Biochemistry Topics 1-4

TOPIC 1

The Central Dogma

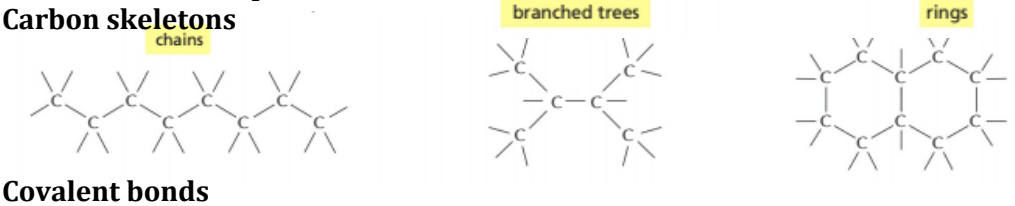
Transcription— Template DNA sequence of nucleotides copied into RNA

Translation— Use of RNA to assemble amino acids into a protein

- Varying sequences provide different 3D confirmations

Chemical Bonds and Groups

1) Carbon skeletons



2) Covalent bonds

- *Sharing* of outer shell electrons between non-metal elements
  - Double bonded atoms can't rotate around the bond axis
  - Alternating double bonds form a *resonance structure*
    - Alternating double bonded rings are highly stable

3) Hydrocarbons (C-H)

- Nonpolar, hydrophobic, insoluble
  - Methyl (CH3), ethyl (CH2CH3), prop, but, pent, hex, hept, oct, non, dec

4) Polar compounds

A) *Alcohol* (Hydroxyl group—OH)

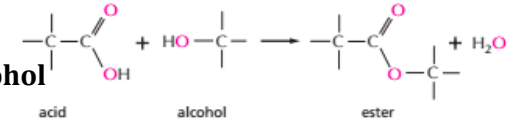
B) *Aldehyde* (Carbonyl—C=O)

C) *Ketone* (Carbonyl—C=O)

D) *Carboxylic acid* (Carboxyl—COOH)

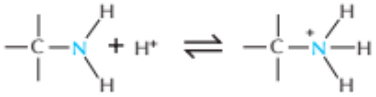
E) Esters

- Formed by combining a **carboxylic acid** and **alcohol**



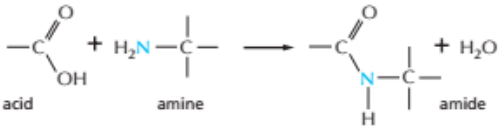
F) Amines

- Combine with H+ ion to become positively charged in water



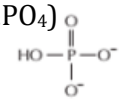
G) Amides

- Formed by combining a **carboxylic acid** and **amine**
  - Uncharged in water

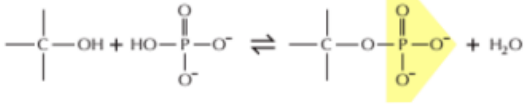


H) Phosphates

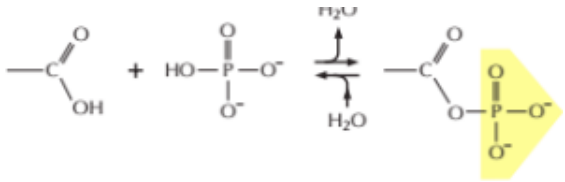
- An **inorganic phosphate** is a stable ion formed from phosphoric acid (H_3PO_4)



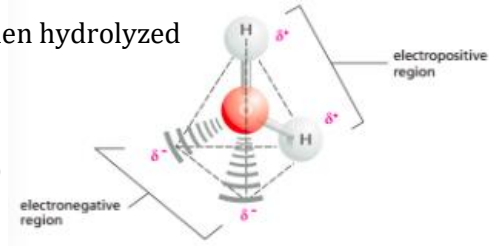
- *Phosphate esters*
  - Formed by combining **phosphate** and **hydroxyl**



- *Acid anhydride*
  - Formed by combining **phosphate** and **carboxyl**



- Contain a high energy bond; release a lot of energy when hydrolyzed



Chemical Properties of Water

Water Structure

- Polar molecule (positively charged hydrogens)
  - *Polar* and *ionic* hydrophilic substances dissolve in H_2O
  - Nonpolar hydrophobic substances are insoluble

pH

- Acids release protons (HCl → H + Cl) to become more negatively charged substances
  - Originally positive, neutral, or negatively charged
- Bases gain protons to become positively charged substances
  - Originally negatively or neutral charged
- Solution acidity defined by concentration of hydronium (H_3O+) ions
  - As pH increases, H+ concentration decreases
    - pH = -log[H+]

<u>Weak</u> Noncovalent Bonds

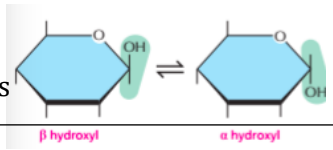
1) Van der Waals (<0.5)—Nonpolar covalent
   - Weak bonding at short distances due to their fluctuating electrical charges
     - Attraction continues until the distance between their nuclei is equal to the sum of their van der Waals radii

2) Hydrogen bond (NOF)
   - Strongest when the 3 atoms are in a straight line; 1/20th of strength of a covalent bond
   - Note: hydrogen bonds between 2 water molecules are weak

3) Electrostatic attractions
   - Attraction between fully charged groups (ionic bond) & partially charged groups on polar molecules
     - Weak in water

Isomers

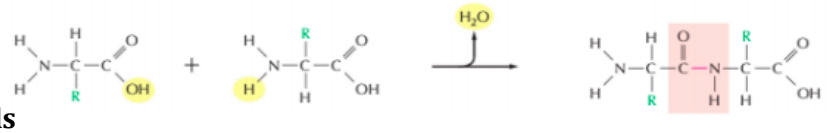
- 2 compounds with the same formula but a different arrangement of atoms
  - ~~Alpha links (Hydroxyl below); Beta link (hydroxyl above)~~

