin G

- IgG functional segments can be separated by proteolysis (break down of protein into smaller segments or amino acids):
    - Proteolysis yields 3 fragments: 2 Fab—Fragment Antigen Binding (recognizes antigen), 1 Fc—Fragment Crystalized (Effector Site – Does the job of the molecule)
- Fc
    - Activates the complement pathway to the antigen it has recognized
    - This portion of the antibody binds to phagocytes, mast cells, basophils, eosinophils, and natural cell killers

Production and Use of Antibodies

- Antibodies are a critical lab tool for biomedical research
- Antibodies are created by injecting an antigen into an animal (rabbit or goat) and then harvesting and purifying the antibodies after the animal's immune response
- IgG's are used in laboratory experiments to identify molecules in mixtures (immunoblot), to diagnose disease (HIV) and clinical therapy (anti-venoms)

Topic 5: Enzyme Catalysis

- Enzymes are biological catalysts responsible for directing the flow of chemical reactions that are the basis for life
- The majority of enzymes are proteins, but some consist of RNA or a complex of RNA and proteins
- Enzymes are remarkably specific, acting on only one or a few types of molecules called substrates in order to form products
- Enzymes speed up the rate of reaction by $<10^{16}$
- There are a few major classes of enzymes which perform different tasks:
    1. Hydrolytic – cleavage using water
        - Nucleases, proteases, phosphatases
    2. Condensation – connect molecules together
        - Polymerases, synthases
    3. Isomerization – rearrange bonds
        - Isomerases
    4. Oxidation-reduction – gain or loss of electrons
        - Oxidases, reductases, dehydrogenases
    5. Group transfer – transfer chemical group
        - Kinases, transferases
- Enzymes act on but are *unchanged* by substrates

- Active sites are the part of the enzyme where the substrate binds and the reaction takes place – the active site is a small part (5% or less) of the surface and is often a cleft or crevice between domains

Transition State

- The state corresponding to the highest energy along the reaction coordinate
- Assuming a perfectly irreversible reaction, colliding reactant molecules will always go on to form products
- Three guidelines which transition state consists of:
    1. Intermediate form between reactants and products
    2. A transient high-energy state
    3. A form different from both reactants and products
- Factors that contribute towards attaining the transition state include:
    a. Bringing substrates together (in multi-substrate reactions)
    b. Orienting substrates in a favourable geography
    c. Supplying proton acceptors/donors, electron donors/acceptors
    d. Excluding water
    e. Stressing the substrate physically or electronically

Enzyme Catalysis

- The speed of reaction is determined by the difficulty of the substrate to reach the transition state
- The greater the activation energy for a particular substrate to reach the transition state, the more difficult it will be for the products to be formed
- Enzymes allow the substrate to reach the transition state much quicker by lowering the activation energy required
- The highest affinity of the enzyme is for the transition state – the topography of the active site is complementary to the transition state

Induced Fit Hypothesis

- The binding of the substrate pushes the enzyme towards the transition state resulting in strain on the substrate – substrate induces small conformational changes in the active site
- The initial reaction between enzyme and substrate is weak, but these weak interactions rapidly become induced

Kinetics & Equilibrium

- Enzymes **do not** change the equilibrium (i.e. the final ratio of substrate to product at equilibrium is unchanged)
- Enzymes allow equilibrium to be achieved quicker by accelerating kinetics (rate) of the reaction
- Both forward and reverse reactions are catalyzed by an enzyme
- Enzymes do not change the free energy difference between substrate and product

Mechanisms for Lowering Activation Energy

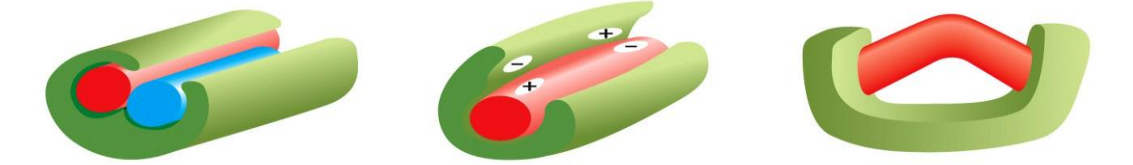


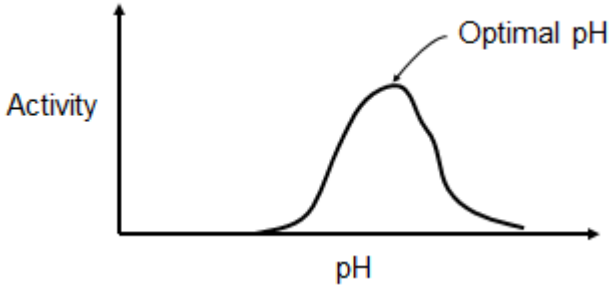
Figure 4-32 Essential Cell Biology 3/e (© Garland Science 2010)

