Biochemistry 2019 Learning Objectives

Introduction to Metabolism and Carbohydrate Metabolism (Exam 2)

- **Draw and interpret a reaction coordinate diagram.**
  - Delta G over time. Goes up to activation energy, then down. If favorable, the product will be lower than reaction side.
- **Define the key characteristics of an enzyme-catalyzed reaction, and identify what aspects of a reaction are, and are not, affected by the enzyme.**
  - Delta G (whether a reaction is favorable or not) is not affected by an enzyme.
  - Speed of reaction is affected by enzyme
- **Write and interpret rate laws for a chemical reaction.**
  - $A + B \leftrightarrow C + D$
  - $k_1[A][B]$
- **Compare and contrast the free energy of a reaction and the activation energy.**
  - DeltaGrxn of the reaction tells you whether a reaction is favored or not, but Ea is required to tell you how fast the reaction will occur
- **Relate the magnitude of Ea to the relative amounts of substrate and transition state.**
  - Decreasing Ea will increase speed of reaction from substrate into transition state
- **Write a general reaction involving enzyme catalysis and include the relevant species that are formed in the process.**
  - $E + S \leftrightarrow ES \rightarrow E + P$
  - Enzymes bind to both substrates and products
  - Enzymes are not consumed
- **Describe how the concentration of the substrate and the enzyme affect the rate of product formation.**
  - Enzyme brings substrate into transition state, which then becomes product
- **Describe how an enzyme accelerates a chemical reaction.**
  - Enzymes decrease the activation energy of a reaction, making it more likely to occur. E.g. using the microenvironments of the active site
  - Stabilizes the transition state
- **Explain how KM and Vmax are determined experimentally for an enzyme.**
  - Test velocity at different substrate concentrations, plot double inverse graph (1/V0 vs. 1/KM)
- **Define and interpret the meaning of KM and Vmax for an enzyme, and estimate both values on a graph.**
  - $k_1[E][S] = (k_{-1} + k_2)[ES]$
  - $K_M = [E][S]/[ES] = (k_{-1} + k_2)/k_1$
  - Vmax is the maximum velocity possible
  - KM is a ratio of rate constants that represents when the reaction's velocity is at half maximal
  - KM is the stability of ES, and the rate of dissociation of what it becomes divided by what you are turning into.
  - Using a double reciprocal plot, KM and V_0 are the axes, but the reciprocals
- **Recognize the Michaelis-Menten rate equation.**
  - $V_0 = V_{max} \frac{[S]}{[S]+K_M} = \frac{[ES]}{[E_{total}]} V_{max}$
  - At high levels of saturation, velocity will be close to VMax.
  - At low levels of saturation, velocity will be a fraction of Vmax, dependent on KM.
  - At [S]=KM, velocity will be half of VMax.
- **Explain how reaction rate is related to the amount of ES complex relative to total enzyme.**
  - $V_{max} = k_2[E\ total]$

- Vmax is when all the enzymes are bound and converting
- **Explain what information about enzymes is provided by KM/kcat.**
  - k2 = kcat, represents catalytic efficiency/turnover
  - $V_0 = k_{cat}[ES] = \frac{k_{cat}}{K_M}[E][S]$
  - Takes into account the speed at which ES turns into E+P divided by how fast ES breaks down
- **Briefly describe what enzyme cofactors are, and what general purpose they serve.**
  - A cofactor is a molecule that is needed for an enzyme's function. Often metals, but can be organic coenzyme as well.
- **Recognize how KM and Vmax are affected by different types of reversible inhibitors (competitive, non-competitive, uncompetitive).**

Type	Explanation	Effect
Competitive	Blocks S from binding to E, but does not prevent product from being formed. Can be overcome with more substrate and still reach Vmax. E, EI, ES	Increase Km
Uncompetitive	Blocks ES from forming product. Km measures dissociation, and ESI keeps it in ES form, dissociating less E, ES, ESI	Decreases Km and Vmax
Noncompetitive	Blocks S from binding to E in addition to blocking ES from forming product E, ES, EI, ESI	Decrease VMax

