Topic 1: Water, pH and Buffers

1. Physical Properties of Water:
   - Tetrahedral shape (~104.3°)
   - Permanent dipole due to electronegativity of oxygen atom (δ-)
   - Polar nature results in hydrogen bonding where each water molecule can form a maximum of 4 H-bonds.
   - Re-orientates themselves at room temperature at a rate of 10^{-12} seconds.
   - Hydrogen bonding in water results in:
     - High heat of fusion (melting point)
     - High heat of vaporization (boiling point)
     - Ability to dissolve most polar compounds via electrostatic interactions
     - An open structure which makes ice less dense than liquid water.
   - Amphiphilic/amphipathic molecules have both hydrophilic and hydrophobic properties.
     - Can be organized in various ways such as micelle or bilayers.
     - Useful as surfactants (soap)
   - A clathrate (cage-like) structure is formed by water molecules around the non-polar solute.
     - Not energetically favored and thus would lead to insolubility of solute.
     - Hydrophobic interactions would cause non-polar molecules to clump together to minimize exposed surface area.
   - Large molecules with both hydrophilic and hydrophobic residues can be soluble in an aqueous environment
     - Hydrophobic residues can be buried inside molecule, maintaining the shape of molecule
     - Hydrophilic regions are exposed to the surroundings.

2. Chemical Properties of Water:
   - Water dissociates poorly: $H_2O \rightleftharpoons H^+ + OH^-$, ionization is about 10^{-7}M
     - 55.5mol water/1L = 55.5M
   - $K = [H^+][OH^-]/[H_2O]$, $K_w = [H^+][OH^-] = 10^{-14}$

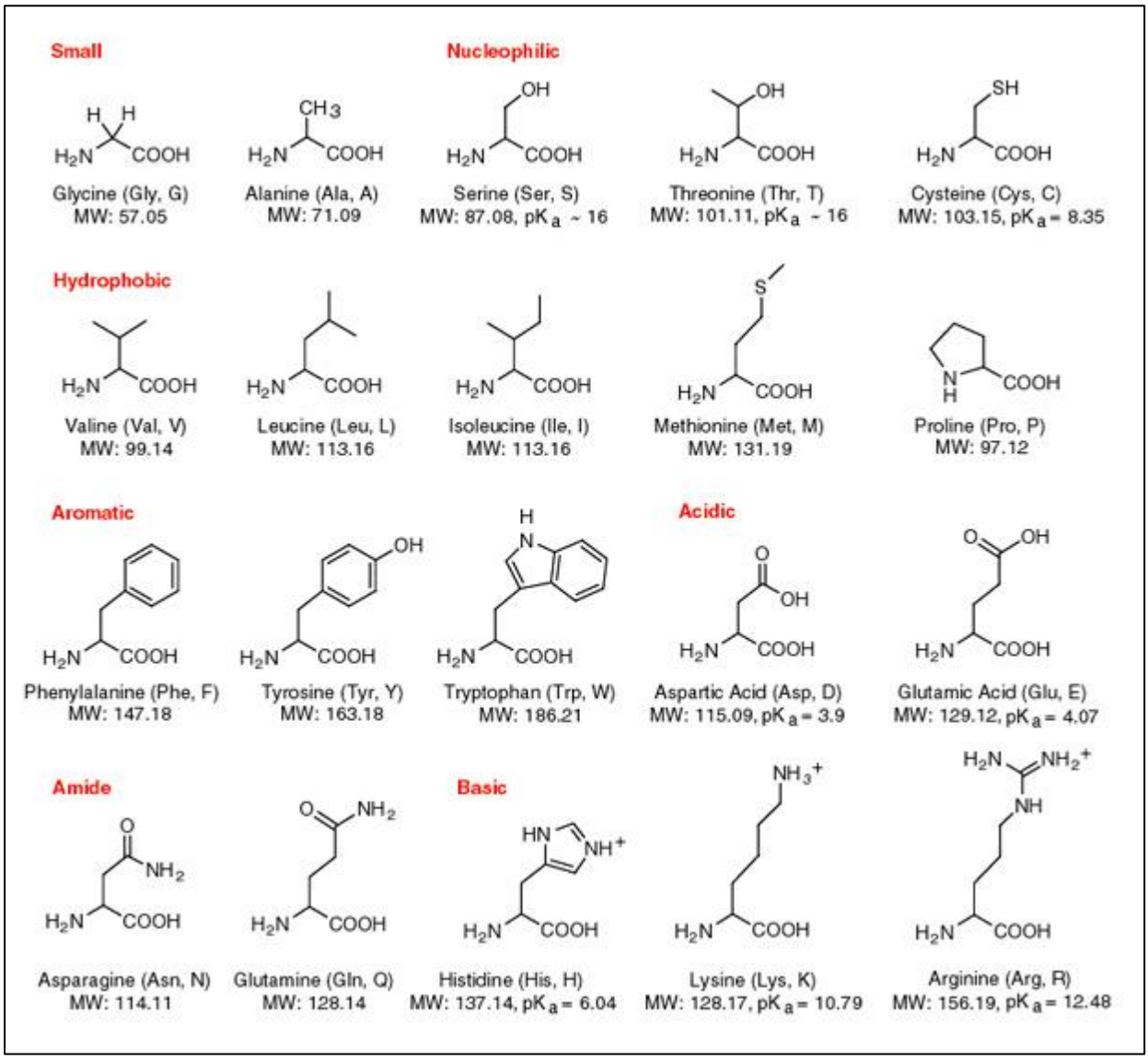
3. pH and Buffers:
   - Bronsted-Lowry: Acids are proton donors while bases are proton acceptors.
   - $pH = -\log_{10}[H^+]$, $pK_a = -\log_{10}K_a$
   - For any weak acid, the strength is measured by $K_a = [H^+][A^-]/[HA]$
     - Smaller the Ka, the weaker the acid.
     - The higher the pKa value, the weaker the acid.
   - Henderson-Hasselbalch Equation: $pH = pK_a + \log_{10}([A-]/[HA])$
   - Buffers resist changes in pH by reacting away excess H^+ or OH^- added.

- Maximum buffering capacity is pH = $pK_a \pm 1$
  - Equal amount of acid and conjugate base are present.
- Buffers present in human body (pH 6.9-7.4)
  - Phosphate system ($HPO_4^{2-}/H_2PO_4^-$): protects pH of intracellular and extracellular environment.
  - Bicarbonate system (HCO_3^-/H_2CO_3): protects pH of blood. Breathing can adjust the equilibrium of the system as CO_2 and H_2O is removed from the body. $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$
  - Amino acids and proteins: acidic and basic amino acids can act as buffers. At neutral pH, only histidine ($pK_a \sim 6.0$) can act as a buffer.
- In drug absorption, un-ionized form of the drug is preferentially absorbed via passive diffusion.
  - Weak acidic and neutral drugs are absorbed from the stomach (pH2) as acidic groups would be fully protonated and thus easily absorbed into the blood stream.

4. Sample calculations:
   - What is the pH of a mixture of 5ml of 0.1M NaOAc and 4ml of 0.1M HOAc where the pKa of acetic acid is 4.76?
     - Moles of NaOAc = $0.1mol/L \times 0.005L = 5 \times 10^4$ mol
     - Moles of HOAc = $0.1mol/L \times 0.004L = 4 \times 10^4$ mol
     - Total volume = 0.009L
     - $[HA] = 4 \times 10^4 \text{ mol} / 0.009L = 4.45 \times 10^{-2}M$
     - $[A^-] = 5 \times 10^4 \text{ mol} / 0.009L = 5.56 \times 10^{-2}M$
     - $pH = 4.76 + \log_{10}([A^-]/[HA]) = \underline{4.86}$
   - When 1mL of 0.1M HCl is added to the mixture, what is the new pH?
     - Moles of HCl = $0.1mol/L \times 0.001L = 1 \times 10^4$ mol
     - Use ICE table here:
     - Moles of NaOAc = $5 \times 10^4 \text{ mol} - 1 \times 10^4 \text{ mol} = 4 \times 10^4$ mol
     - Moles of HOAc = $1 \times 10^4 \text{ mol} + 4 \times 10^4 \text{ mol} = 5 \times 10^4$ mol
     - Total volume = 0.01L
     - $[HA] = 5 \times 10^4 \text{ mol} / 0.01L = 5 \times 10^{-2}M$
     - $[A^-] = 4 \times 10^4 \text{ mol} / 0.01L = 4 \times 10^{-2}M$
     - $pH = 4.76 + \log_{10}(0.04/0.05) = \underline{4.66}$
   - Drug HX has a pK_a of 6.5 and is absorbed into the blood through the stomach (pH 1.5) and small intestine (pH 6.0). Calculate the amount of X absorbed in each of the sites.
     - $\log([X^-]/[HX]) = pH - pK_a = -5$, thus $[X^-]/[HX] = 10^{-5}$ (stomach)
     - $\log([X^-]/[HX]) = pH - pK_a = -0.5$, thus $[X^-]/[HX] = 0.32$ (intestine)
     - %HX in stomach = $1/(1+10^{-5}) \times 100\% = 100\%$
     - %HX in intestine = $1/(1+0.32) \times 100\% = \underline{75.7\%}$.
     - Very little amount of dissociated form is found in the stomach and thus major absorption route is likely to be from the stomach.