Transition State Analogues

- Compounds that resemble the transition state with similar geometry and charge distribution but do not undergo a chemical reaction – these act as excellent inhibitors because they bind tightly to the active site
- Many drugs and antibiotics are enzyme inhibitors, and are most effective if they are transition state analogues

Optimal Conditions for Enzyme Function

- Enzymes function best at optimal temperature or pH – enzymes are unstable at extremes of temperature or pH



Lysozymes as Catalytic Enzymes:

- A small protective enzyme found in tears, saliva, mucus and eggs – lysozymes hydrolyze polysaccharides found in cell walls of some bacteria which breaks down their bacterial wall using water
- Lysozymes are a component of the innate immune system and is stabilized by disulfide bonds
- The cleft between the two domains of lysozyme is the active site which is where the polysaccharide chain is cleaved (between acidic residues of each domain) and the two products are released

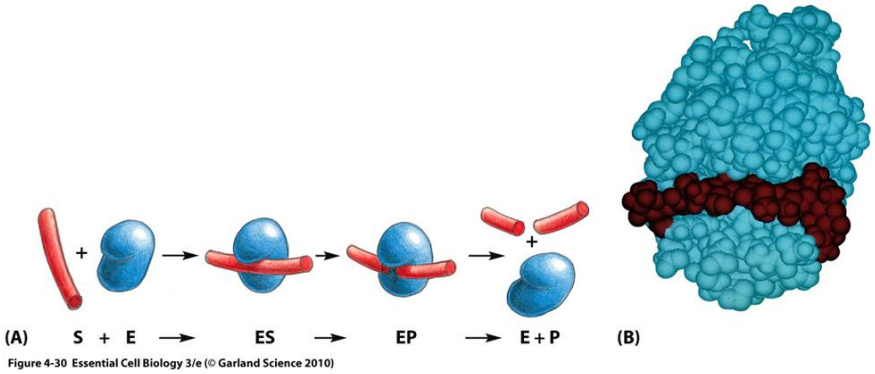


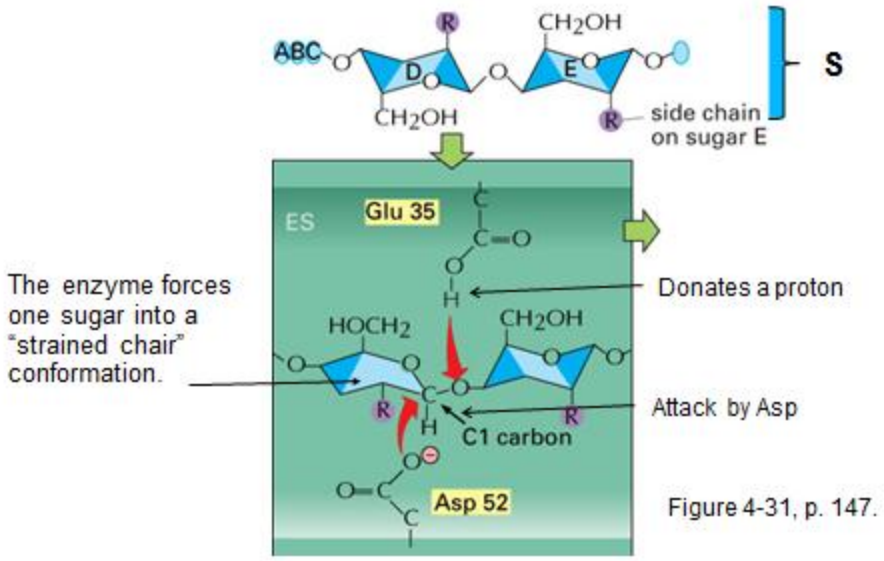
Figure 4-30 Essential Cell Biology 3/e (© Garland Science 2010)

