This exam has **12 pages** and is out of **230 points**. On questions with choices, all of your attempts will be graded and you will receive the best grade. Use the space provided, or the back of the preceding page.

1. (6 pts) Entropy plays an important role in biochemistry. Discuss the role of entropy in **two** of the following:
   a) the solubility of hydro**philic** compounds, such as ions and other polar molecules.
   b) lipid bilayer formation
   c) protein-DNA interactions (non-sequence specific)

a) In both cases the solute (salts, pure liquids) is going from an ordered state (crystal, pure liquid) where the entropy is zero to a solution where the solutes are more disordered, therefore $\Delta S > 0$, which is favorable.

b) The non-polar tails of the monomeric lipids will order water, when the bilayer forms this water is released, and $\Delta_{H2O}S > 0$ which is favorable. (Otherwise known as the hydrophobic effect).

c) Non-sequence specific protein-DNA interactions typically involved a positively charged residue (lysine or arginine) interacting with a negatively charged phosphate on the DNA. The phosphate has Na and K ions ordered around it, and when the protein binds to the DNA these ions become disordered when they are released to the solvent, therefore $\Delta S > 0$ which is favorable.

2. (12 pts) Please do **one** of the following two choices:
   Choice A: Describe how you would make a buffer solution with the following characteristics. pH=5.0, buffer concentration 0.1 M, volume = 0.5 L. You have the following monoprotic weak acids available, only in their fully protonated form: a) acetic acid (pKa=4.8), b) pyruvate (pKa=3), c) imidazole (pKa=7).
   Choice B: Determine the isoelectric pI of the following peptide: Alanine-Glycine, within 0.1 pH unit.

Choice A:

i) You would select acetic acid because the desired pH is within 1 unit of the pKa of acetic acid (3 pts)

ii) Calculate $f_{A\_} = R/(1+R)$ and $f_{HA} = 1/(1+R)$. $R=10^{pH-pKa} = 10^{5.0-4.8} = 1.58$

$f_{a-} = 0.61$ $f_{HA} = 0.39$ [this is consistent with the pH > pKa, more should be deprotonated]. (3 pts)

iii) moles of [HA] (fully protonated form) = 0. 1 moles/L x 0.5 L = 0.05 moles. (3 pts)

iv) moles of NaOH to add: $f_{A-} \times [A_T] \times V = 0.61 \times 0.1 \times 0.5 = 0.03$ moles. (3 pts)

Choice B:

$q=\Sigma (f_{HA}q_{HA} + f_{A-}q_{A-})$

Since neither residue has ionizable sidechains, you only need to consider the amino terminus (pKa = 9) and the carboxy terminus (pKa = 2). (4 pts)

For pH values between 4 and 7 the carboxy terminus is fully deprotonated (q= - 1) and the amino terminus is fully protonated (q = +1), so the net charge is zero. (8 pts)

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3. (8 pts) Briefly explain why solutions of weak acids resist pH changes and are therefore buffers.

Weak acids are only partially dissociated (for pH values 1 below to 1 above their pKa). (4 pts)
If a strong acid is added to the solution the deprotonated weak acid will become protonated, so the pH (-log H+) won't change very much. (2 pts)
If a strong base is added to the solution, the protonated form of the weak acid will release a proton that will neutralize the added base. Otherwise the added base would neutralize H+ in solution, raising the pH. (2 pts)

4. (8 pts) An enzyme has a lysine residue in its active site that is involved in substrate binding. The enzyme also contains an aspartic acid residue in its active site that must be deprotonated for activity. Please do **one** of the following choices:

Choice A: The normal pKa for Aspartic acid is 4.0. Do you expect the pKa of the Asp residue in the active site to be higher or lower? Briefly justify your answer.

Choice B: Sketch the pH dependence for the K_M. Briefly justify your answer.