Topic 2: Amino Acids and Proteins

1. Amino acids: There are 20 naturally occurring alpha amino acids where the amino group is attached to the alpha carbon.
   - Alpha carbon is chiral for all amino acids except for glycine and is found in the L form in cells.
   - D (clockwise)/L (anticlockwise) or R/S configuration can be used to determine chirality.
   - Amino acids can condense to form peptides and proteins via the formation of an amide bond.
   - Amino acids are amphoteric and will always have a charge in aqueous solution depending on the pH of the solution and the pK_a of the amino acid.
   - A zwitterion occurs when the amino acid has both a positive and negative charge but is electrically neutral.
   - 20 Standard Amino Acids:



- Amino acids can also be grouped as “essential” and “non-essential”

Essential (cannot biosynthesize)	Non-essential
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic Acid
Leucine	Cysteine
Lysine	Glutamic Acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

- The frequency of amino acids vary in proteins with Leu, Ala, Ser and Gly being the most common and Met, His, Cys and Trp being the most uncommon.
- The pK_a of amino acids are similar:
  - The alpha amino group $pK_a \approx 9$
  - The alpha carboxyl group $pK_a \approx 2$
  - Acidic side chain (Gln, Asp) $pK_a \approx 4$
  - Basic side chain (Lys, Arg) $pK_a \approx 11$, (His) $pK_a \approx 6$
- The isoelectric point is where the amino acid is electrically neutral, and it is affected by any charges on the R group.
  - $pI = (pK_{a1} + pK_{a2})/2$ for acidic or uncharged amino acids
  - $pI = (pK_{a2} + pK_{a3})/2$ for basic amino acids
- Other naturally occurring amino acids found in proteins include:
  - Selenocysteine
  - Pyrrolysine
- Post-translational modifications include:
  - Phosphorylation ($-PO_3^{2-}$) on Ser, Thr, Tyr for hormone receptors/regulatory enzymes
  - Acetylation ($-CH_2COO^-$) on Lysine in histone proteins
  - Methylation ($-CH_3$) on Lysine and Arginine in histones
  - Acylation ($-COCH_3$) on Cysteine in G-protein-coupled receptors
  - Prenylation ($-CH_2CH=CHMe_2$) on Cysteine in Ras p21
  - ADP-ribosylation (ADP-ribose) on Histidine and Arginine in G proteins and eukaryotic elongation factors
  - Adenlylation (AMP) on Tyrosine in glutamine synthesis
  - Myristoylation ($-(CH_2)_{13}COOH$)
- Examples of modified amino acids: 3-methylhistidine, 5-hydroxylysine, 4-hydroxyproline, phosphothreonine, γ-Carboxyglutamic acid etc.
- Derivatives of amino acids that are not found in proteins include: γ-Aminobutyric acid(GABA) - E, Histamine - H, Serotonin – W
- Polymers of amino acids is called a peptide:
  - Dipeptide, tripeptide, oligopeptide, polypeptide

2. Proteins
   - Proteins are the agents of many biological functions:
     - Enzymatic (amylase)
     - Regulatory (insulin)
     - Transport (hemoglobin
     - Structural (collagen)
     - Contractile (myosin, actin)
   - Proteins can have different overall conformations such as fibrous (collagen), globular (myoglobin) and membrane (bacteriorhodopsin)
   - Proteins can be modified e.g. Lipoproteins, Glycoproteins, Phosphoproteins, Hemoproteins, Flavoproteins (Iron) and Metalloproteins (Zn, Cu, Ca etc)
   - Proteins can be found in different locations:
     - Intracellular proteins
     - Extracellular proteins
     - Membrane proteins such as peripheral proteins which are associated to the membrane and integral proteins which are bound to the membrane. Peripheral proteins can be removed using NaCl while Integral proteins can be removed using SDS-PAGE.
   - Proteins are a polymer of amino acids and have 4 levels of organization;
     - Primary (linear arrangement)
     - Secondary (local folds: α-helix, β-sheet)
     - Tertiary (global folding)
     - Quaternary (aggregation of global folds)
   - Primary structure of proteins can be determined via:
     - Direct sequencing through protein digestion and analysis of amino acid fragments
     - Mass spectrometry to determine mass of protein fragments
     - Prediction from DNA/RNA sequence.
   - A proteins needs to be correctly folded to allow:
     - Binding of specific substrates
     - Catalysis of specific reactions
     - Structural proteins to have the mechanical strength
   - Folding is driven by weak interactions, assisted by molecular chaperones:
     - Covalent bonds (disulphide linkages)
     - Hydrophobic interactions
     - Hydrogen bonds (gives rise to secondary structures)
     - Ionic bonds (between acidic and basic amino acids)
     - Van der Waals interactions
   - Under specific conditions, proteins can renature itself by settling into various conditions before settling into the most energetically favorable configuration.

- Unfolded proteins may aggregate, forming insoluble products that lead to cell death due to hydrophobic interactions and thus molecular chaperones are present to prevent or reverse misfolding.
- Secondary structures are determined by hydrogen bonds between peptide bonds and the rotation of the amide bond which limits the number of conformations about the α-Carbon –amide plane.
- Alpha Helix (e.g. keratin)
    - 3.6 amino acids per turn
    - Intra-Hydrogen bonding is 4 residues apart
    - R-groups extend outwards
    - Right handed helix as R groups will cause steric hindrance in a left handed helix.
    - Glycine and Proline are rare in as glycine is too flexible and proline lacks the NH group to extend the helix.
    - Commonly found in globular proteins
- Beta Sheet (e.g. silk fibroin)
    - Alternating amino acids point in the opposite direction where the C_α – C bond is 180° different from the C_α – N bond.
    - Hydrogen bonds occur between strands in the beta sheet
    - R groups project above or below the plane of the beta sheet.
    - Can be parallel (both strands in N→C direction) or antiparallel (N→C and C⟵N directions)
    - Amino acids with compact R groups are favored.
- Beta Turns
    - Found between antiparallel beta sheets strands
    - Often contain proline and glycine
- Triple helix (tropocollagen – fibrous protein)
    - Characteristic amino acid sequence of –(Gly-X-Pro/Hypro)$_n$–
    - One inter-chain H-bond for each triplet of amino acid between NH of Gly and CO of X in adjacent chain.
    - Composed of 3 left-handed helices wound together to give a right-handed super-helix.
- Function of protein depends on structure of protein which depends on both amino acid sequence and weak, non-covalent forces.
    - Slight changes to the structure of proteins can affect its function
- Motifs are a combination of alpha helices and beta sheets with beta turns in specific geometric arrangements.
- Domains (super-secondary structure) are discreet, independently folded globular units which consists of about 100-200 amino acid residues and a combination of motifs

    - Domains have specific functions (e.g. Glyceraldehyde-3-phosphate dehydrogenase has 2 distinct domains where NAD^+ binds to the first domain and G-3-P the second).
    - Immunoglobin domains contain about 70-110 amino acids and are from via 2 beta sheets held together by interactions between cysteines and other charged amino acids.
- Quaternary structures are formed when multiple proteins are bound together by non-covalent interactions (e.g. hemoglobin)
    - Oligomers are more stable than dissociated subunits thus prolong protein life in vivo
    - Active sites are formed by residues from adjacent chains
    - Errors in synthesis will be greater for longer/bigger chains, thus smaller chains are made and then assembled
    - Subunit interactions allow for cooperativity/allosteric effects.
- Proteins can be denatured by heat, SDS, organic solvents and pH which interferes with hydrophobic interactions and hydrogen bonds.
    - 2°, 3° and 4° structures are destroyed, but 1° structure remains intact.
    - 1° structure dictates the 3-D structure for the protein.
    - Refolding can be done in vitro with the following conditions:
        i. Buffer + denaturant + reducing agent
        ii. Low temperature
        iii. Large volume (low concentration of denatured protein <0.1mg/ml)
        iv. Slow dialysis to remove denaturant and allow gradual refolding
- Prion is an infectious protein which can cause transmissible spongiform encephalopathy (TSEs)
    - Prion is native to humans, but misfolding can cause the disease phenotype as misfolded PrP is protease insensitive and thus will form insoluble fibers which will lead to death.
    - Diseased PrP can induce normal PrP to misfold.

