Instructions. This exam consists of 208 points, 27 questions, on 9 pages. On questions with choices, all of your answers will be graded and you will be given the best grade. Use the space provided or the back of the previous page.

1. (5 pts) Many drugs have chiral centers.
   i) What are chiral centers? What bonding properties of carbon result in chiral centers?
   ii) Briefly discuss the biological consequence of a chiral center in a drug.

i) A chiral center is created when a carbon is bound to four different atoms in a tetrahedral geometry. This means there is a mirror image of the molecule and the two mirror images cannot be superimposed.

ii) One of the mirror images could be the effective drug and the other mirror image could be ineffectual, or worse, toxic.

2. (5 pts) A drug binds to DNA via a lysine side chain. The pKa of lysine is 9.0. Will the binding of the drug to DNA become better or worse if the pH is changed from 7.0 to 10.0? Briefly justify your answer.

At pH = 7 the lysine will be positively charged, therefore it will bind strongly to the negative charge on the DNA due to electrostatic interactions.

At pH = 10 the lysine will be deprotonated (pH >> pKa) so it will no longer be charged and it will not bind to the DNA very well.

3. (10 pts) Describe the hydrophobic effect and explain its role in the spontaneous assembly of biological structures. Illustrate you answer with an example from any section of the course (protein stability, lipid membrane formation, micelle formation, drug binding).

The hydrophobic effect is a change in the order of the water molecules due to the presence of a non-polar group. The water molecules will order themselves around a non-polar group. This decreases the order of the water which is unfavorable. Therefore non-polar groups do not interact with water.

Protein stability; The hydrophobic effect drives the non-polar residues into the core of the protein, stabilizing the folded form.

Membrane formation: The two fatty acid tails in a phospholipid are driven into the center of the bilayer by the hydrophobic effect.

Micelle formation: Fatty acids from spherical micelles because the hydrophobic effect causes the burial of the non-polar part of the fatty acid.

Drugs that have non-polar functional groups will bind to non-polar parts of the protein, because the release of ordered water when the binding occurs is favorable.

4. (12 pts) Briefly describe the following levels of protein structure:
a) primary, b) secondary, c) tertiary, d) quaternary.

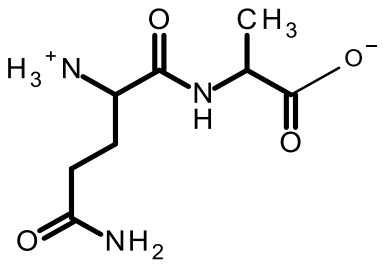
Primary – sequence of amino acids.

Secondary – conformation of mainchain atoms, hydrogen bonded structures, α-helix/β-sheet

Tertiary – conformation/structure of all of the atoms in a single chain

Quaternary – Structure/assembly of multiple chains.

5. (8 pts) Select any two amino acids and draw the dipeptide that would result from the formation of a peptide bond between the two. Indicate the sequence of the dipeptide that you have drawn.



This is Glutamine-Alanine (amino terminus to carboxy)

6. (10 pts) Enzymes are biological catalysts. How do they accelerate the rate of reactions?

They lower the energy of the transition state, which increases the concentration of the transition state.

The more transition state, the faster the reaction.

The energy of the transition state is lowered because of pre-ordering of functional groups in the enzyme.

7. (6 pts) Many drugs are either competitive or allosteric inhibitors. What is the difference between these two modes of inhibition?

Competitive inhibitors look like the substrate and bind to the active site, blocking the substrate from binding

Allosteric inhibitors bind elsewhere (not at the active site) and cause a change in the shape of the enzyme, the new shape is no longer active.

8. (8 pts) It is important that enzymes are regulated within metabolic pathways. We discussed the regulation of phosphofrutokinase in glycolysis, and glycogen synthase and glycogen phosphorylase in glycogen metabolism. Pick any **one** of these three examples and briefly discuss:

i) How the enzyme is regulated

ii) Why this form of regulation makes sense with regard to optimum performance of the organism.

a) Phosphofructose kinase: Inhibited by ATP, since glycolysis produces ATP it should be turned off when there is sufficient ATP. Activated by ADP/AMP – glycolysis needs to be turned on to convert these back to ATP.