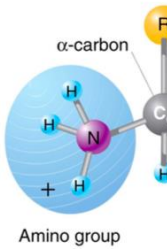


TOPIC 2

The structure of amino acids

- The properties of the R side chains distinguish amino acids which project from the backbone
  - Hydrophobic/hydrophilic, physical size, ability to participate hydrogen bonds
- Of the D & L stereoisomers, L-forms are found in proteins
- A polypeptide chain of amino acids is held together by a peptide bond formed by condensation reactions

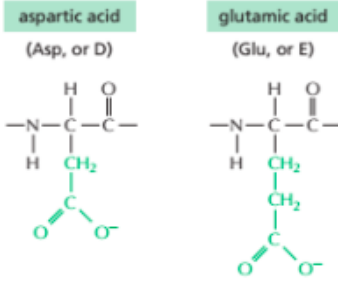




4 classes of amino acids

Hydrophilic

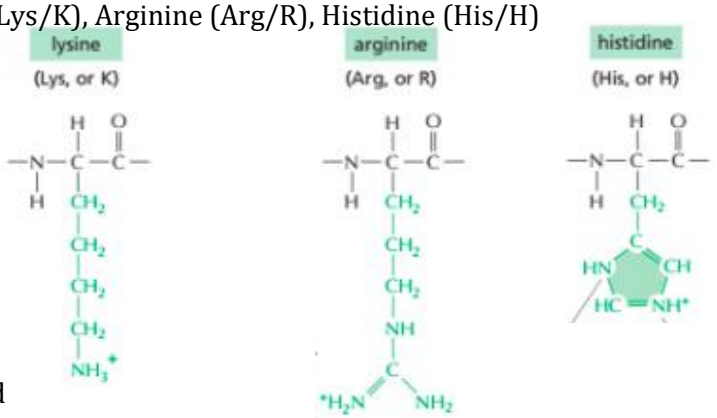
1) Polar acidic (negative charge)
   - Aspartic acid (Asp/D), Glutamic acid (Glu/E)



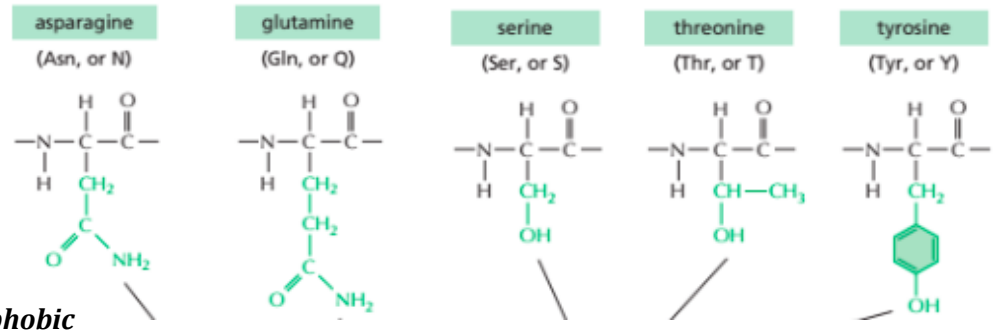
AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negatively charged
Glutamic acid	Glu	E	negatively charged
Arginine	Arg	R	positively charged
Lysine	Lys	K	positively charged
Histidine	His	H	positively charged
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine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

POLAR AMINO ACIDS

2) Polar basic (positive charge)
   - Lysine (Lys/K), Arginine (Arg/R), Histidine (His/H)

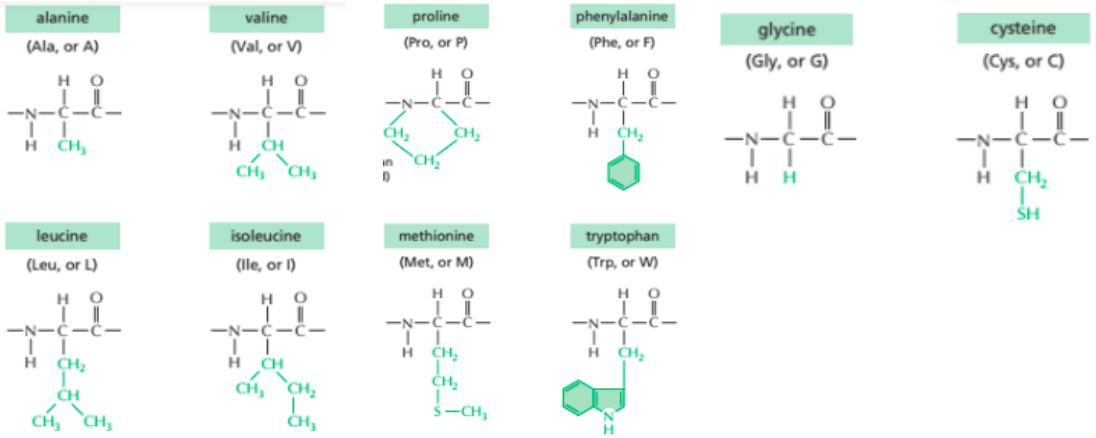


3) Polar uncharged
   - **Asparagine (Asn/N), Glutamine (Gln/Q)**, Serine (Ser/S), Threonine (Thr/T), Tyrosine (Tyr/Y)
     - Although the amide N isn't charged at neutral pH, it's polar



Hydrophobic

1) Nonpolar
   - Alanine (Ala/A), Valine (Val/V), Leucine (Leu/L), Isoleucine (Ile/I), Proline (Pro/P), Phenylalanine (Phe/F), Tryptophan (Trp/W), Methionine (Met/M), Glycine (Gly/G), Cysteine (Cys/C)



Amino acids contain ionisable groups

- Amino acids contain weak acid and weak basic groups that ionize when placed in water
- Ionization state (acquiring charge by gaining or losing electrons) is a function of
  - pH (because of the association/dissociation of proton)
    - For acids, as pH increases, ionization increases

        - For bases, as pH increases, ionization decreases
    - The affinity of the ionisable group for its proton (pKa)
        - For acids, the stronger the acid the lower the pKa (lower affinity)
        - For bases, the stronger the base the higher the pKa