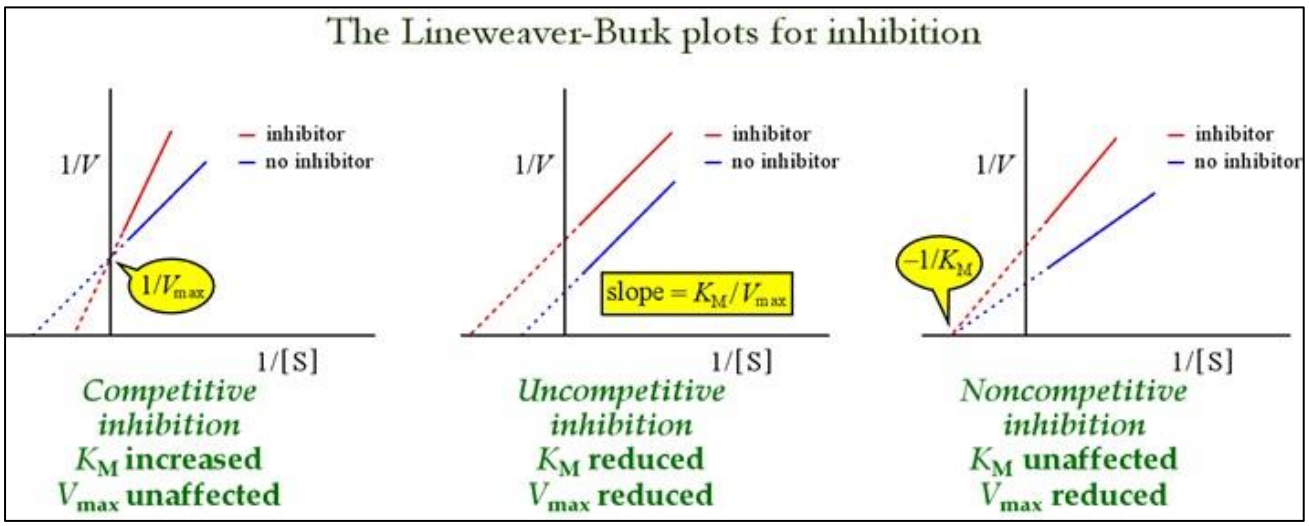
Topic 3: Enzymes

1. Enzyme is a protein catalyst that increases the velocity of a chemical reaction without being consumed during the reaction
   - Has an active site where substrate can bind for catalysis.
   - Allow for higher reaction rates, milder reaction conditions and greater specificity thus giving high product yields (>95%) which is important in many biological reactions and it will give a high overall yield over the large number of steps.
   - Have the capacity for regulation as the activities can change in response to the concentrations of substances besides their substrates and products.
2. Enzymes fit their substrate by either:
   - Lock and key model: substrate has exact shape of active site
   - Induced fit: binding of substrate induce a conformational change in active site
3. Enzymes require cofactors to function:
   - Metal ions (Mg^{2+}, Zn^{2+}, Fe^{2+}/Fe^{3+})
   - Coenzymes (organic molecules)
   - Prosthetic groups are cofactors that are covalently attached to proteins
   - Apoenzyme + Cofactor = Holoenzyme
   - E.g. Alcohol dehydrogenase catalyzes the following reaction: $CH_3CH_2OH + NAD^+ \rightleftharpoons NADH + CH_3CHO + H^+$, where NAD^+ is the coenzyme and Ethanol is the substrate.
4. Enzymes reduce the activation energy ($\Delta G^{\ddagger}$) of the reaction, thus accelerating both the forward and reverse reactions
   - $\Delta G_{reaction}$ is not altered, direction of equilibrium doesn't change.
   - Equilibrium favors products when $\Delta G_{reaction} < 0$ and favors reactions when $\Delta G_{reaction} > 0$.
5. Enzymes catalyze reactions in 3 ways:
   - Acid-base catalysis (e.g. RNase A, histidine residue)
     - Enzyme adds or accepts a proton from the functional group of the substrate, making it more reactive
     - Acidic (Glu, Asp) and basic (Lys, Arg, His) amino acids can do this.
     - Cys, Ser and Tyr can do this to a certain extent due to OH/SH group.
   - Covalent catalysis (e.g. GAPDH with nucleophilic SH on Cys residue)
     - Involves transient formation of catalyst-substrate covalent bond due to nucleophilic attack of enzyme nucleophile to electrophile substrate.
   - Metal Ion catalysis (e.g. hexokinase where Mg^{2+} shields phosphate group)
     - Metal ions retain their positive charge at neutral pH and can have higher charges than amino acid side groups (+2, +3 etc)
     - Metal ions bind to both substrate and enzyme, orientating the substrate.

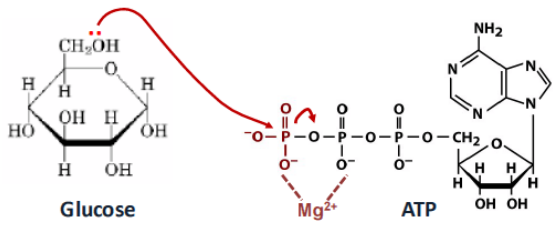
- Metal ion stabilizes/shield negative charges during transition state and thus helps promote nucleophilic attack.
- In carbonic anhydrase $CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$, Zn^{2+} mediates deprotonation of H_2O, forming the OH^- nucleophile which attacks CO_2.

6. Enzymes can be affected by several factors:
   - pH affects the ionization of R-groups in the amino acid residues involved in the catalytic activity of the enzymes.
   - High temperature can denature proteins and thus will decrease enzyme activity.
7. Enzyme Kinetics: step with the higher activation energy is the rate-determining step.
   - For a simple reaction S→P, velocity is given by $\nu = d[P]/dt$ or $\nu = - d[S]/dt$
   - For $E + S \rightleftharpoons ES \rightarrow E + P$, use the Michaelis-Menten equation:
     - $\nu_o = (V_{max}[S])/(K_m + [S])$
     - Assumptions are that $[P] \approx 0$ when initial velocity is measured, steady state is achieved and enzyme only exists in two forms, E and ES, thus $[E_T] = [E] + [ES]$
     - $V_{max} = k_2[E_T]$
     - When $[S] << K_m$, $\nu_o = (V_{max}/K_m)[S]$ (first order reaction)
     - When $[S] >> K_m$, $\nu_o = V_{max}$ (zero order reaction)
     - $K_M = (k_{-1} + k_2)/k_1$ = substrate concentration at $V_{max}/2$
   - K_M measures the affinity of the enzyme for its substrate when $k_2/k_1 << k_{-1}/k_1$
     - k_{-1}/k_1 is K_d (dissociation constant)
     - Small K_M means high affinity
     - High K_M means low affinity
   - Use Lineweaver-Burk plot to find K_m as Michaelis-Menten plot requires large substrate concentrations to find V_{max} and K_m
     - Plot $1/\nu$ against $1/[S]$: $\frac{1}{\nu} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$
     - Y-intercept is $1/ V_{max}$ and x-intercept is $-1/K_M$.
     - Gradient of the slope is K_M/V_{max}
   - K_{cat} is the turnover number, number of substrate molecules converted into product per enzyme molecule per unit time when the enzyme is saturated with substrate.
   - $V_{max} = K_{cat}[E_T]$ and thus $V_o = K_{cat}[ES]$
     - Ratio of K_{cat}/K_M defines the catalytic efficiency of an enzyme.
     - When $[S] << K_M$, $V_o = (K_{cat}/K_M)[E_T][S]$, thus reaction velocity is dependent on $[E_T]$ and [S]. For same concentration of enzyme and substrate, enzyme with larger K_{cat}/K_M ratio will produce a higher velocity.
   - Irreversible inhibition occurs when a covalent bond is formed with functional groups at the active site of the enzyme. The amount of active enzymes is decreased and thus V_{max} is decreased. The dilution/dialysis of the solution containing the EI complex will not restore enzyme activity.

- Reversible inhibition occurs when the inhibitor associates reversibly with the enzyme at various sites:
  - Competitive inhibitors: binds at active site, increases K_M but does not affect V_{max} as concentration of free enzyme decreases → [ES] decreases. Effect of competitive inhibitor can be reduced by increasing substrate concentration. $\nu_0 = \frac{V_{max}[S]}{\alpha K_M + [S]}$ where $\alpha = 1 + \frac{[I]}{K_I}$
  - Uncompetitive inhibitors: binds to the enzyme-substrate complex which affects the catalytic function of the enzyme. Some of the substrate is bound in the ESI complex and thus K_M decreases. V_{max} decreases because inhibitor binding hinders product formation. K_M decreases proportional to V_{max} and thus the K_M/V_{max} ratio remains the same. $\nu_0 = \frac{(\frac{V_{max}}{\alpha'})[S]}{(\frac{K_M}{\alpha'}) + [S]}$ where $\alpha' = 1 + \frac{[I]}{K_I'}$
  - Noncompetitive inhibitors: can bind to both free enzyme and ES complex. V_{max} decreases as ESI affects catalytic activity while change in K_M depends on affinity of inhibitor with E and ES. Assuming that the affinity is equal, then it is a pure non-competitive inhibitor and thus there will be no change in K_M. $\nu_0 = \frac{(\frac{V_{max}}{\alpha'})[S]}{(\frac{\alpha K_M}{\alpha'}) + [S]}$ where $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K_I'}$



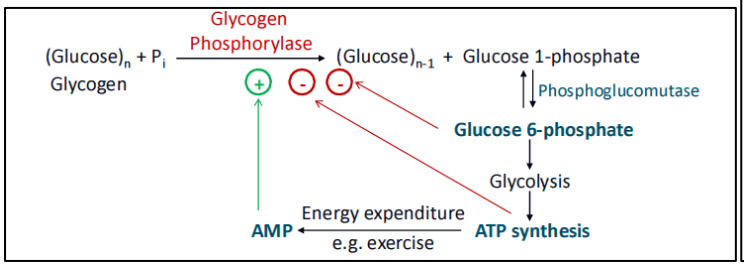
- Sample calculations:
  - The K_M for an enzyme in the absence and presence of 3μM of competitive inhibitor is 8μM and 12μM. Find K_i.
    - $\alpha = K'_M/K_M = 12/8 = 1.5$
    - use equation: $\alpha = 1 + [I]/K_i$ to solve for K_i
  - Hexokinase utilizes Mg^{2+}-ATP as a substrate. When uncomplexed ATP was used the activity was decreased as Mg^{2+} promotes nucleophilic attack by masking the negative charge on the phosphate.