Lysozymes Mechanism of Hydrolysis

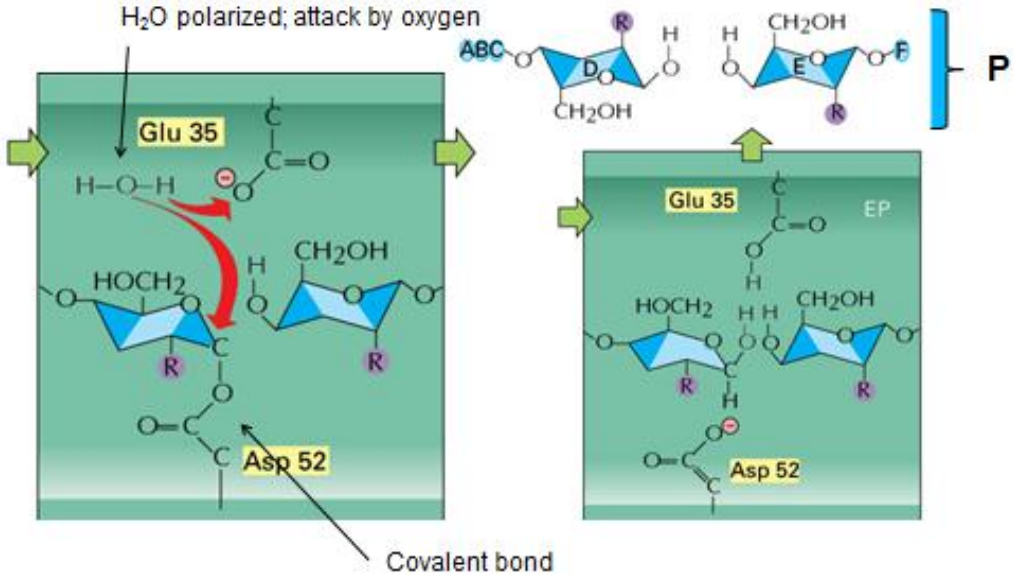
- A chain of sugar molecules is the substrate which the lysozyme acts upon – forming the enzyme substrate complex (ES complex)

Process:

i. A proton (H^+) from glutamic acid (at position 35) is donated to the oxygen holding the two sugars (D and E) together and transforms it into a hydroxyl group (–OH)
ii. This protonation cleaves sugars D and E apart
iii. Aspartic acid (at position 52) acts as a nucleophile and donates a pair of electrons to the hydrogen (electrophile) in order to form a bond between oxygen and carbon 1 of sugar D
iv. The substrate is now covalently bonded to the lysozyme at the aspartic acid at position 52
v. Water donates a proton to both the carbon 1 of sugar D and the glutamic acid at position 35
vi. The lysozyme is restored to its original state and completes the catalytic reaction

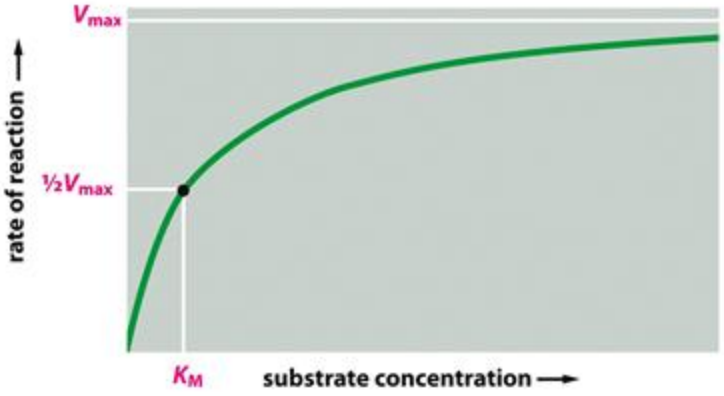


The substrate is an oligosaccharide of six sugars, labeled A-F.
Only the sugars D & E are shown.