Net Charge on Amino Acids/Proteins

- Amino acids are *amphoteric* (possess both acidic (COOH) and basic (NH_2) groups)
- Can exist as *zwitterions* (carrying both positive and negative charges)
- Net charge is the sum or positive and negative charges of *all* ionisable groups present at the given pH
    - As pH decreases, net charge becomes more positive (basic ionization increases)
- Net charge of a *protein* is the sum of charges of all groups in the amino acid chain

Ionization of side chains

- Acidic side chains lose proton when ionized
- Basic side chains gain proton when ionized
- Non-polar side chains do not have ionisable groups
    - *Exception*: Cysteine can lose proton attached to sulphur (think orgo)
- Polar-uncharged side chains do not have ionisable groups
    - *Exception*: Tyrosine can lose proton attached to oxygen (think orgo)

Acid dissociation constant (Ka)

- Ka relates the affinity of a group to a proton (strength of an acid)

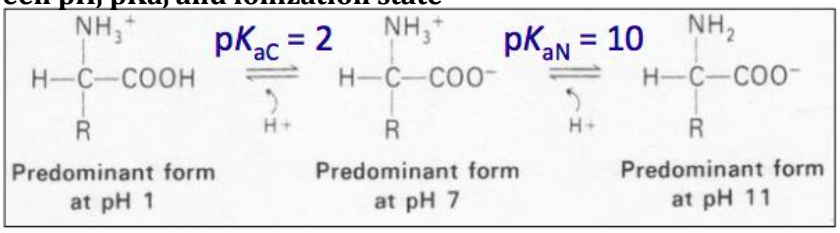
Amino acids contain weak acidic and basic groups that ionize when placed in water:

Weak acid		**Weak base**	
Dissociation equation	*Equilibrium expression*	*Dissociation equation*	*Equilibrium expression*
$RCOOH \rightarrow H^+ + RCOO^-$	$Ka = \frac{[H][RCOO]}{[RCOOH]}$	$RNH3^+ \rightarrow H + RNH2$	$Ka = \frac{[H][RNH2]}{[RNH3]}$

$pH = -\log[H^+]$ $pKa = -\log[Ka]$ $pH = pKa + \log\frac{[A]}{[HA]}$

$pH = pKa + \log\frac{R-NH2}{R-NH3}$

Relationship between pH, pKa, and ionization state



CALCULATING NET CHARGE [Net charge = (%prot.)(charge prot.) + (% depro)(charge depro)]

CASE 1 (pH = pKa)

- When the concentration of weak acid & conjugate base are equal (50% protonated & 50% deprotonated), pH = pKa

E.g. Determine the charge on the carboxyl group at pKa of 2, where pH = pKa

Net charge = (0.5)(0) + (0.5)(-1) = **-0.5**

CASE 2 (pH < Pka)
E.g. Determine the charge on the carboxyl group at pKa of 2 when pH = 1

$pH = pKa + \log\frac{[A]}{[HA]}$ $\quad 1 = 2 + \log\frac{[A]}{[HA]}$ $\quad 10^{-1} = \frac{[A]}{[HA]}$

- Therefore a 10:1 ratio of protonated to deprotonated form

Net charge = 0.9(0) + 0.1 (-1) = **-0.1**

∴ At a very low pH, everything is protonated; at a very high pH, everything is deprotonated

CALCULATING OVERALL NET CHARGE

- Add up net charge of all ionisable groups at a given pH
    - *If the difference between the pKa and the pH is 2+ units away, assume 100% for the given ionisable group*

TOPIC 3

Peptides and proteins

- Proteins are linear polymers of amino acids
- Can range in size from 50 to >30000 amino acids
- **Nomenclature**
    - **Dipeptide**: a peptide chain composed of 2 amino acids
    - **Tripeptide**: a peptide chain composed of 3 amino acids
    - **Oligopeptide**: a peptide chain composed of approx.. 3-10 amino acids
    - **Polypeptide**: a single peptide chain composed of many amino acids
    - **Protein**: a molecule composed of one or more polypeptide chains
        - All proteins contain polypeptide chains, but not all polypeptide chains make up a protein
        - A protein is a unit with a specific function, and requires all of it's polypeptide chains to function

Peptide Bond

- Removal of water through condensation reaction resulting in a peptide bond between *a*-amino group and *a*-carboxyl group
- Individual amino acids in a polypeptide chain are known as *amino acid residues*
    - They have lost their carboxyl and amino groups due to the loss of water
- The bond is directional (chain is read from the N-terminus to the C-terminus)
    - The two ends have different polarity
    - There is only ONE amino terminus, and it is always on the left for nomenclature
    - There is only ONE carboxyl terminus, and it is always on the right for nomenclature
        - **Therefore, each polypeptide chain only has one amino group and one carboxyl group**
- Polypeptides are elongated by the addition of amino acids to the *C-terminal* end of the chain

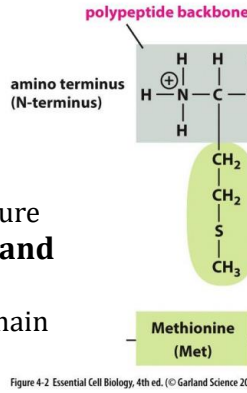
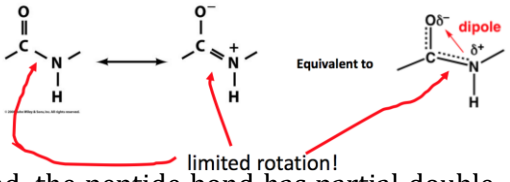


Figure 4-2 Essential Cell Biology, 4th ed. (© Garland Science 20

Reading Polypeptide Chains

- Read from the N terminus to the C terminus

- i.e. in the image to the right, we'd read the polypeptide as Met-Asp-Leu-Tyr (the other way around gives a completely different polypeptide)