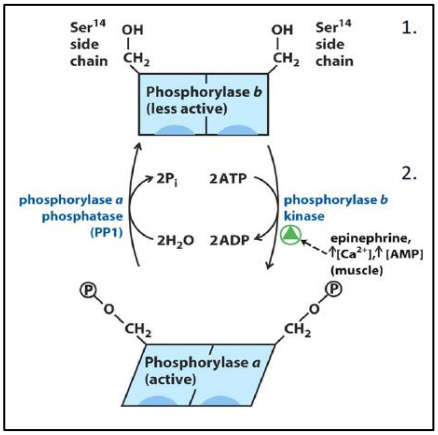


8. Enzyme Regulation: Enzyme activity is dependent on their concentration and affinity for the substrate and can be regulated by controlling the amount of enzyme or the enzyme activity.
    - E.g. Glucose levels in the cell are controlled by hexokinases I, II, III and IV (isoenzymes). Hexokinase IV is found in the liver while hexokinase I, II and III are found in most tissues.
        - Phosphorylation of glucose via Hexokinase prevents the transport of glucose out of the cell.
        - Hexokinase I to III have low K_M for glucose and thus allow for utilization of glucose when blood glucose is low by trapping the glucose in the cell. V_{max} is achieved at low glucose concentration and thus allow the tissue to utilize glucose efficiently.
        - Hexokinase IV (glucokinase) has a high K_M to allow for the phosphorylation to occur mainly at high blood glucose concentrations. High V_{max} allows the uptake of large amounts of glucose from the blood. Thus this buffers the level of blood glucose by removing excess glucose and storing them in the liver. Portal vein allows glucose to enter liver in high concentrations so that glucokinase can approach V_{max} quickly after a meal.
    - Enzyme activity can be regulated by localization by isolating the enzyme from the substrate
        - Glucokinase activity in liver cells is regulated by glucokinase regulatory protein (GKRP) which competitively inhibits the binding of glucose to glucokinase. Once bound, GKRP inactivates glucokinase by bringing it into the nucleus.
        - When blood glucose is high, glucose will cause GKRP to dissociate from glucokinase which moves into the cytoplasm, increasing phosphorylation to keep glucose in the cell.
        - When blood glucose drops, F-6-P induces GKRP to bind to glucokinase, preventing the liver from competing with other organs for glucose.
    - Enzyme activity can be regulated by transcription:
        - When blood glucose level rises, β-cells in pancreas increase insulin release and about half is extracted by the liver. Insulin promotes transcription of the glucokinase gene which leads to an increase of glucokinase synthesis.
    - In all, when $[S] << K_M$ and $\nu_o = (K_{cat}/K_M)[E_T][S]$, ν_o can be regulated by:
        - Tissue-specific expression (hexokinase I and glucokinase) with different k_{cat} and k_M.
        - Controlling the synthesis of the enzyme $[E_T]$ in the cell/organ (insulin regulates the expression)
        - Supply of the substrate [S] (portal vein supplying glucose).

- Enzyme activity is also regulated by cleavage activation (proteolytic cleavage)
  - Some enzymes are synthesized in their proenzyme (zymogen) form which is inactivated and requires cleavage to be activated.
  - E.g. pepsin, trypsin and chymotrypsin are synthesized as pepsinogen, trypsinogen and chymotrypsinogen in the pancreas. At low pH, inhibitory domain of pepsinogen unfolds and the active catalytic site of pepsinogen will cleave its own inhibitory domain resulting in pepsin.
  - Pepsin can active another pepsinogen via cleavage of the inhibitory domain, which leads to a positive feedback loop.
  - Digestive proteolytic enzymes are produced as proenzymes to prevent cell destruction as enzymes can digest the proteins inside the cell.
- Enzyme activity can be regulated through allosteric regulation where molecules called effectors/modulators bind to the enzyme via non-covalent binding at a regulatory site other than the active site
  - Homotropic modulators: substrate serves as a modulator
    - Enzymes which display substrate cooperative binding usually contains 2 or more subunits where binding of substrate to an enzyme subunit makes it easier for additional substrate molecule to bind to the other subunits.
    - Sigmoid curve results when active site affinity keeps switching depending on number of substrates already bound
  - Heterotropic modulators: modulator is different from the substrate
  - Allosteric modulators can activate or inhibit the target enzyme.
  - E.g. allosteric activation of cAMP-dependent protein kinase by cyclic AMP (cAMP). cAMP binds to the regulatory subunits, releasing the catalytic subunits which expose the catalytic site, thus making it active.
  - E.g. allosteric inhibition of threonine dehydratase by L-isoleucine. Conversion of L-threonine to L-isoleucine is a five step enzymatic reaction. Final product L-isoleucine is a negative modulator that inhibits threonine dehydratase upon binding. This negative feedback mechanism allows the cells to fully utilize the end product to prevent wasteful over production.
  - E.g. ATCase catalyzes the reaction between carbamoyl phosphate and aspartate to produce N-carbamoyl aspartate which is a precursor for CTP synthesis. CTP is a negative allosteric effector which inhibits ATCase while ATP is a positive allosteric effector. When use of CTP is low, [CTP] increases to inhibit ATCase and thus CTP is conserved. When [ATP] is high, ATCase is activated which increases [CTP], leading to DNA synthesis.

- Enzyme activity can be regulated by covalent modification of the enzyme
  - An amino acid residue (Ser/Thr/Tyr) on the regulatory domain can be modified via phosphorylation, thus inducing conformational change which allows access to active sites on the catalytic domain.
- Glycogen phosphorylase is controlled via both allosteric regulation and covalent modification
  - When [AMP] is high, glycogen phosphorylase is activated which leads to glycogen degradation to produce glucose which will undergo glycolysis to produce ATP.
  - When [G6P] and [ATP] are high, it indicates low energy expenditure and thus they inhibit glycogen phosphorylase to conserve glycogen.
  - Epinephrine, calcium and AMP levels increase during exercise which stimulates the kinase that phosphorylates the enzyme, thus activating the breakdown of glycogen. Phosphorylated enzyme is less sensitive to allosteric regulation.

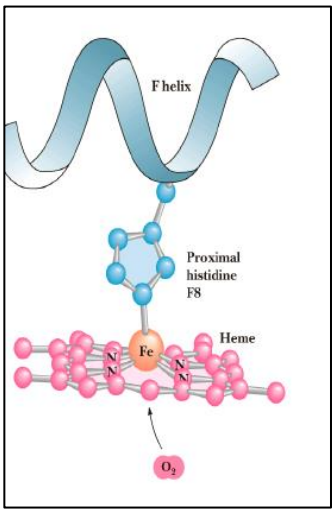




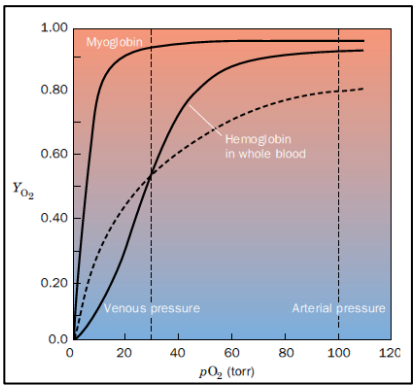
- Applications of enzymes:
  - Intracellular enzymes are released into plasma due to increased cell death, and this can serve as a biomarker to identify which organ is affected.
    - E.g. Creatine Kinase (CK) has 3 isoenzymes. Skeletal muscle contains 98% CK3 and 2% CK2 while cardiac muscle contains 70% CK3 and 30% CK2.
    - Aminotransferases are present in liver only and thus can serve as a biomarker if the liver is damaged.
  - When the product and substrate of a reaction cannot be readily measured, the reaction can be coupled to a second reaction which contains a measurable biomolecule.
    - E.g. Glucose + ATP → G6P + ADP via glucokinase
    - G6P + NADP → 6-phosphogluconolactone + NADPH + H^+ via G6P dehydrogenase. NADPH can be measured using A_{340}.

Topic 4: Hemoglobin

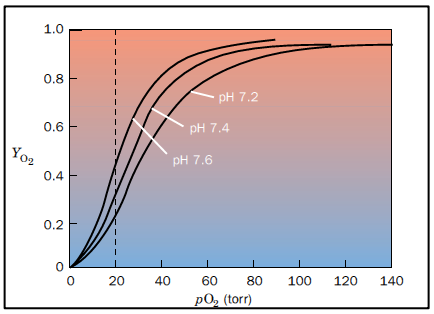
1. Heme is a complex of protoporphyrin IX and ferrous iron Fe^{2+} where the iron is held in the center by bonds to the four nitrogens on the porphyrin rings.
   - In myoglobin, 4 bonds on the same plane are taken up by the porphyrin ring, 1 bond with His F8 on myoglobin and 1 bond with O_2.
   - Heme itself can bind to oxygen without myoglobin, but the myoglobin helps to protect the heme iron atom from oxidation and decrease the affinity of heme for carbon monoxide due to steric hindrances via His E7 residue.
   - Without oxygen bound, the Fe^{2+} is located above the heme porphyrin ring plane towards the direction of His F8. Oxygen binding pulls the Fe^{2+} towards the heme plane and this causes a conformational change in the F helix of myoglobin.
   - Myoglobin and the subunits in hemoglobin are structurally similar. Myoglobin is found in muscle fibers while hemoglobin is found in red blood cells.