Enzyme Kinetics

- The study of how the rate of reaction is influenced by substrate concentration, inhibitors etc. – can help us understand how the enzyme works in a quantitative manner
- The rate of reaction will depend on these factors:
    a. Affinity of the enzyme for the substrate
    b. Substrate Concentration
- The rate (V) of an enzymatic reaction increases as [S] increases until a maximum rate (V_{max}) is reached – at V_{max} all substrate-binding sites on the enzyme are fully occupied
- At [S] where V_{max} is at its half (½ V_{max}) it is referred to as K_m and is a measure of binding force (affinity) between the enzyme and substrate
- If the value of K_m is larger, affinity is lower. If the value of K_m is lower, the affinity is higher

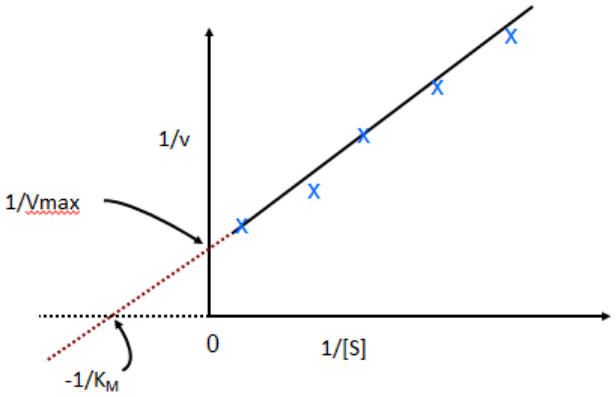


Michaelis-Menten Equation

- Describes the kinetics of the enzyme substrate complex (i.e. how the concentration of substrate and rate of reaction relate to each other)

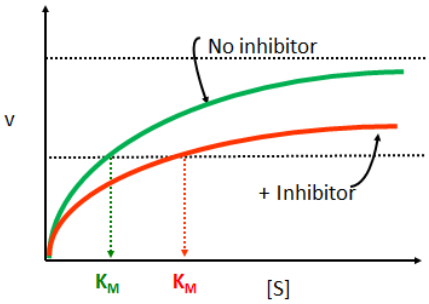
$$V = \frac{V_{max}[S]}{K_m + [S]}$$

- Where V is the initial velocity (before any product is produced), V_{max} is the maximum velocity, [S] is the concentration of substrate, and K_m is the Michaelis constant (independent of enzyme concentration)
- The Michaelis-Menten equation allows the determination of K_m and V_{max} from velocity vs. substrate concentration data. For convenience, a double-reciprocal plot is used where the equation is the inverse of the Michaelis-Menten equation with a plot of 1/v vs. 1/[S]: *1/v = (1 / [S]) (K_m / V_{max}) + 1/V_{max}*
- Gives a double reciprocal or Lineweaver-Burk plot (gives straight line)



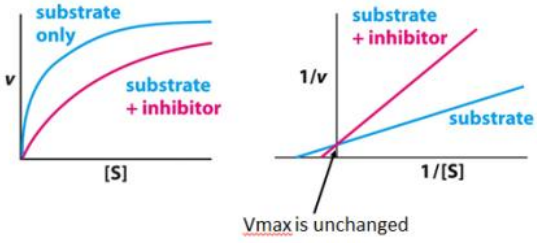
Enzyme Inhibition

- There are two general types of enzyme inhibition:
  - Reversible: Binding of inhibitor to enzyme non-covalently (inhibitor can be removed)
  - Irreversible: Covalent bond formed with enzyme; permanently blocks activity



Reversible Inhibitors

- Competitive Inhibitors: the inhibitor binds in the active site because it share similar structure to substrate or product – blocking the enzymes access to the substrate
- Increases K_m but never increases V_{max} – reduces concentration of available enzyme for substrate binding
- Large excess of substrate overcomes this inhibition



Topic 6: Protein Purification

- It is often necessary to obtain a preparation containing protein molecules of only a single type – 3D structure, binding affinities, or amino acid sequences can be determined when a protein is purified
- Protein purification has a few practical applications:
    1. Medical Use
        - Human growth hormones, insulin, chemotherapy, auto-immune disorders, Beano (α-galactosidase), Lactaid® (glycoside hydrolase)
        - Investigate which protein your body might require
    2. To study structure and function
        - Difficult to understand protein function in mixtures
        - Must be pure to determine structure
        - Must understand structure and function to purify proteins for pharmacological use
    3. For use as a reagent in the laboratory
        - Enzymes for manipulating DNA