The regulation makes sense because the pathway (glycolysis) is only on when ATP needs to be generated.

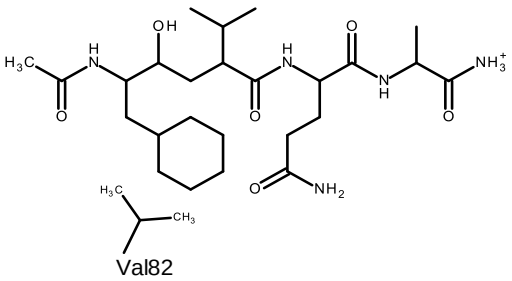
b)Glycogen synthase is active when dephosphorylated. Protein phosphatases are active when blood glucose levels are high, due to insulin signaling by tyrosine kinase receptors.

This regulation makes sense because excess glucose will be stored in glycogen.

c) Glycogen phosphorylase is active when phosphorylated – protein kinases are active with the blood glucose levels are low, due to glucagon signaling via G-protein coupled receptors.

This makes sense because glucose will be released from glycogen, increasing the blood glucose levels.

9. (14 pts) The structure of an HIV protease inhibitor, complexed to HIV protease, is shown on the right. Val82 is in the active site of the enzyme. An individual that is infected with the HIV virus has developed a drug resistant strain of virus. You isolate the virus bearing the mutation and sequence it. The sequence of the wild-type and mutant protease gene are shown below:



Wildtype DNA Seq: CCTACACCT**GTC**AACATAATT

Wildtype Protein Seq: **ProThrPro<u>Val</u>AsnIleIle**$_{85}$

Mutant DNA Sequence: CCTACACCT**GAT**AACATAATT

i) What is the role of HIV protease in the lifecycle of the HIV virus (1 pt)?

ii) Why do mutations frequently arise in the HIV virus (1 pts)?

iii) What amino acid was Val_{82} changed to in the mutant virus (4 pts)?

iv) How might you modify the drug to make it bind more effectively to the mutant HIV protease (4 pts)?

v) Assuming that you would like to study the mutant HIV protease by X-ray crystallography, list the steps that you would need to take to produce the protein in E. Coli, beginning with the viral RNA (4 pts).

i) This enzyme cleaves immature viral proteins into mature proteins, which form the virus.

ii) The reverse transcriptase has no proofreading function, so errors are generated copying the viral RNA to DNA.

iii) The GTC codon was replaced by GAT, which codes for Aspartic acid.

iv) Aspartic acid has a negative charge, so I would replace the cyclohexane ring with a positively charged amine – NH_3^+

v) The steps are:

a) convert the viral RNA to dsDNA using reverse transcriptase.

b) Use PCR to amplify the HIV protease gene

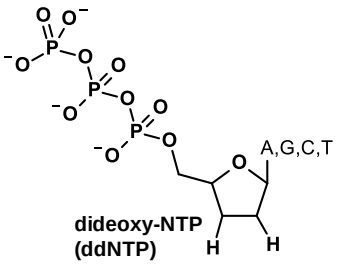
c) Use restriction enzymes to insert HIV protease gene into the expression vector.

d) Transform bacteria, induce expression with IPTG, purify protein, crystallize

10. (8 pts) Please do **one** of the following choices.

Choice A: What is the role of dideoxyNTPs in DNA sequencing? The general structure of a dideoxyNTP is shown on the right.

Choice B: How does PCR lead to DNA amplification of a targeted DNA sequence.



Choice A: These cause chain termination by the polymerase at a known base because they lack a 3'-OH, so once they are incorporated, it is not possible to add new bases.
Thus we know that a particular base is at that position.

Choice B: Two primers are used that are the sequence of the upper strand (left primer) and the lower strand (right primer) at the boundary of the amplified region. During PCR, the template is denatured, primers anneal, and then DNA polymerase extends the primers.
The left primer produces a DNA molecule that begins with the primer. During the 2nd PCR cycle, this is primed by the right primer, and replication by the polymerase produces one strand of the amplified product. A similar reaction occurs in the 3rd cycle to produce the double stranded PCR product. This PCR product doubles in each subsequent cycle.