Polypeptide Backbone

- Repeating pattern of NCC-NCC…
- The R side chains project outwards
- Due to the resonance between the C-O and C-N bond, the peptide bond has partial double bond character… two consequences:
    - There is a partial dipole between the positive nitrogen and negative oxygen
    - The C and N of the peptide bond are sp2 hybridized, making the 6 atoms of the backbone coplanar (all in the same plane)
        - Double bond character prevents rotation around the double bond → constrains flexibility
- Rotation IS possible around the *a*-carbon

4 levels of protein structure

Primary structure:

Sequence of amino acids forming a polypeptide (linear)

- Native conformation is dictated *solely* based on the primary structure
- The native conformation is the lowest free energy state of the protein
    - The lower the free energy, the more stable the molecule
    - The unfolded primary protein has the highest free energy

Secondary structure:

Local folding pattern of the polypeptide *backbone*

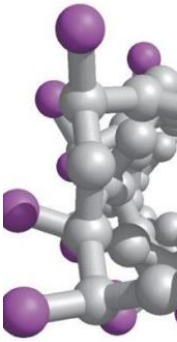
- Stabilized by hydrogen bonds between *backbone* N-H and C=O groups

Two major types: *a*-helix and β-strand

- Many other types exist (turns, different helices, loops), but these two are the most common
- Proteins are made up of many different secondary structures
- Some parts of protein structure do not have any "regular" secondary structure (*random coils*)
    - Random coil means that there is no recognizable pattern, *however* it is still a specific discrete structure encoded by genes

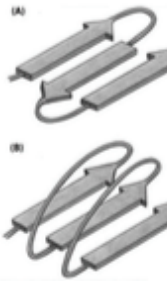
Alpha helix

- Coil shape formed when hydrogen bonds occur between **backbone** N-H of 1 amino acid & backbone C=O group of another amino acid located 4 residues away
    - All bonding opportunities are taken advantage of making a rigid cylinder
        - C=O and N-H groups are parallel to the axis, therefore the hydrogen bonds are as well
    - Side chains project outwards
    - Right handed (turns right as it goes up)
    - Dimensions: 3.6 residues & 0.54nm per turn



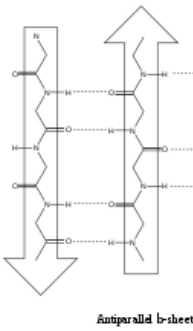
Side chains in the *a*-helix

- All the side chains are radiating outwards from the spiral
- They do not come out at right angles to the helix, resulting in a pinwheel shape
    - This is evidence that only one chiral form of amino acids is present in the body (L-amino acids) as all the side chains point in the same direction



Beta Sheet

- Formed by side-by-side hydrogen bonding between **backbones** of adjacent beta strands
    - Side chains project alternately upward and downward
    - Charged side chains are mainly on the exterior
- Hydrogen bonding is intra-chain (can happen between many beta strands within the chain)
- It is pleated due to inability of all bonds in the backbone to rotate
- The polypeptide chain folds back on itself in two ways:
    - Antiparallel arrangement (*a*)
        - All H-Bonds are maximized
        - Bonds are straight → the straighter the arrangement, the stronger the sheet
    - Parallel arrangement (*b*)
        - Hydrogen bonds are bent → weaker arrangement



Determinant of whether a sequence will fold into a confirmation?

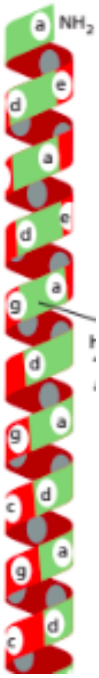
- Interactions between R-side chains
    - Steric hindrance between large side chains
    - Charge repulsion between like-charged side chains
    - Presence of proline and glycine
        - **Proline** contains a ring that constrains bond angles & kinks α-helix or β-sheet
            - There is no H on one peptide bond when proline is present, so a hydrogen bond cannot form (look at proline structure)
            - Acts as a signal for the protein to stop forming an alpha helix and beta strands
            - Considered a "helix breaker"
        - **Glycine**, with the smallest possible side chain, imparts flexibility into the polypeptide backbone, which can disrupt α-helix or β-sheet

Tertiary structure:

- Folding of the polypeptide into 3D shape due to **interactions between side chains** or **side-chain to backbone**
- Four major *weak force* interactions between R groups:
    - *Ionic bonds* between ionized side chains
    - *Hydrogen bonds*
        - Backbone to backbone (same as for secondary structures)
        - Backbone to side chain
        - Side chain to side chain
    - *Van der Waals forces*
    - *Hydrophobic interactions* → most important
        - Hydrophobic residues are buried in the core while hydrophilic residues are on the surface exposed to water
        - The attraction is caused by a *repulsion of water*
            - Water forces hydrophobic, nonpolar side chains together to minimize their disruptive effects on the water network formed by the H bonds between water molecules
    - *Covalent bond*
        - Disulfide bridge formed between *cysteine* residues
            - This bond is important only in non-cytoplasmic proteins since there are enzyme systems present in the cytoplasm to remove disulfide bonds

- Only form in oxidizing environments

Quaternary structure:



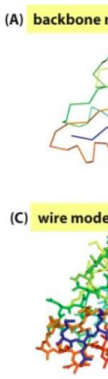
Arrangement of polypeptide chains in a protein formed by multiple tertiary structures

- Held together by the same forces/bonds that hold tertiary structures
- Each polypeptide is a subunit of the protein
- Very important for protein function

E.g. *Coiled-coils*

- 2 alpha helices come together to form a helix to minimize exposure of hydrophobic amino acid side chains to aqueous environment
- **Heptameric** repeat pattern of hydrophilic/hydrophobic residues
    - Every 7 residues (abcdefg); every "a" and "d" position is hydrophobic, "e" and "g" are charge hydrophilic amino acid
- E.g. Myosin → found in muscles

Visualization of protein structures



- *Backbone model*: trace of backbone atoms, no side chains
- *Ribbon model*: secondary structure indicated clearly with ribbons, no side chains
- *Wire model*: shows all the bonds, side chains added
- *Space-filling model*: shows the space occupied by all atoms
- *Electrostatic potential map*: shows distribution of charges on the surface of the molecule (negative being red, positive charged being in blue)