Purification Process

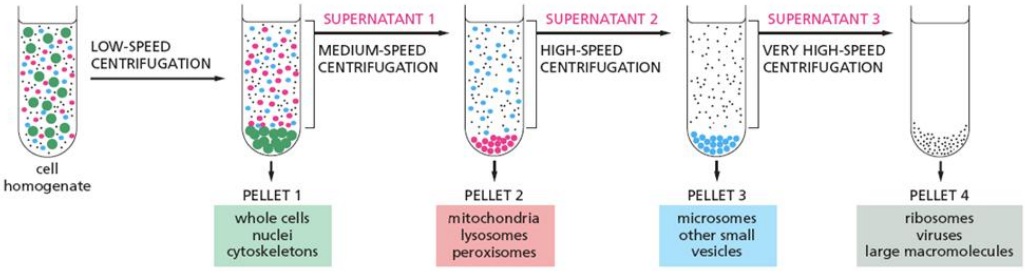
- Proteins are purified/isolated form their environment by using their unique properties to differentiate them from everything else
    - These properties include: size and shape, charge, location, and surface hydrophobicity
- An assay method is needed prior to the purification process to detect the protein in a mixture as well as measure some property of the protein of interest
- A starting material (human tissues, plants, or bacteria) which contains the protein of interest
- Throughout the process, the protein must be isolated (preparative method) and its purity determined (analytical)
    - Preparative Method: used to homogenize ("bust up") material at a large scale (mg-kg) and divide mixture into "fractions"
    - Analytical Method: used to detect specific protein of interest at a smaller scale (≤mg) with small samples of fractions for analysis

Step 1: Homogenization

- The first step of the protein purification process utilizes different forms of homogenization in order to break open tissue or cell to release the protein
- Many ways to homogenize are: sonication (high frequency sound waves), grinders of various types, blenders/homogenizers, exertion of pressure, and detergents (poke holes in cell membranes)
- A compatible buffer solution is also essential for the protein to maintain its native conformation

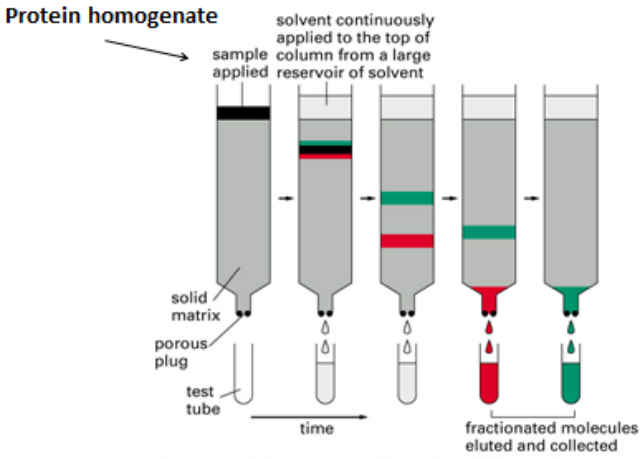
Step 2: Centrifugation

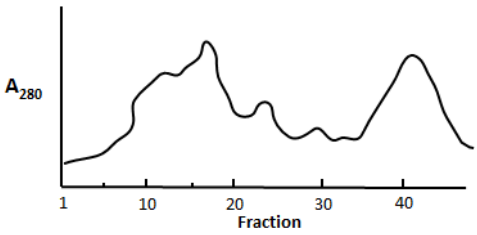
- Samples are placed in a chamber that spins at high speeds – this chamber separates particles by size and density
- Differential Centrifugation: the samples spins at various high speeds – different debris will pellet out or sink to the bottom
    - At low speed nuclei and whole cells pellet
    - At medium speed slightly smaller organelles pellet
    - At maximum seed viruses, ribosomes and large macromolecules pellet
- Centrifugation is a quick way to get rid of things that you don't need



Step 3: Column Chromatography

- Further separates based on characteristics such as charge and size
- Column Chromatography requires two components:
  - Mobile phase (an appropriate mobile buffer solution)
  - Solid phase (usually small beads)
- Solution is held in a cylinder or column
- Proteins in mobile phase interact (or not) with matrix as a continuous flow of the buffer solution carries unbound proteins along
- The eluted molecules are collected in "fractions" and are tested to detect the specific protein of interest and measure the total amount of protein
- Spectrophotometry is used to measure the absorbance at 280nm measures total protein since aromatic acids (W,F and Y) absorb at 280nm
- A_{280} can show you the absorbance of all fractions and your assay method can be used to determine which fraction your protein is in