11. (10 pts) A single stranded nucleic acid is shown on the right.

i) This sequence is both RNA and DNA. Circle the RNA part (2 pts).

ii) Circle, and label, the linkage between the monomeric units (2 pts).

iii) Indicate the carbohydrate portion(s) of the molecule, and label one (2 pts).

iv) What is the sequence of this nucleic acid (1 pt)?

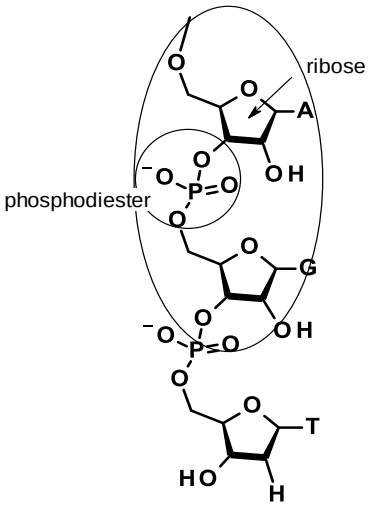
The 5' end is at the top, sequence is AGT

v) What is the sequence of the complementary strand? (1 pt)

5'-ACT-3'

vi) In what biological process would you routinely see such a molecule (specifically a DNA – RNA hybrid) (2 pts)

The RNA would be the primer in DNA replication, the DNA part would be added during replication.



12. (12 pts) It is important for both eukaryotics and prokaryotic organisms to regulate the production of mRNA:

i) Briefly discuss **one** mechanism of regulation that is used in both types of organisms.

ii) Briefly discuss **one** mechanism of regulation that is **exclusive** to eukaryotic organisms.

i) Common features:

a) repressors – block the production of mRNA
b) activators – enhance the production of mRNA
c) polyA addition – alter stability of mRNA, decreasing it (prok) or increasing it (euk)

ii) exclusive

a) histone modification – changes accessibility of DNA for RNA polymerase.
b) miRNA – microRNAs can interfere with the translation of mRNA
c) Alternate splicing can be regulated, affect which type of protein is made in different tissues.

13. (8 pts) Please do one of the following choices:

Choice A: Pick any drug that inhibits protein synthesis (you do not need to give the name of the drug). Indicate where it binds on the ribosome and how its binding inhibits protein synthesis.

Choice B: Pick any drug that inhibits the synthesis of bacterial cell walls and briefly discuss its mechanism.

Choice C: Briefly discuss one application of how antibodies can be used to treat a medical condition.

Choice A:

Binds in A site, ribosome can still form initiation complex, but no new amino acids can be added since the charged tRNA cannot bind to the A site.

Binds in the exit tunnel. First few amino acids could be joined, but the protein exit is blocked so the protein cannot be completed.

Choice B:

Penicillin binds to the enzyme that crosslinks the protein component of the bacterial cell wall, inhibiting its function (6 pts). The penicillin actually chemically modifies the enzyme (2 pts)

Clavulanic acid: Forms a chemical bond with the enzyme that degrades penicillin (β-lactamase), enhancing the effectiveness of penicillin.

Choice C:

Drug detoxification – antibody can bind to a drug and prevent it from reacting with the body – e.g. binding cocaine to prevent the cocaine from interacting with the central nervous system.

Cancer treatment – Breast cancer – antibody binds to growth factor receptor, preventing growth factor from binding and causing the cell to proliferate.

14. (8 pts) The protein that is produced from the mRNA that encodes the heavy chain of the antibody contains both a signal sequence and stop transfer sequence. Select **either** of these and describe its role in directing the heavy chain to the cell membrane of a B-cell. You answer should include a description of the role of cellular organelles in the protein export process.

The **signal sequence** will cause the ribosome that is synthesizing the protein to attach to the membrane of the rough endoplasmic reticulum (ER) and the protein will be exported into the lumen of the rough ER. The protein will then travel to the golgi in a membrane vesicle, and then the vesicle will fuse with the plasma membrane, and the protein will now be outside the cell.