- **Distinguish and plot different types of enzyme inhibition using double-reciprocal plots, and interpret relevant points on the plots.**
  - See above
- **Apply general knowledge of enzymes and inhibitors to new examples.**
- **Explain what the term "alpha" describes for an inhibitor using words, an equation, and graphically.**
  - Alpha is the level of inhibition
  - $_{\alpha} = 1 + \frac{1}{K_I}$where $K_I = \frac{[E][I]}{[EI]}$
  - Furthermore, it is the change in slope
- **Describe the general catalytic mechanism of serine proteases, including the role of the catalytic triad.**
  - Peptide recognition by serine
  - Histidine abstracts hydrogen, forming oxyanion hole (-) stabilized by 2 hydrogen bonds (+)
  - Acyl enzyme intermediate
  - C terminus leaves (NH3), H2O takes its place
  - Histidine abstracts H2O
  - Product release
- **Compare and contrast different strategies to regulate enzyme function that were discussed in class.**

Membranes

- **Describe how the molecular structure of a cellular membrane prevents polar molecules and ions from crossing membranes.**
    - Phosphlipids contain hydrophilic head and hydrophobic tail. Phospholipids form bilayers by the hydrophobic effect.
    - Small nonpolar and small uncharged polar molecules can pass through easily, but large nonpolar and charged molecules can't.
- **List the characteristics of different types of membrane transporters (channels, pumps, and carriers).**
    - Down the gradient - moving from higher to lower concentration.
    - Against the gradient - moving from lower to higher concentration.
    - Channels - molecule diffuses down the gradient through pore in the membrane that is open to both sides.
    - Pumps - molecule binds to enzyme that is open only on one side, enzyme hydrolyzes ATP to change conformation and open to the other side. Pump moves molecules against the gradient
    - Carriers - molecule binds to enzyme that is open only on one side, does not use ATP. Moves molecules either (down, down down, down against)
        - Uniporter - moves molecule down the gradients.
        - Symporter - moves two molecules in the same directions.
        - Antiporter - moves two molecules in opposite directions.
        - Note that down/against is independent of the direction, since these are different molecules
- **Analyze the thermodynamic and kinetic characteristics of the transport of molecules across membranes.**
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    - $M_{out} \leftrightarrow M_{in}$
    - $\Delta G^{\circ} = 0$, since molecules inside and outside are inherently the same, this tells us that at equilibrium, there are equal concentration inside and outside the cell (think diffusion).
    - $\Delta G = G_{inside} - G_{outside} = 0 + RT\ ln\ \frac{[In]}{[Out[}$, this tells us whether molecules will move inside or outside the cell, and how much energy.
    - This does not apply for charged molecules
- **Predict the movement of molecules across a membrane through a given type of transporter (channels, pumps, or carriers).**

Metabolism (general)

- **Define metabolism in your own words.**
- **List the general characteristics of a metabolic pathway.**
    - Metabolite - products and reactants of metabolism, anything that is produced or consumed
    - Enzymes - catalyze chemical reactions