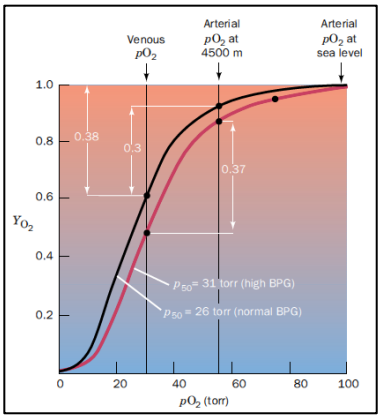
2. Hemoglobin is a tetramer consisting of 2 alpha chains and 2 beta chains (α1β1 and α2β2 dimers) which contains a heme each. Thus, one Hb molecule can bind to four O_2.
   - Hemoglobin exhibits cooperative O_2 binding and thus exhibits a sigmoidal curve. Oxygen can be released when hemoglobin reaches the tissues and the steepness allows it to respond to small changes in tissue oxygen pressure, releasing more or less oxygen when needed.
   - When one oxygen molecule binds to a myoglobin subunit, the hemoglobin changes from the T (tense) state to the R (relaxed) state which has a higher affinity for O_2.



3. Hemoglobin's affinity for oxygen can be affected by:
   - pH as H^+ decreases the affinity of Hb toward O_2 by shifting the equilibrium to the T state by protonating the His146 residue.
     - When CO_2 is produced via metabolism, H^+ is generated via H_2CO_3. Thus T state of Hb is induced which promotes the release of oxygen.
   - Carbon dioxide can form carbamates with N-terminals of subunits which would form ionic interactions that can stabilize the T state and promote the release of oxygen.
   - 2, 3–bisphosphoglycerate (2, 3-BPG) is a metabolite synthesized from the glycolytic pathway which binds to a positively charged cavity form by the β-chains of deoxyhemoglobin (T state). 2, 3-BPG stabilizes the T state and decreases the O_2 affinity of hemoglobin.



- In high altitude adaptations, 2, 3-BPG level is high which enables hemoglobin to release the normal amount of O_2. The curve is shifted to the right and thus there is a greater unloading of oxygen in the capillaries of the tissues.
- Fetal hemoglobin contains a different subunit that has a lower affinity for 2, 3-BPG and thus this increases its affinity for oxygen which allows fetal tissues to be supplied with oxygen from maternal oxyhemoglobin.



4. Sickle cell anemia is caused by a mutation in the gene coding for hemoglobin where a Glu is substituted for a Val in the β-subunit.
   - A protrusion in the β-subunit is the result of this mutation.
   - The protrusion can only fit in a hydrophobic pocket present in deoxyhemoglobin which will cause polymerization of HbS, forming insoluble fibers when oxygen is released.
   - Fibers deform the RBC which hinders its movement through blood capillaries, reducing blood flow and preventing efficient oxygen transport.

Topic 5: Nucleic Acids

1. Experiments that demonstrate that DNA is the genetic material:
   - Griffith (1928) with *S. pneumonia*.
     - Used S (virulent) and R (non-virulent) bacteria to infect mouse.
     - Conclusion: genetic material from type S bacteria could transform type R to type S bacteria.

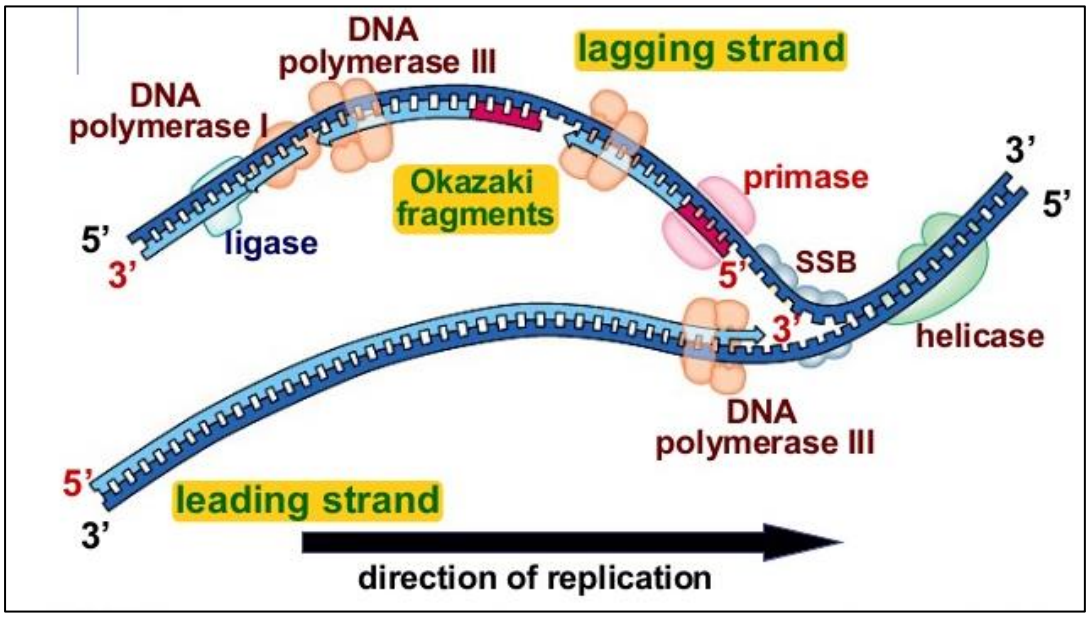
S strain	R strain	Heat killed S strain	Heat killed S strain + R strain
Mouse died	Mouse survived	Mouse survived	Mouse died
Type S isolated	No bacteria	No bacteria	Type S isolated

   - Avery, MacLeod and McCarty (1944) *S. pneumonia*.
     - Prepared cell extracts from type S cells and antibodies for type R bacteria were bounded to them. Non transformed R cells will die and only transformed S cells will be seen. Concluded that the transforming substance is DNA.

R cells	R cell + S DNA extract	R cells + S DNA extract + DNAse	R cells + S DNA extract + RNAse	R cells + S DNA extract + protease
No growth	Growth	No growth	Growth	Growth

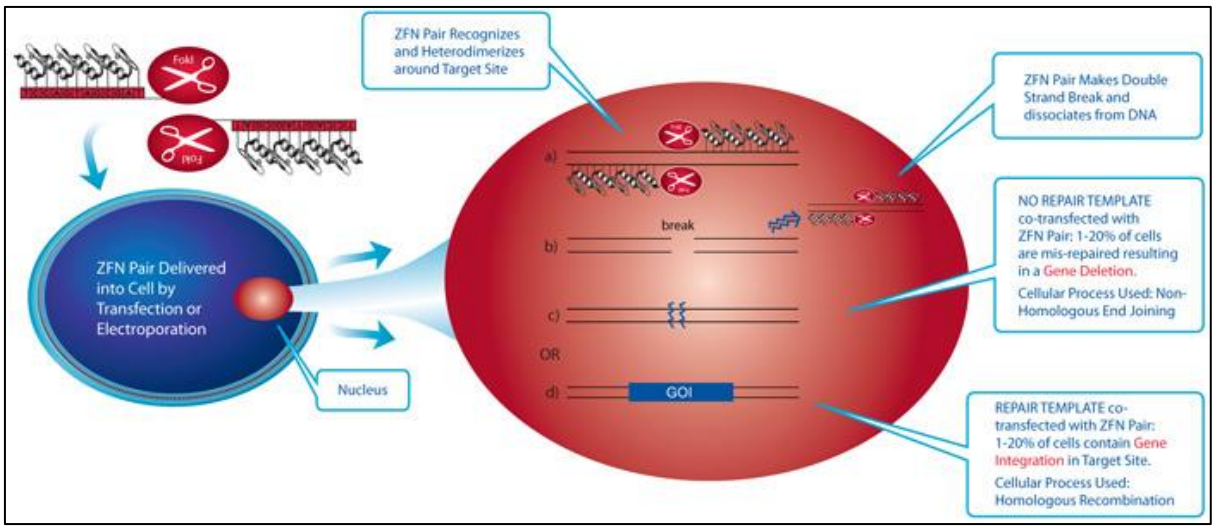
   - Hershey and Chase (1952) with Bacteriophage T2 (blender experiment)
     - T2 only contains DNA and protein, no RNAs are present.
     - ^{32}P was used to label DNA and ^{35}S to label protein to distinguish DNA from proteins. The radioactively-labeled phages were used to infect nonradioactive E.coli cells and after a period of time, the phage particles were sheared off the cells using a blender. Radioactivity was then monitored using a Geiger counter.
     - Supernatant should contain ^{35}S while the pellet should contain ^{32}P.
2. Structure of nucleic acids
   - Double stranded DNA (146-147bp) is wrapped around histone octamer (2 of H2A, H2B, H3 and H4) which forms a nucleosome. Linker regions consist of H1 (linker histone) which play a role in the organization and compaction of chromosomes.
   - Primary form of DNA found is B-DNA which is right handed with 10 base pairs per turn.
   - A nucleotide contains a phosphate group (PO_4^{3-}), pentose sugar (2' deoxyribose) and nitrogenous base (ATCG or U in RNA). Nucleotides are covalently linked together by phosphodiester bonds where a phosphate