The **stop-transfer-sequence** will halt the transfer of the secreted protein and the stop-transfer sequence will remain in the membrane. The protein will then travel to the golgi in a membrane vesicle, and then the vesicle will fuse with the plasma membrane, and the protein will now be outside the cell, but anchored in the cell membrane by its stop transfer sequence.

15. (10 pts) Please do one of the following:

Choice A: A unique problem with linear chromosomes is the replication of the ends of each chromosome. Briefly describe how this is accomplished.

Choice B: What are tandem repeats and how are they used to identify individuals (give details about how you would actually determine the number of tandem repeats).

Choice A:

The reverse transcriptase, telomerase, has a RNA molecule bound to it that can bind to the DNA of the unreplicated lagging strand at the end of the chromosome. The RNA serves as a template, allowing telomerase to elongate the DNA. Eventually it becomes long enough such that primase can lay down a primer and normal DNA synthesis can occur, this copies the end of the chromosome and removes the RNA primer from the Okasaki fragment

Choice B: Tandem repeats are a repeated DNA sequence that are found at different places in the chromosome.

Different people have the same repeats at the same location, but they differ in the number of repeats.

PCR can be used to amplify the repeated region, using primers that anneal to outside the region.

The length of the DNA can be measured using gel electrophoresis.

16. (14 pts)
   i) Outline the major metabolic pathways that are responsible for the complete oxidation of carbohydrates, beginning with monosaccharides and ending with the reduction of water. You answer should focus on the fate of carbon as well how the energy released by these oxidations is captured for ATP formation (8 pts).
   ii) Briefly discuss how ATP is formed in the mitochondria (6 pts).

i)
Glucose is converted to pyruvate in the glycolysis pathway. Oxidation produces NADH.
The pyruvate enters the TCA cycle, where the carbon is released as CO2. Oxidation produces NADH and FADH2

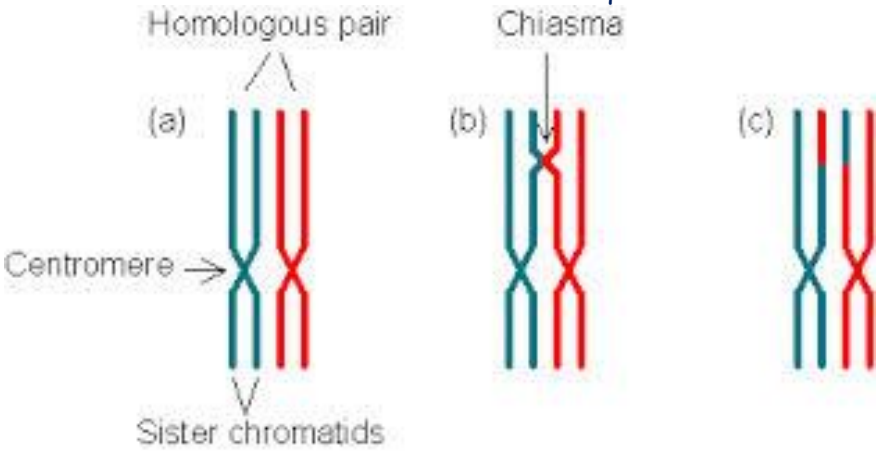
The NADH and the FADH2 give their electrons to the electron transport chain. As the electrons move through the complexes in the membrane, protons are pumped from a low to a high concentration across the membrane.

The electrons eventually end up on oxygen to form water.

ii) As the hydrogen ions flow from high concentration to low, through the ATP synthase, they cause rotation of the gamma subunit.
As the gamma subunit rotates, it changes the conformation of the three beta subunits.
As the conformation changes, bound ADP + Pi is converted to ATP due to conformational changes in the beta-subunit.

17. (8 pts) DNA sequences can be found in the chromosomes of gametes (sperm and egg) that were not present in either parent, how does this occur?

DNA recombination occurs between homologous chromosomes during meiosis I. This cause part of one chromosome to be transferred to the equivalent location in the other chromosome.