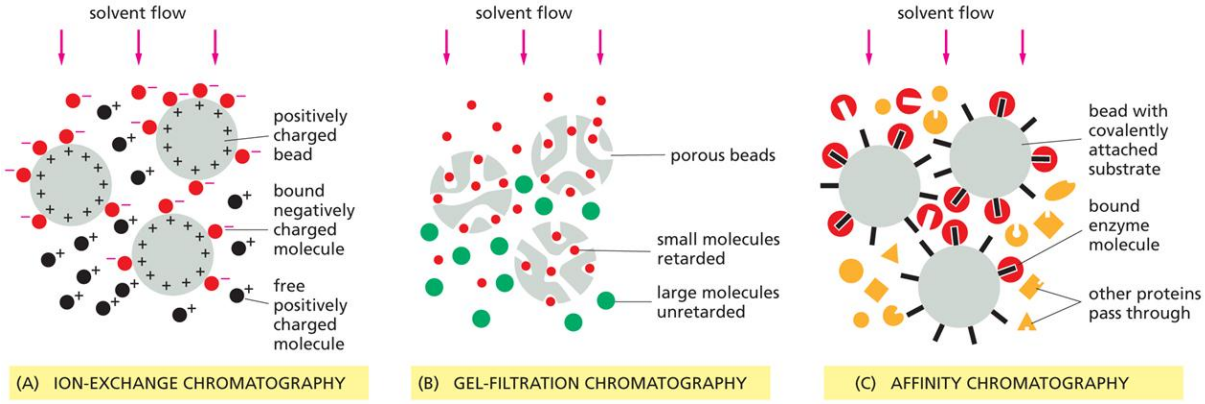




Types of Chromatography

- Ion-Exchange Chromatography
  - Relies on the attraction of opposite charges
  - A positively-charged bead will bind to negatively charged ions (anions) – if the protein is negatively charged, it will bind to the beads, then the salt concentration is increased (increases concentration of negatively charged ions Cl^- to compete)
  - The negatively charged ions will overtake and bond to all of the positively charged beads and the protein will be eluted to the fraction
  - This process can work both ways (i.e. with negatively charged beads and positively charged proteins)
  - Isoelectric Point: The charge on proteins vary with pH – the point where a protein's net charge is 0 is known as its isoelectric point
  - If the pH is raised above *pI* then the net charge will be negative, if the pH is lowered below the *pI* then the net charge will be positive

- Gel-Filtration
  - The solid phase beads contain holes and smaller proteins will be captured by the holes – larger proteins will run out the bottom
  - This is known as “size exclusion”
- Affinity Chromatography
  - Relies on a specific reaction between a protein and a ligand
  - If a ligand is attached to column matrix, certain proteins will bind to it, others will not – flow of buffer will wash away unbound protein, leaving specifically bound proteins
  - Most powerful method of chromatography however it requires the knowledge of what ligand to use



SDS Polyacrylamide Gel Electrophoresis

- SDS-PAGE is the most commonly used analytical method that separates on the basis of polypeptide chain size – used to estimate polypeptide chain size
- Charged molecules migrate in an electric field
- The gel acts as a molecular sieve (filter) that allows smaller molecules to go faster
- Polyacrylamide is the gel that is a hydrophilic polymer in long chains
- SDS is used to denature the proteins’ quaternary and tertiary structures and give the chains a negative charge
- Before electrophoresis, the protein samples are heated in the presence of SDS and β-mercaptoethanol, a reducing agent is added to break disulfide bonds by exchanging cysteines in bonds – this can occur because disulfide bonds can only form in an oxidizing environment

Molecular Weight from SDS-PAGE and Immunoblot

- The distance of how far the desired protein migrated in the gel, the measurements are compared to the standards of known size and how far they migrated
- There is a logarithmic relationship between distance migrated and molecular weight
- Proteins are transferred onto a membrane according to their negative charge – transferring to a positive charge and use an antibody (high specificity)
- This is the immunoblot (western blot) – it is a way of confirming the results are actually the protein you desire