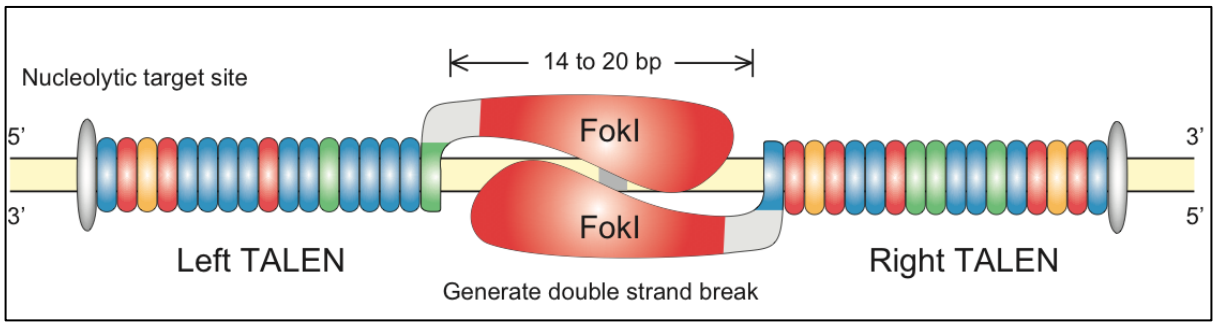
connects the 5’ carbon of one nucleotide to the 3’ carbon of the other which results in a 5’ to 3’ direction e.g. 5’-ATCG-3’.
  - Bases in opposite strand hydrogen bond according to AT-CG rule. AT has 2 hydrogen bonds while CG has 3 hydrogen bonds.
  - Each base pair traverses about 0.34nm.
3. DNA replication is semiconservative as proven by Meselson and Stahl (1958).
  - Conservative model: both parental strands stay together after DNA replication
  - Semiconservative model: dsDNA contains one parental and one daughter strand following replication
  - Dispersive model: Both parental and daughter DNA is interspersed in both strands following replication.
4. DNA replication is bidirectional and begins at an origin of replication.
  - Step 1: DnaA proteins bind to the origin of replication, resulting in the separation of the AT-rich region.
  - Step 2: DNA helicase breaks the hydrogen bonds between the DNA strands, topoisomerases alleviate positive supercoiling, and single-strand binding proteins hold the parental strands apart.
  - Step 3: Primase synthesizes one RNA primer in the leading strand and many RNA primers in the lagging strand which are complementary to the template strand. DNA polymerase III then synthesizes the daughter strands of DNA. In the lagging strand, many short segments of DNA (Okazaki fragments) are made. DNA polymerase I removes the RNA primers and fills in with DNA, and DNA ligase covalently links the Okazaki fragments together.
  - Step 4: The processes described in steps 2 and 3 continue until the two replication forks reach the ter sequences on the other side of the circular bacterial chromosome.
  - Step 5: Topoisomerases unravel the intertwined chromosomes, if necessary.



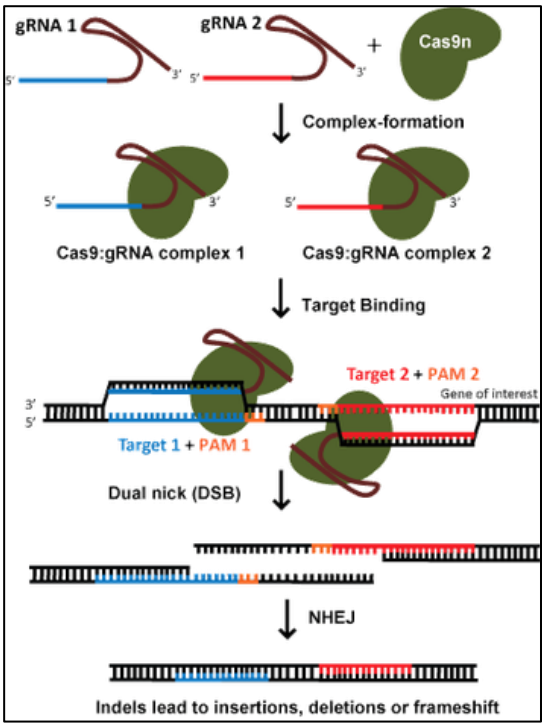
  - DNA polymerase III synthesizes DNA in the leading and lagging strands. Has 5’ → 3’ polymerization and 3’ → 5’ exonuclease capabilities (proofreading).

5. Telomeres are found at the ends of eukaryotic chromosomes as DNA polymerase is unable to replicate the 3' ends of the DNA strands since a primer cannot be made upstream
    - This causes the DNA to get shorter with each round of replication.
    - Telomerase uses a short RNA molecule complementary to DNA sequence found in the telomeric repeat to bind to the 3' overhang region. The RNA sequence will serve as a template to add repeat sequences onto telomeres, preventing shortening of the chromosome.
6. DNA repair mechanisms correct mutations:
    - Base excision repair replaces a single damaged base
    - Nucleotide excision repair replaces a section of DNA for bulky pyrimidine dimers.
    - Mismatch repair corrects incorrectly paired bases that were missed during DNA replication using the template parent strand which was methylated.
    - Recombinational repair doesn't use a template which may result in errors.
7. DNA can be shuffled around by recombination processes:
    - Homologous recombination can only occur when there is a homologous chromosome present after the S phase.
        - A double strand break followed by a strand invasion results in the formation of a Holliday junction.
        - Branch migration occurs where the Holliday junction moves further away, causing DNA to be swapped. Resolvase will then cleave the holiday junction and DNA ligation will occur.
    - Transposons are mobile genetic elements that can change their position within the genome.
        - Simple transposition occurs via cut and paste method
        - Retrotransposons use reverse transcription to produce a DNA molecule complementary to the RNA.
8. Mutations in DNA occurs due to mutagens or errors
    - Silent Mutation: change in a single base but does not affect the mRNA.
    - Nonsense mutation: causes a STOP codon
    - Missense mutation: changes the amino acid.
    - Transition is when a purine/pyrimidine changes to another purine/pyrimidine (e.g. A to G or C to T)
    - Transversion is when a purine changes to a pyrimidine (e.g. A to C or G to T)
9. Genome can be edited via endonucleases (cleave phosphodiester bond)
    - Zinc Finger Nucleases (ZFNs): each ZF module recognizes 3 DNA bases
    - Transcription Activator Like Effector Nucleases (TALENs): each TALE module recognizes a single DNA base
        - Both nucleases are used to make a double strand break (for gene knock out or insertion) and stimulate homologous recombination.





- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
  - Consists of guide RNA (gRNA) and Cas9 (endonuclease).
  - gRNA is composed of a sequence necessary for Cas9 binding and a user-defined 20 nucleotide "targeting" sequence which defines the genomic target to be modified.
  - Targeting sequence must be unique compared to the rest of the genome and be present immediately upstream of a Protospacer Adjacent Motif (PAM).
  - Genomic target of Cas9 can be changed by simply changing the targeting sequence in the gRNA.
  - CRISPR/Cas9 can be used to make precise modifications using homology directed repair (HDR) or activation/repression of target genes using dCas9 (dead) tagged with transcriptional repressors/activators.



- Viruses form dsRNA when they replicate, animal and plant cells perform RNA interference to cut them into pieces (~21bp) called small interfering RNA (SiRNA) by the protein Dicer.
  - A non-natural interfering RNA can used to destroy viral mRNA.

Topic 6: Lipids

1. Classification
   - Fatty acids: even number of carbons between 14 to 24.
     - Two numbers m:n where m is the number of carbons and n is the number of double bonds
     - Double bonds are mostly cis which introduces kinks in the hydrocarbon tail and thus lowers melting point.
   - Triacylglycerols: formed via the esterification between three fatty acid molecules and one glycerol molecule.
     - Functions as energy storage compound in adipose tissue and can be broken down by lipases through hydrolysis to produce energy.
     - Not found in lipid membranes
   - Phosphoglycerides: consists of a glycerol-3-phosphate with 2 fatty acid chains.
     - Hydrophobic fatty acid tails and polar phosphoryl group makes it a good component for lipid membranes as it is amphipathic.
     - Phosphoryl group can be bonded to another group which gives rise to different forms of glycerolphospholipids.
   - Sphingolipids: are derivatives of sphingosine (18C amino alcohol)
     - Amino group forms an amide bond with a fatty acid molecule..
     - Major components of membrane
     - Functions:
       - Cell-cell recognition
       - Tissue immunity
       - Impulse transmission
   - Steroids: derivatives of cyclopentanoperhydrophenanthrene
     - Influences gene expression
     - Cholesterol is a major sterol in animals and is both a structural component of membranes and precursor to a wide variety of steroids.
2. Membrane: cellular membranes are made from lipid bilayers
   - Amphipathic lipids will form spontaneous structures in water such as a micelle or bilayer vesicle
   - Lipids can move about within the membrane, can diffuse laterally rapidly but transversely very slowly.
   - Increase in temperature will cause the lipid to behave more fluidly
   - Proportions of different lipids will affect fluidity
     - Unsaturated fatty acids increase fluidity while cholesterol increase order and rigidity by stabilizing the straight chain arrangement of saturated fatty acids
   - Functions of membrane:
     - Delineation and compartmentalization
       - Act as a barrier for hydrophilic molecules

        - Keep essential substances inside the cell and keeps the undesirable substances out
        - Intracellular membranes serve to compartmentalize functions within eukaryotic cells
    - Organization and localization of functions
        - Inner membranes of mitochondrion allow for respiration
        - Thylakoid membranes in chloroplasts allow for photosynthesis
        - ER membrane is the site of synthesis of biomolecules
    - Transport
        - Regulate movement of substances in and out of the cell and organelles.
    - Cell to cell communication
        - Gap junctions (animal) and plasmodesmata (plants)
    - Signal detection
        - Electrical and chemical signals are received at the outer surface of the cell and the plasma membrane plays a key role in signal transduction through ligand-receptor interaction.