18. (2 pts) A cell is said to be aneuploid if it has more (trisomy) or less (monosomy) than two homologous chromosomes.

19. (2 pts) What is the difference between a trait and phenotype?

A trait is an observable characteristic – e.g. pea shape.
A phenotype is a particular instance of the trait – e.g. wrinkled, smooth.

20. (2 pts) The alleles of a gene are R and r. In order to observe an autosomal recessive phenotype, the genotype(s) can be: _rr is the only possibility._____.

21. (2 pts) The alleles of a gene are R and r. In order to observe an autosomal dominant phenotype, the genotype(s) can be:Rr or RR, both will show the phenotype._____.

22. (10 pts) Please do one of the following choices:

Choice A: The cancer drug taxol stabilizes microtubules by preventing the release of tubulin subunits from the end. How does this interfere with the growth of cells?

Choice B: Briefly describe the role of kinesins in the separation of chromosomes/chromatids during anaphase.

Choice A:

Chromosome movement in anaphase requires the release of tubulin monomers from the end of the microtubule. If this is prevented by taxol, then the chromosomes cannot move and the cancer cell cannot divide.

Choice B:

Kinesins are motor proteins that use the energy released by ATP hydrolysis to "walk down"microtubules. The motor proteins keep the chromosome attached to the microtubule as it shrinks due to the loss of subunits from an end.

23. (8 pts) Please do one of the following choices.

Choice A: Explain why type A blood should not be given to a type B individual.

Choice B: Describe how rhogam works to protect developing Rh^+ fetuses if the mother is Rh^- and previously had an Rh^+ child.

Choice A: A type B individual will have antibodies against type A blood. The antibodies arise due to prior exposure to bacteria with similar carbohydrates as the blood group antigen.
The incoming red blood cells with the type A antigen will be crosslinked by the antibodies, leading to clumping of cells and the blockage of capillary beds.

Choice B: When an Rh^- mother has an Rh^+ child, the mother will be exposed to the Rh antigen during childbirth because some of the blood cells from the child will enter the mother (childbirth is messy). Since the mother is Rh-, she will view the Rh protein on the baby's blood cells as foreign and generate antibodies against that protein. These antibodies can harm the second baby. Rhogam binds to the Rh antibodies and prevents the mother's immune system from generating antibodies.

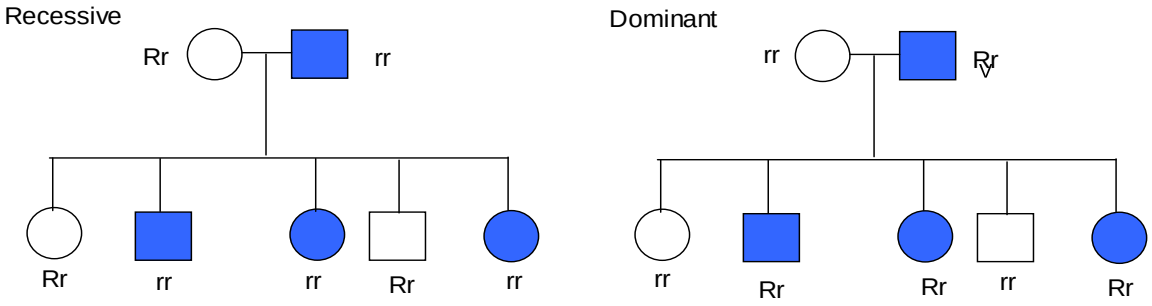
24. (10 pts) The following pedigree was obtained for a family.

i) What pattern(s) of inheritance (e.g. autosomal/X-linked, dominant/recessive) are consistent with this pedigree? Briefly justify your answer. Two copies of the pedigree are provided for labeling purposes.

ii) What are the possibilities for Susan's genotype? Briefly justify your answer.

The inheritance is autosomal, since both males and females are affected.

It can be either dominate or recessive.



Sally's genotype will be rr if the phenotype is recessive, or Rr if the phenotype is dominant.

25. (4 pts) Recessive phenotypes generally lead to disease conditions, yet these alleles persist in the population. Why?