- **Distinguish between anabolic and catabolic pathways.**
  - Catabolic - macromol → mol building blocks and energy (ATP), C for cut
  - Anabolic - mol building blocks and energy → macromol, A for add
- **Explain how ATP acts as a cellular energy source.**
  - $ATP + H_2O \leftrightarrow ADP + P_i + H^+$
  - Cells keep concentrations of ATP relatively high
  - ATP can phosphorylate another molecule or hydrolysis
  - ATP hydrolysis is thermodynamically favorable (Delta G knot) but kinetically slow (Delta G activation). Specifically, ATP releases a lot of energy, but it requires a high activation energy. Use enzymes to control energy usage!
  - We can couple an unfavorable reaction with ATP: $A + ATP + H_2O \leftrightarrow B + ADP + P_i + H^+$
- **List the number of each type of molecule that is consumed or generated by a particular metabolic pathway or combination of pathways.**
  - In glycolysis, the reaction takes glucose (6C), splits and generates (net) two pyruvates (3C), 2 ATP (2 consumed, 4 generated), 2 NADH
- **Discuss the free energy of metabolic pathways and reactions.**
  - Irreversible reactions have high delta G
- **Identify irreversible reactions in a metabolic pathway.**
  - Unidirectional arrow, rather bidirectional
- **Identify (or predict) the regulated enzymes in each metabolic pathway.**
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- **Describe the regulation of the mostly highly regulated enzyme in each metabolic pathway.**
  - Committed step
- **Predict allosteric enzyme regulation for a metabolic reaction or pathway based on your knowledge of the reaction/pathway or information provided.**
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- **Evaluate the roles of kinases and phosphatases in the regulation of enzyme activity (either an example provided in class or given sufficient new information).**
  - Kinases attach ATP, phosphatases dephosphorylate
- **Interpret experimental data measuring enzyme activity or the levels of metabolites or energy molecules.**
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- **Apply knowledge of general enzyme characteristics (structure, mechanism, cofactors, thermodynamics, kinetics, regulation) to specific metabolic enzymes.**
  - Structurally
    - Deep cleft or binding site for susbtrate
    - Active site takes up small part of volume of protein
    - Active site has unique microenvironments
    - Substrates bound by weak interactoins
    - Lock and key model, or induced fit, change shape slightly when substrates bind
  - Cofactors - coenzymes or metal ions required for optimal activity
  - Thermodynamics - Michaelis Menten
- **Explain how specific molecules cross specific cellular membranes during metabolic processes.**
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- **Describe the reciprocal regulation of two opposing metabolic pathways (for example: glycolysis/gluconeogenesis).**
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- **Draw, analyze and interpret diagrams of metabolic pathways.**
    - Arrow in is a substrate, arrow out is a product
- **Identify the molecules that connect metabolic pathways.**
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- **Predict the activity of enzymes or metabolic pathways under specific physiological, disease, or experimental conditions.**
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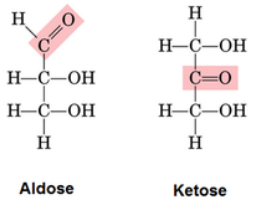
Carbohydrate Metabolism

- **Categorize a carbohydrate based on the number of carbons and presence of a ketone or aldehyde group.**
    - Hexose, pentose, tetrose, triose.



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    - Aldose ends with R-C=O, ketose R-C=O attached to R'.
- **Label the carbons atoms in a carbohydrate using the standard numbering convention.**
    - Look for the carbon that has two oxygens attached to it, that is the ketose/aldose end (lower number) end.
- **Recognize major features of carbohydrate structures in different forms including linear, cyclic, modified, and polymers.**
    - Linear/cyclical are equivalent, use numbering convention to convert between them, OH attacks C=O.
    - Modifications with phosphates or amines.
    - Form polymers with glycosidic bonds, between hydrolyzed monosaccarides.
- **List the biological functions of carbohydrates.**
    - Energy source - complex carbs digested into monosaccharides, converted to energy
    - Nucleotide synthesis - ribose is precursor of nucleotide
    - Physical structure - cellulose in plants and chitin in arthropods
    - Protein and lipid modification - cell-cell recognition, protection, structure, signaling
- **Compare and contrast glycolysis and gluconeogenesis.**
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- **Predict whether glycolysis or gluconeogenesis would occur under a set of conditions at the cellular or organismal level.**
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- **Explain how blood glucose levels are regulated by insulin and glucagon including the role of glycolysis, gluconeogenesis, glycogen synthesis, and glycogen breakdown.**
    - Insulin goal is to break down glucose, removing it from the bloodstream, promotes glycolysis, glycogen synthesis
    - Glucagon goal is to form glucose, adding it to the bloodstream, promotes gluconeogenesis and glycogen breakdown