* Wire and space-filling model are not all that useful, ribbon model is usually the best as it shows us the distribution of secondary structures of the protein

Protein folding

- The native conformation is the lowest free energy state of the protein
    - The lower the free energy, the more stable the molecule
    - Likely stepwise (hydrophobic interaction, secondary structure, tertiary structure)
- Correct folding is *facilitated* by **chaperone proteins**
    - Acts by reversibly masking exposed hydrophobic (protect them) regions to prevent aggregation (misfolded proteins accumulate and clump together—causing disease)
        1) Partially folded protein enters chaperone
        2) 1 polypeptide chain is sequestered by chaperone; chamber cap closes
        3) Isolated polypeptide folds correctly & cap opens
    - Proteins that cross membranes must stay unfolded & chaperones protect them

Denaturing

- A loss of both the structure and function of a protein due to extreme conditions—often aggravate and precipitate
    - pH, high temperature, alcohol, heavy metal salts, detergents—E.g. Urea, guanidine hydrochloride
- Disrupts the **weak bonds** of the secondary and tertiary structure
    - Since denaturation reactions aren't strong enough to break the covalent peptide bonds, the primary structure remains the same

Denaturation is reversible (renaturing)

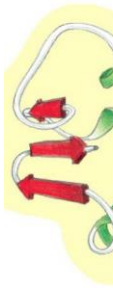
- Upon removal of the denaturating agent, some proteins go back to their native conformation
    - Evidence that the native conformation is dependent on primary structure
- Sometimes cross-links (i.e. disulphide-bonds) are formed during denaturation, which prevent the protein from renaturing (i.e. when we cook eggs)

Protein domains

- Distinct region that can fold independently of other domains & have different functions
    - Many large proteins are made up of multiple domains
- Related domains are often found in different proteins (evolution has mix and matched domains)

If you were to separate domains, they would still function and wouldn't denature

Note: The overall tertiary structure of a protein is made up of one or more domains

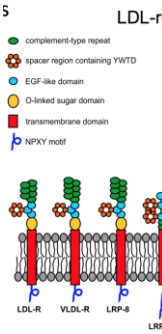


Conformational Flexibility & Changes of Proteins

- Proteins are not rigid structures
- Often, protein domains are connected by flexible linkers (clefts), which allows them to move relative to each other → overall protein structure is maintained
- Known as **conformational changes**

Protein families

- Classify proteins based on their evolutionary relatedness
    - *Homologous proteins*: derived from the same ancestor
        - Can have similar primary sequence, structures, functions, domains
- *Conserved residues*: amino acids that're critical for function & don't change on an evolutionary timescale
    - Note: the amino acid sequence *does* differ, even in similar domains; however, within domains there are conserved residues that're always there and are maintained in a particular spot in the domain as they are critical for function
    - Recognizing conserved residues allow us to identify the function of the protein



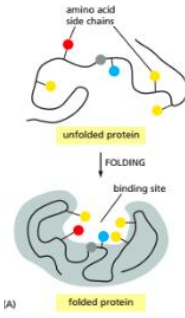
TOPIC 4: PROTEIN FUNCTION

Protein function

- Enzymes
- Structural proteins (Provide mechanical support)
- Transport proteins
- Motor proteins (Generate movement)
- Storage proteins
- Signal proteins (Carry extracellular signals from cell to cell)
- Receptor proteins (Detect signals and transmit)
- Gene regulatory (Bind to DNA to switch genes on/off)

Ligand binding proteins

- Contains substrate binding/active sites to which specific ligands bind weakly or tightly to and form a complex with the protein
    - *Ligand*: molecule that binds to a receptor & signals a cell response (NOT catalyzed or converted into a product!)
- Binding occurs with extreme specificity due to the formation of ***weak, noncovalent*** bonds & hydrophobic forces
- Binding site consists of a cavity formed by amino acid side chains



The dissociation constant (K_d)

- A measure of the **strength or affinity** of a ligand binding interaction
- Values range from femtomolar (high affinity) to millimolar

$$K_d = \frac{[Protein][Ligand]}{[Protein\ \&\ ligand\ complex]}$$, units mol/L

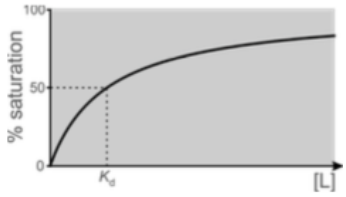
Exponentials

- 10^0
- 10^{-1} -- deci
- 10^{-2} -- centi
- 10^{-3} -- milli
- 10^{-6} -- micro
- 10^{-9} -- nano
- 10^{-12} -- pico
- 10^{-15} -- femto

Binding isotherm

- We take a fixed concentration of protein and vary the concentration of ligand, then we measure the extent to which the binding is saturated
  - K_d is equal to the ligand concentration (x-axis) at which the binding sites on the protein are 50% saturated (y-axis)
  - 100% is the point at which all ligands are bound—never occurs, plateaus before
- High affinity = tight bonding = low K_d value

$$\%\ saturation = 100\ x\ \frac{[L]}{Kd+[L]}$$



COLLAGEN

- Extracellular fibrous protein that forms strong fibrils that strengthen bones, skin, tissues—found in skin & bones
- **Triple helical** quaternary structure
- A family of proteins: collagen I – XIV
- Thin (1.5 nm), long (300) nm, strong molecule that's *hydrophobic & insoluble* due to many exposed hydrophobic side chains

Collagen Polypeptide Sequence

- Collagen has a characteristic sequence
  - Every 3rd residue is glycine (rich in glycine)
  - Lots of prolines (imino acid) and modified *hydroxyprolines*
    - Hydroxylated to have hydroxyl group
- Presence of so much proline prevents alpha helix; instead forms a **poly-proline type II helix**
  - More extended than alpha helix
  - 3 residues per turn
  - No hydrogen bonds between **backbone** N-H of 1 amino acid & backbone C=O group of another amino acid (too far apart)
    - Instead stabilized by steric repulsion of proline side chains