The recessive phenotype must confer some advantage to the species under certain conditions. (For example, sickle cell anemia provides resistance to malaria.)

26. (4 pts) What is meant by the term "linkage" and how would it affect the inheritance of different genetic markers.

It means that two genes are on the same chromosome, so the phenotype for each gene are likely to remain together during the formation of gametes (unless there is recombination). The two phenotypes for each gene will be inherited together. Example, if pea shape is linked to pea color, the allele for a smooth pea will be associated with the allele for yellow color.

27. Presentation Questions (8 pts). **Two** are required; **two** additional can be answered for bonus. Only the first four that you answer will be graded (choose wisely).
    1. What is the cause of Huntington's disease and what is its inheritance pattern?
    2. What is the cause of hemophilia and what is its inheritance pattern?
    3. Achondroplasia (dwarfism) is due to an overactive fibroblast growth factor receptor, why is this lethal for a homozygous (DD) individual.
    4. What is fish odor disease and why is it inherited as an autosomal recessive manner?
    5. What protein is affected in cystic fibrosis and how does this defect lead to the disease?
    6. What cells in the body respond to high blood sugar, and how is this response affected in type I diabetes?
    7. How does a deficiency in Adenosine Deaminase interfere with the production of B and T-cells?
    8. In what way should individuals with PKU disease modify their diet? Why?
    9. What is the relationship between Hutchinson-Gilford Progeria disease (dramatic aging) and RNA splicing?
    10. What are the clinical symptoms of Canavan disease?

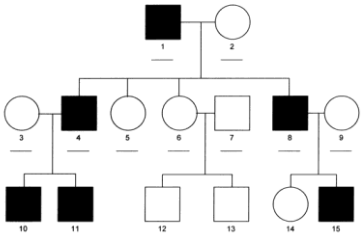
1. The amplification of a repeat, it is autosomal dominant.
2. A deficiency in a clotting factor, sex linked (X) recessive.
3. The bones are so short that there is not enough room in the chest cavity to accommodate the organs.
4. The lack of an enzyme that converts the fish smelling compound (trimethyl amine) to another compound. It is recessive because a single copy of the correct gene is sufficient to hide the fish smell.
5. A chloride channel is non-functional, thus chloride is not pumped out of the cell. This prevents other ions, and then subsequently water, from leaving the cell. Hence secretions in the lung and pancreas are thick.
6. Beta cells in the pancreas respond by secreting insulin. These cells are destroyed by the immune system.
7. This enzyme is required to remove excess A, which leads to the build up of toxic chemicals that kills rapidly dividing B and T cells.
8. PKU disease is a deficiency in the metabolism of the amino acid phenylalanine. People should have a low protein diet.
9. The disease introduces a new RNA splice site in the protein that codes for the nuclear lamina.
10. The brain develops large defects in its structure.

Bonus (4 pts each): *(Answer on the back of the previous page)*

B1. Gene therapy can be used to correct genetic diseases. It is often easier to treat recessive alleles with this method, why? The location of insertion of the corrected gene is not defined. For a recessive allele, putting a good copy anywhere will often fix the problem. If the allele is dominant, it is necessary to replace the mutant protein, i.e. the replacement gene must insert at a single location.

B2. Why are excess calories, when consumed as carbohydrates, stored as fat in humans? The step in the pathway that enters the TCA cycle is one-way in humans, once carbon from excess glucose goes past this step, it can only be stored as fats.

B3. The inheritance pattern for Retinitis pigmentosa is shown on the right. What is the most likely mode of inheritance? Briefly justify your answer. Y-linked. It only occurs in males, and is passed from father to son.



B4. Manx (tail-less) cats are heterozygous, Tt. Homozygous dominant cats (TT) have normal tails. Two manx cats are mated and 1/3 have normal tails and 2/3 have no tails. Explain this result. [Hint. Creating a Punnett square may be helpful.]. A cross between two heterozygous individuals has the following expected outcome: (Tt x Tt) = 25% TT, 50% Tt, 25% tt, or a ratio of 1:2:1. In this case the homozygous recessive are lethal, so the ratio becomes 1:2 (TT:Tt)