3. Fluid mosaic model
    - Allows freedom of motion of membrane components especially lipids and proteins
    - Homeoviscous adaptation allows organisms to compensate for temperature changes by changing the lipid composition.
    - Membrane proteins may move laterally, but some may be restricted if they are anchored to the cytoskeleton.
    - Integral (intrinsic) proteins span the entire membrane through hydrophobic segments
        - Possess hydrophobic regions which interact with interior of lipid bilayer while hydrophilic regions extend outward to the aqueous environment.
        - Not easily removed, must use SDS to remove
        - Hydropathy plot can be used to measure the hydrophobicity of a region of the protein via amino acid residues
        - Acts as selective transport channel, cell surface identity marker, cell surface receptor, cell adhesion, enzyme or attachment to cytoskeleton.
        - Transmembrane domains are usually alpha helices or beta sheets
    - Peripheral (extrinsic) proteins have no discrete hydrophobic sequences and do not penetrate into the lipid bilayer.
        - Have weak interactions with membrane and are easily removed.
        - Can associate directly or indirectly with polar groups of lipids or integral proteins
    - Lipoprotein contains lipids bound to proteins, allowing fats to be carried in the blood stream. Can be classified as HDL(high density) or LDL(low density)

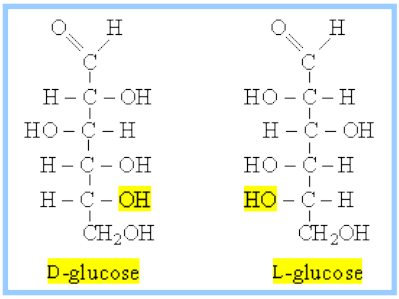
- An erythrocyte is a RBC which membrane contains several proteins. Presence of A or B antigens on the surface sphingoglycolipids help to determine blood group.

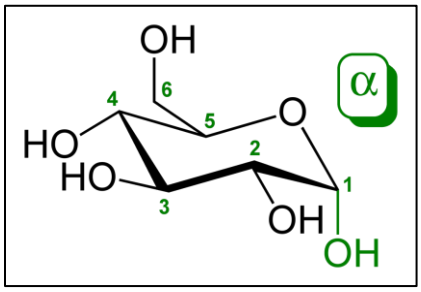
4. Lipid asymmetry: distributions of lipids are different between the different sides of the membrane.
   - Exoplasmic region contains primarily choline, sphingomyelin and glycolipids while cytosolic regions contain serine and ethanolamine.
   - Lipids can be redistributed by flippase (out to in)/floppase (in to out) using ATP or scrambase, using Ca^{2+} to randomly flip lipids transversely.
5. Lipid microdomains: cholesterol-sphingolipid microdomains in the outer monolayer of the plasma membrane are thicker and more ordered, thus behaving like a "raft"
   - "Raft" has high specificity for signaling factors
6. Transport across membrane
   - Material can get transported across membranes through gap junctions in animal cells or plasmodesmata in plant cells.
   - Some material can get transported in transport vesicles (ER vesicle, secretory vesicle)
   - Some proteins get transport in a cage like vesicle made from the protein Clathrin.
   - When foreign material enters the cell via endocytosis, they carry part of the plasma membrane with them which can be recognized by lysosomes which will facilitate degradation.
7. Types of transport
   - Membrane transport can be either passive/active or simple/mediated
     - Simple passive – simple diffusion of solutes which are readily permeable through the membrane down a concentration gradient.
     - Passive mediated – facilitated diffusion, assisted by membrane proteins to mediate passive diffusion down a concentration gradient for solutes which are too large or too polar.
       - Carrier proteins binds and shields the polar groups from the non-polar interior of the membrane (valinomycin for K^+ transport)
       - Channel proteins are solute specific hydrophilic channels through the membrane (gramicidin A for protons and alkali cations)
       - 'Gated pore' mechanism occurs when conformation of protein changes. (e.g. GLUT1 in RBC)
     - Active mediated – active transport, movement against concentration or electrochemical gradient.
       - Requires hydrolysis of ATP as energy source.
       - Uniport: one molecule at a time
       - Symport: two different molecules in the same direction

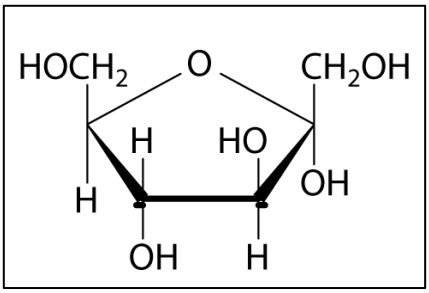
- Antiport: two different molecules in opposite directions
- Na^+/K^+ pump transports Na^+ out of the cell and K^+ into the cell.
- Na^+/glucose symport in the intestines allows glucose to be transported against its concentration gradient through the Na^+ gradient that is maintained by the Na^+/K^+ pump into the cell. Uniport then allows glucose to be transported into the blood.

Topic 7: Carbohydrates

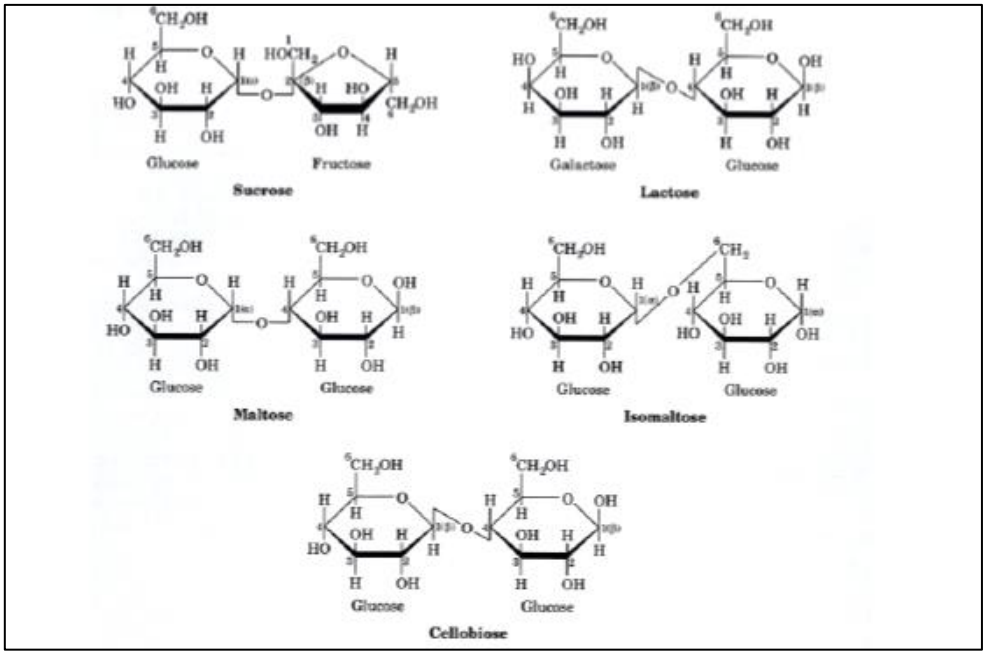
1. Carbohydrates have the formula $(CH_2O)_n$ where n > 3.
   - Functions as energy source, structural materials, metabolic precursors and signal recognition
   - Consists of monosaccharides, disaccharides, oligosaccharides and polysaccharides
2. Monosaccharides can be classified as an aldose (aldehyde) or ketose (ketone).
   - Chiral in nature and can be differentiated as L (left) or D (right) isomers.
   - Number of stereoisomers for aldose and ketose is 2^{n-2} and 2^{n-3} respectively.
   - Epimers are isomers that differ at 1 chiral center.
3. Sugars with 4 or more carbons exist in cyclic forms.
   - 5-member sugar is called furanose and 6-member sugar is called pyranose
   - In the cyclic form, the carbonyl carbon becomes a new chiral center, resulting in the C_1/C_2 –OH being anti to C_6 and anomers (diastereomers) being formed
   - 6 membered rings can exist in either boat (β-D-glucopyranose) or chair (α-D-glucopyranose) conformation.