Hydroxylation of Proline and Lysene

- Hydroxylation of proline plays a role in triple helix stabilization of collagen molecules
    - *Hydroxyproline*: OH group can form hydrogen bonds to keep triple helix together
    - *Hydroxylysine*: OH groups serve as sites for sugar addition (**glycosylation**) to increase solubility and water absorption outside of the cell
    - *Lysines* participate in cross-linking reactions that create covalent bonds between collagen chains

Role of Glycine in Collagen

A) Allows chains to wind tightly to each other since it has the smallest side chain
    - When the three chains come together, the area between them is very tight, and glycine is the only one small enough (shortest side chain) that can fit within

B) Donates peptide bonds for hydrogen bonding

Collagen Quaternary Triple Helix

- Held together by hydrogen bonds
    - Main HB donors are the amino groups of glycine residues
    - Hydrogen bonds only exist between different strands, not within one strand
    - OH groups on hydroxyproline also form hydrogen bonds

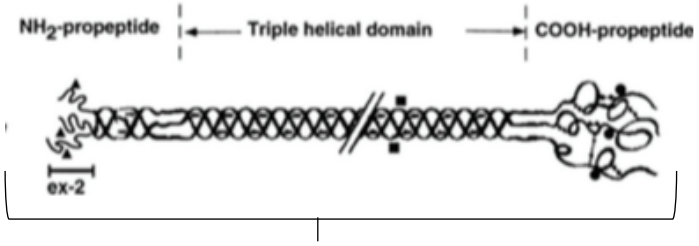
Construction of a collagen fiber

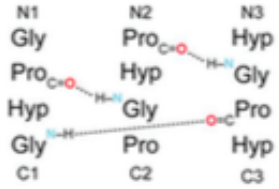
1) Individual polypeptides contain N- and C- terminals that're removed to form **propeptides** that prevent premature fibre formation and *increase solubility*

2) Polypeptide chains are hydroxylated in the endoplasmic reticulum by 2 enzymes

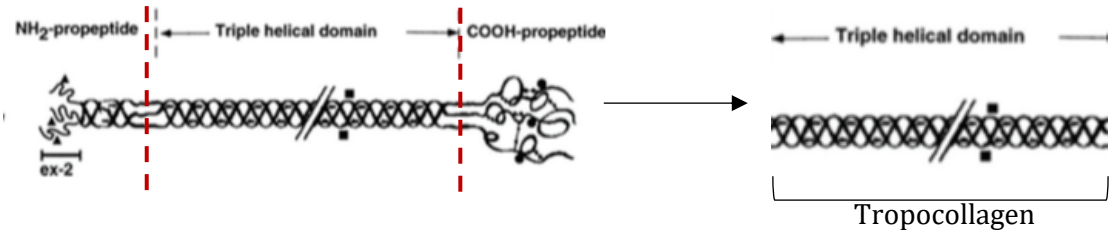
3) 3 collagen strands tightly wind together, starting at the C-terminal, to form **procollagen** in the *cytosol* of the cell

- 3 chains are H-bonded *to each other* by the peptide NH group of glycine and peptide C=O of proline residues



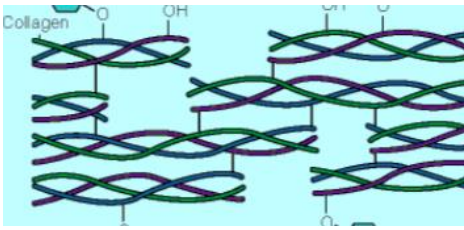


4) Procollagen is secreted from cell
- Propeptides don't form triple helices ∴ are trimmed off by pro-peptidase enzyme to leave only the triple stranded helix to form **tropocollagen**



5) Tropocollagens assemble into **collagen fibrils**

- Join end to end to create long microfibrils AND bundle laterally
- Lysine residue ends are converted to aldehydes by lysyl oxidase
    - Allows them to form covalent bonds with each other, covalently cross-linking collagen molecules in the fibril (making the fibril stronger)
- NOTE: Procollagen *<u>contains</u>* pro-peptides and is soluble; tropocollagen is hydrophobic and insoluble



Diseases from collagen gene mutations

1) Scurvy

- Lack of vitamin C (absorbic acid) in diet
    - Vitamin C is required for enzymes that hydroxylate proline
    - Non-hydroxylated proline results in an unstable collagen

2) Osteogenesis imperfecta

- Glycine mutations that prevent proper assembly of triple helix
    - Leads to a lack of collagen which results in malformed or absent bone

3) Ehlers-Danlos Syndrome (EDS)

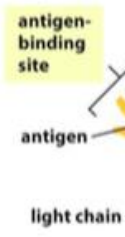
- Inherited connective tissue disorders caused by a defect in collagen synthesis
    - Caused by mutations in collagen genes or processing enzymes such as procollagen I, N-proteinase or lysyl oxidase
    - Results in loose joints and stretchy skin

ANTIBODIES (IMMUNOGLOBULINS)

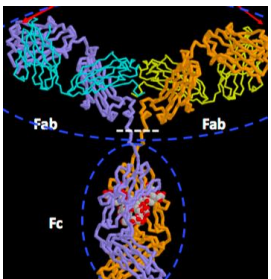
- Soluble proteins produced by **B lymphocytes** of the immune system that selectively recognize & bind tightly to foreign **antigens** (high affinity) to either inactivate it directly or mark for destruction

A) Immunoglobulin G (IgG)

- Found in blood
- *Tetrameric molecule with a "Y" shape*
    - Consist of 2 long "heavy" chains and 2 small "light" chains held in the center by *disulfide bonds*
    - Contains "Ig" domains
        - Hydrophobic center between sheets
        - β-sheets connected by **loops** to form a globular region
            - Stabilized by di-sulfide bonds
        - **Hypervariable loops** (in terms of amino acid sequence)
            A) Gives antibodies variability in antigen specificity
            B) Allows for a single antibody to bind to different sites on the same antigen
                - 1 variable domain & 3 constant domains for **heavy chains (V_H)**
                - 1 variable domain & 1 constant domain for **light chains (V_L)**
    - Antigen-binding site at the tips of the Y



Proteolysis of IgG functional segments



Yields 3 fragments

1) 2 FAB (Fragment Antigen Binding)

- Binds to the antigen
- Antigen makes contact with both heavy & light chain

2) 1 FC (Fragment Crystallized)
   - Acts as the effector site to mediate function
   - Signals/activates the immune response pathway
     - Binds to phagocytes, mast cells, basophils, eosinophils, NK cells

Artificial production and use of antibodies (in labs)

A) Polyclonal

- Created by injecting purified antigen in a host animal
  - After immune response, isolate the accumulated antibodies from the <u>blood</u>
- Produces a collection of antibodies (with different amino acids sequences) that bind to *different sites* on the same antigen

B) Monoclonal

- Created by injecting purified antigen in a mouse
  - After immune response, isolate the accumulated antibodies from the <u>spleen</u>
- Fuse isolated cells to an immortalized cell line such that they continue replicating outside of the body
- The cloned cells produce a unique antibody (with the exact same amino acid sequence) that bind to a *single site* on an antigen

Extreme specificity of IgG makes it a valuable tool for

1) Laboratory experiments: identification of molecules in mixtures
2) Clinical: diagnosis of disease
3) Clinical: therapy (E.g. Antibody drugs)

Therapeutic antibodies

A) Humira

- Binds to the signalling molecule that caused the inflammation, or a receptor molecule
- Rheumatoid arthritis, Crohn's disease, Psoriasis, etc.

B) Herceptin

- Prevents cancer cells growth

C) Reopro

- Anticoagulant often used in surgery

D) Praluent

E) Stelara

- Must be injected—can't be taken as pills since the amino acids would be degraded before entering blood stream

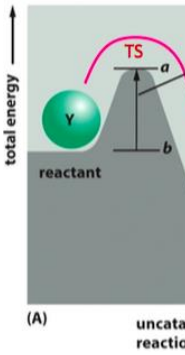
ENZYME CATALYSIS

Enzyme

- Catalyst that increases the rate of reactions by decreasing activation energy and providing an alternative pathway
  - Don't *provide* energy, rather facilitate the interaction

- Ratio of the substrate to product at equilibrium (Equilibrium constant—K_{eq}) isn't affected; only the *rate* at which equilibrium is reached

Transition State Confirmation(‡)—THEORETICAL



- An intermediate form between reactants and products wherein bonds have sufficient energy to break and form products
  - Has bonds in the process of forming *and* breaking
- Highest energy state along the reaction coordinate

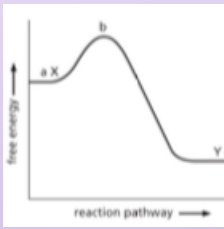
Activation Energy (EA)

- Minimum amount of energy required to undergo a reaction and change from one conformation to transition state

Enzyme mechanisms to lower activation energy

1) Binding 2 substrates and orienting them to encourage a reaction between them
2) Rearranging the electrons in the substrate to create a partial negative & positive charge that favor a reaction
3) Straining substrates into a favorable geometry that *resembles* the transition state
   - An enzyme has the highest affinity for the transition state, *not* for substrates or products
     - The shape of the active site (topography) is more complimentary to the transition state
4) Supplying proton acceptors/donors, electron donors/acceptors
5) Excluding water

Thermodynamics



- *Spontaneous (-ΔG):* Energy change for reactant to product is energy c – a = negative value
  - Energetically favorable; increase entropy
- *Nonspontaneous:* Energy change for product to reactant is energy b – c = positive value

Equilibrium (ΔG=0)

- Reactions proceed until the rate of forward and backward reactions are equal, no further change in concentrations of substrate or product
- K_{eq} directly related to ΔG^o by $\Delta G° = -RT\ln(K_{eq})$

Transition state analogues

- Compounds that structurally resemble the transition state and bind tightly to the active site—but aren't substrates for the enzyme
  - Act as enzyme inhibitors

Active site

- Binding of substrate to activate site results in substrate attaining transition state conformation
- Makes up 5% of the enzyme *surface* (crevice between domains)
- Upon catalyzing a reaction, enzymes are released unchanged and further catalyze other substrates
  - Unlike ligands that come on and off, substrates are chemically transformed
- Rapid diffusion of substrates results in collisions that allow enzymes to find their substrate

Cofactors

- Compound bound to an apoenzyme vital for its function

    - Proteins, metal ions (E.g. Iron) or coenzymes (complex organic molecules derived from vitamins)
- **Apoenzyme** (protein portion—inactive) + **cofactor** (nonprotein portion—activator) = **holoenzyme** (whole enzyme)

Characteristics of enzymes

1) Specificity—Bind to specific substrates
2) Fidelity—Never make a mistake (I.e. Recognizing improper substrate or producing incorrect product)
3) Rapidity—Accelerate reactions
4) Work under mild conditions—don't require high temperatures or pressure

Types of chemical reactions carried out by enzymes

1) Hydrolytic—Bond breaking using water (E.g. Nuclease, protease)
2) Condensation—Bond making (E.g. RNA polymerase)
3) Isomerization—Rearranging bonds
4) Oxidation-Reduction—Gain/loss of electrons (E.g. Oxidase)
5) Group transfer—Transferring chemical groups (E.g. Phosphorylation)

Relationship between activation energy and reaction rate

- $\Delta\Delta G^{\ddagger}_{cat}$ represents the change in uncatalyzed activation energy and catalyzed activation energy
- Rate enhancement = $e^{-\Delta\Delta G\ddagger cat/RT}$
- A single weak interaction between active site and substrate lowers activation energy by 4-30 kJ/mol