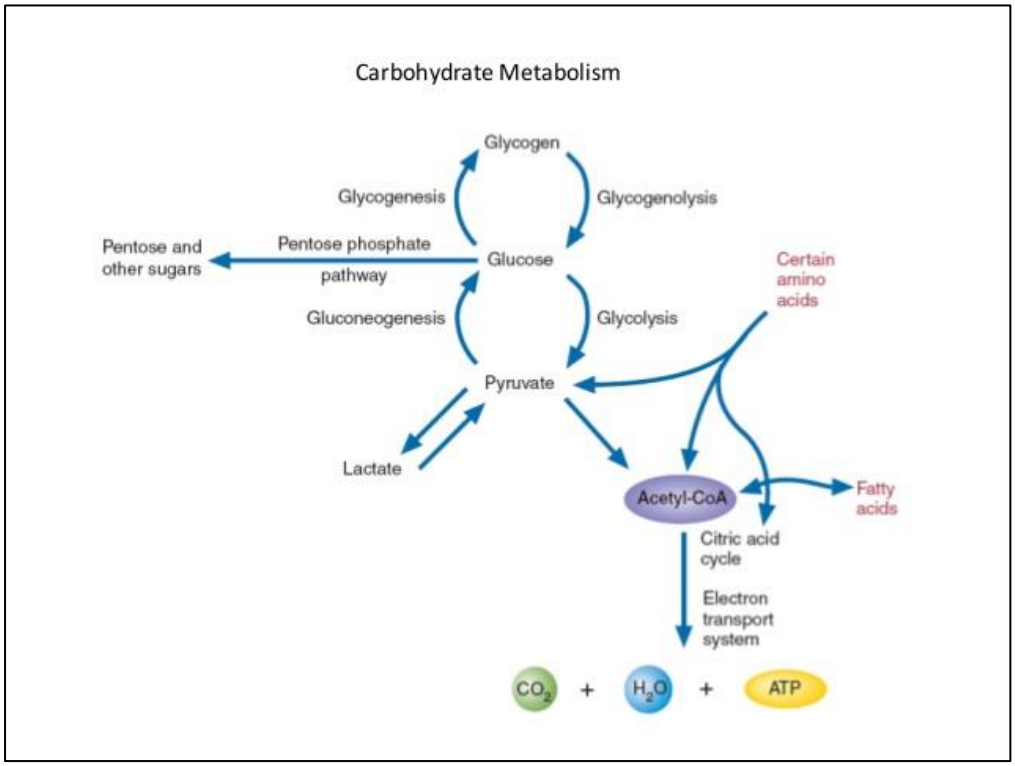


   - Glycosidic (C-O) bonds form when an anomeric carbon bonds to oxygen: $C_1OH + CH_3OH \rightleftharpoons C_1OCH_3 + H_2O$.
4. Disaccharides consist of 2 monosaccharides.

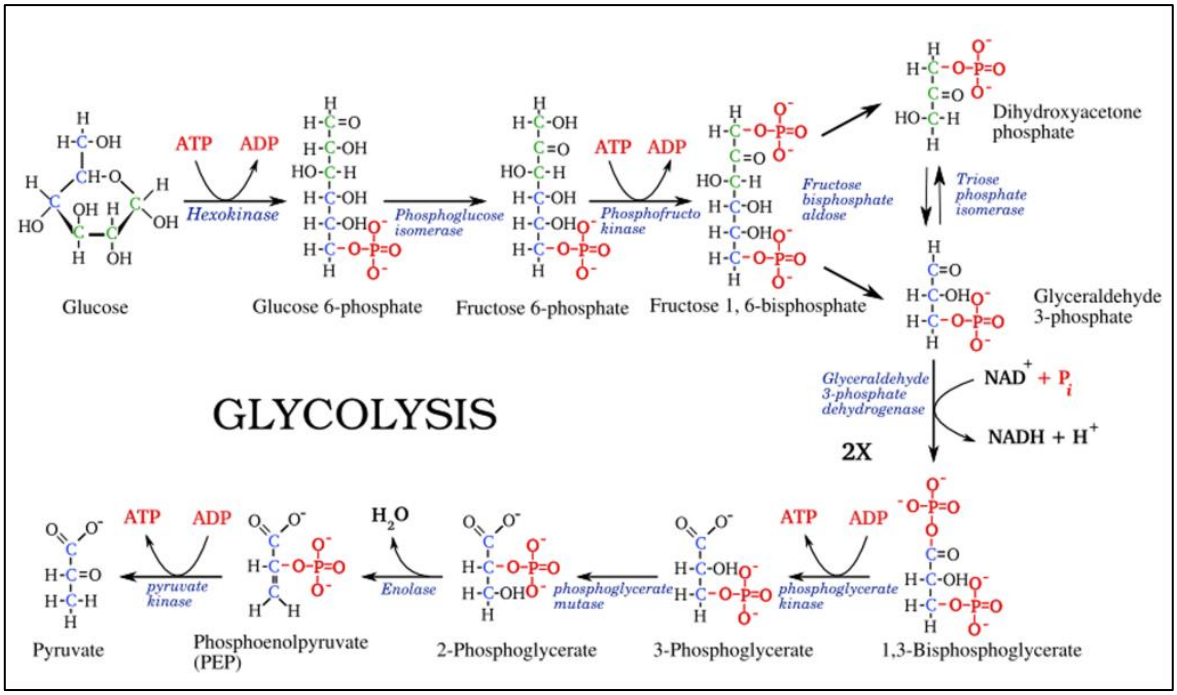


- α-Glycosidic bond causes the other monosaccharide to have the hydroxyl group on anomeric carbon to be on the same side while β-Glycosidic bonds have the hydroxyl group on the opposite site.

5. Polysaccharides are formed by the linkage of multiple monosaccharides and their derivatives.
   - Branching can be present via glycosidic bonds on the terminal carbon (1,2: 1,3: 1,4: 1,6)
   - Linear amylose can form helical structures, 1, 6 linkages result in branching in amylopectin.
   - Cellulose is formed via β-1, 4 glycosidic bonds which result in the formation of sheets.
6. Glycoproteins are proteins that are covalently associated with carbohydrates.
   - Peptidoglycans are formed when polysaccharides are linked with peptides.
   - Peptidoglycans are found in bacterial cell wall.
     - Gram positive bacteria is when the peptidoglycan is exposed
     - Gram negative bacteria have the peptidoglycan sandwiched between two lipid layers.
     - Glycoproteins have many functions which include:
       - Cell growth
       - Cell-cell adhesion
       - Differentiation
       - Cytokine action
       - Viral infection
       - Metastasis
7. Metabolism of glycogen:



- Glycogenesis:
  - Glucose + ATP → G6P + ADP (Enzyme: Hexokinase)
  - G6P → G1P (Enzyme: Phosphoglucomutase)
  - G1P + UTP → UDPG (Uridine diphosphate glucose) + PP_i (Enzyme: UDPG pyrophosphorylase)
  - Glycogen(n) + UDPG → Glycogen(n+1) (Enzyme: Glycogensynthase)
  - Glycogen branching (1,4 → 1,6) (Enzyme: 1,4-glycosyl transferase)
- Glycogenolysis:
  - Glycogen(n) + P_i → Glycogen (n-1) + G1P (Enzyme: Glycogen phosphorylase)
  - Glycogen debranches via 1,4-glycosyl transferase and amylo-(1,6)-glucosidase
  - G1P → G6P (Enzyme: phosphoglucomutase)
  - G6P → Glucose (Enzyme: gluco-6-phosphatase)
- Glycolysis:
  - 2 molecules of pyruvate are produced along with 2NADH + 2ATP.



- Pyruvate will undergo alcohol fermentation to form ethanol or lactate fermentation to form lactate depending if no oxygen is present.
  - Fermentation regenerates NAD^+ which is used for glycolysis.
- Aerobic oxidation of pyruvate (Krebs cycle) converts pyruvate into Acetyl CoA and produces 4 CO_2, 6NADH, 2 $FADH_2$, 2ATP and $6H^+$
  - Electrons which are generated go through the electron transport chain in the membrane via proton gradient.
  - Proteins complexes found in the membrane of the mitochondria facilitate the electron transport to ATP synthase which forms ATP.

Useful information from Practicals

1. Effect of buffer pK_a on buffering capacity: maximum buffering capacity is $pK_a \pm 1$ and thus the correct buffer must be chosen to counter either acid or base.
   - Tris-HCl (pK_a = 8.1) is an effective buffer against bases while potassium phosphate (pK_a = 6.8) buffer is effective against acid.
2. Effect of temperature on the pH of buffer: when temperature decreases, the pH of buffer solutions will increase as the forward equation ($HA \rightleftharpoons H^+ + A^-$) is endothermic (absorb heat).
3. UV absorbance of protein: Tyrosine and tryptophan absorb at λ_{max} =280nm, Nucleic acids absorb at 260nm.
   - Dye binding method such as "Bradford assay" can help to quantify the amount of proteins more accurately. Coomassie Brilliant Blue binds to positively charged amino acids such as arginine and lysine and cause a shift in λ_{max} from 465nm to 595nm.
   - $E^{1\%}$ value = Absorbance of 10mg/mL of the substance.
4. Enzyme Kinetics: Lactate dehydrogenase catalyzes the following reaction:
   - Lactate + $NAD^+ \rightarrow$ Pyruvate + NADH + H^+
   - NADH has a λ_{max} of 340nm while λ_{max} NAD^+ is at 260nm.
   - Initial velocity of enzyme can be determined by tangent line at t=0 for graph of absorbance against time.
5. DNA Gel Electrophoresis: Restriction enzymes can be used to cleave DNA at specific nucleotide sequences.
   - Advantages and disadvantages of using different gels:

Agarose Gel	Polyacrylamide Gel (PAGE)
Used for separating sizes >100bp	Used for separating sizes <100bp
Easy to prepare	Complex to prepare
Formation of pores is dependent on temperature which may lead to non-uniform pores	Formation of pores is a chemical process which result in uniform pores
Fragile	Stronger than agarose
Able to load ~20µL of sample	Able to load larger volume ~50µL
Gel ingredients are often not suitable for recovery purposes	Gel ingredients are relatively pure, which allows for purer recovery of samples
Gel without DNA stain is safe	Unpolymerized gel is neurotoxic