Optimal conditions for enzyme function

- There exists an *optimum temperature* & pH at which enzyme catalysis peaks
    - Temperature: if you shift from the optimum point, enzyme denatures and growth rate is low
    - pH: If you shift from the optimum point, certain side chains can get protonated and deprotonated
        - Represented by a bell-shaped curve

Lysozyme

- *Hydrolyzes* bonds between polysaccharide chains of bacteria cell walls → thought to be ancient antibiotic
- Present in saliva, tears, egg white
- Stabilized by disulphide bonds
- 1st enzyme for which detailed mechanism of catalysis was determined
- 1st enzyme whose structure was determined by X-ray crystallography

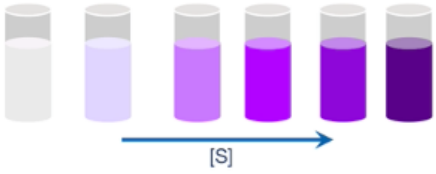
Lysozyme hydrolysis mechanism

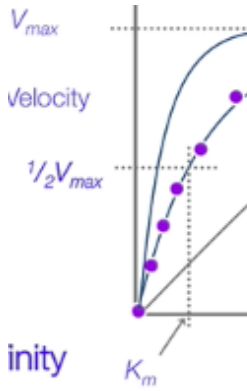
1) Lysozyme-substrate binding induces a distortion of 1 sugar into a strained chair conformation (resembles transition state), weakening the bond
2) Two acidic residues are precisely positioned adjacent to the bond where they receive and donate protons
    - Glu 35 & Asp 52
    - Glu donates proton to one of the sugars, while Asp forms a covalent bond with the other sugar

3) Result is a transient covalent complex of an enzyme-sugar linkage at the same time as the sugars are separated
    - *Transient covalent complex* (enzyme-substrate complex with the substrate in its transition state)
4) 1 product is free to leave, water comes in to donate a proton to the deprotonated Glu, while the OH attacks C1 carbon that is bonded to Asp, which hydrolyzes the enzyme linkage, releasing the remaining the product and regenerating the enzyme

Enzyme kinetics as a function of substrate concentration

- Keep enzyme concentration constant and adjust the substrate concentration in each vial
- Measure the rate of product formation (enzyme velocity), dictated by a change in colour (Dark purple—more product)





V vs. [S] plot

1) No substrate, no velocity
2) *Substrate limited portion*
    - As substrate concentration increases, velocity increases linearly
3) *Enzyme limited portion*
    - Velocity remains constant despite increase in substrate concentration
    - The rate of reaction is limited by enzyme concentration
        - Ability of enzymes available to bind to substrate is finite

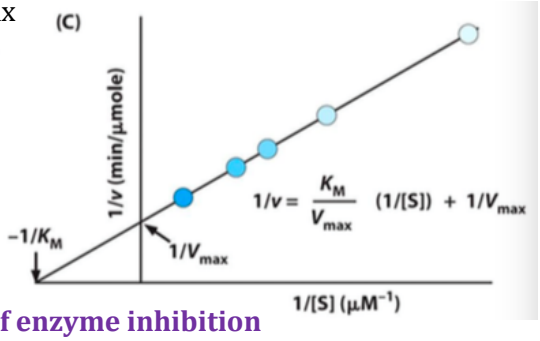
Michaelis-Menten Equation

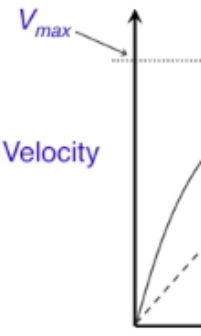
$$v = \frac{Vmax\,[S]}{Km+[S]}$$

- V_{max} – Maximum rate of the reaction at which it saturates (All available enzymes bound to substrates)
    - Directly dependant on enzyme concentration (doubling concentration doubles Vmax)
- K_m – Substrate concentration that reaches ½ V_{max}
    - Independent of enzyme concentration
    - Measure of affinity between enzyme and substrate
    - The higher the affinity, the less time required to reach Vmax
        - High K_m = Low affinity
        - Low K_m= High affinity

Double-reciprocal/Lineweaver-Burk plot is used (1/v vs. 1/[S])
Inverse of Michaelis-Menten: $\mathbf{1/v = (1/[S])\,(KM\,/Vmax) + 1/Vmax}$

- Intercepts give Km & Vmax





Enzyme kinetics as a function of enzyme inhibition

Reversible inhibition

- Noncovalent binding of inhibitor to enzyme

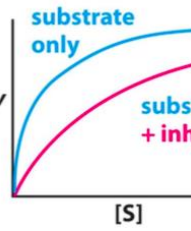
- Inhibitor can be removed

Irreversible inhibition

- Covalent binding of inhibitor to enzyme (covalent complex formed)
  - Permanently blocks activity (enzyme disabled)

***1) Competitive inhibition* (Reversible)**

- Binding of an inhibitor, that resembles substrate, to the active site on the enzyme prevents binding of the substrate
  - Overcome competitive inhibition by increasing substrate concentration ($\therefore$ Vmax unaffected)
- V_{max}, despite the number or type of inhibitors, remains constant
- K_m increases as competitive inhibitors increase, affinity decrease
  - Km increases, -1/Km decreases



Post-translational modifications

- Allows us to have much more proteins in the body then are coded for in the genome
- Phosphorylation, Ubiquitination, Acetylation, Sumoylation
  - Alters stability or signaling of proteins
- Glycoslyation
  - Affects protein folding, secretion, solubility, binding to other biomolecules
- Myristoylation, Farnesylation
  - Alters location
- Lipoproteins binds lipids
- Metalloproteins bind metal ions
- Hemoproteins have an attached heme group

Phosphorylation

- Modification of serine, threonine or tyrosine by a phosphate group (all have a free hydroxyl group)
  - Two negative charges are present on that phosphate, which is important since none of the other side chains have a charge of -2
  - These negative charges allow for new bonds to form between protein residues, ultimately modifying the structure of the polypeptide