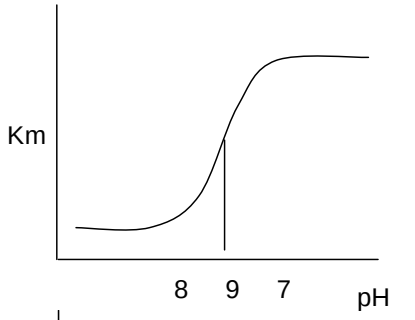
Choice C: Sketch the pH dependence for k_{CAT}. Briefly justify your answer.

Choice A:

The lysine will have a positive charge that will affect the ionization of the aspartic acid by electrostatic effects.
The positive charge will stabilize the deprotonated form of the aspartic acid, making it more likely to deprotonated, or a stronger acid with a lower pKa.

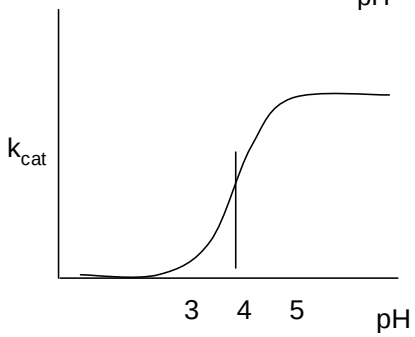
Choice B:

Assuming that the lysine must have a positive charge for good binding, the K_M will be low (good binding) for pH<pKa and increase (poorer binding) for pH> pKa. The shape of the curve is shown on the right. Note that Km is not likely to go to zero.



Choice C:

Since the Asp residue must be deprotonated, there will be low activity at low pH and maximal activity at high pH. The curve is shown on the right, note kcat is zero at low pH.



5. (10 pts) Draw the chemical structure of a tetra (4 residue)-peptide that has the following sequence properties:

a) the sequence would be found in a solvent exposed β-strand of a soluble globular protein.
b) one of the residues can be phosphorylated.
c) one of the residues absorbs UV light.

a) Should be alternating non-polar (Ala, Val, Leu, Ile, Phe), polar/charged
b) Should be Ser, Thr, or Tyr
c) Trp or Tyr (phe is also acceptable).

Please answer the following questions:

i) Give the name (sequence) of your tetra-peptide: (1 pt) - list of residues from amino terminus.

ii) What type of enzyme would add a phosphate to the residue you drew to satisfy b)? What is the source of the phosphate? (2 pts) A kinase would add a phosphate, from ATP

iii) Which residue would be released in the first cycle of Edman degradation (used for protein sequencing)? (1 pt) The amino terminal residue

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6. (10 pts) DNA is stabilized in its double stranded form by molecular interactions that also stabilize the tertiary structure of proteins.

i) Compare the relative importance of enthalpic factors (van der Waals, H-bonds, electrostatics) on the stability of proteins and double stranded DNA. Which factors stabilize folded proteins and double stranded DNA? Which destabilize them?

- van der Waals stabilize DNA through base stacking and proteins due to interactions in the core of the folded protein. (1 pt)
- van der Waals are more important for DNA(1 pt)
- H-bonds stabilize both DNA (Watson-Crick Hbonds) and proteins (secondary structure) (1 pt)
- Electrostatics have little role in protein stability. (1 pt)
- Electrostatic repulsion between the negatively charged phosphates destabilizes DNA(1 pt)

ii) Compare the relative importance of entropic factors (chain entropy, hydrophobic effect) on the stability of proteins and double stranded DNA. Which factors stabilize folded proteins and double stranded DNA? Which destabilize them?

When proteins unfold, or DNA becomes single stranded, there is a large increase in the entropy of the polymer, this destabilizes the folded form. (2 ½ pts)

The hydrophobic effect stabilizes the folded form of proteins (due to the non-polar core), but have little effect on the stability of the DNA. (2 ½ pts)

7. (6 pts) The peptide bond is has unique properties. What are those properties and how do they affect protein folding?

It is planer and trans (4 pts)

The partial double bond character means that there is only one configuration in the folded and unfolded form, therefore rotation about the peptide bond does not contribute to chain entropy (2 pts).

8. (4 pts) You have two unlabeled samples, one is a globular protein and the second is a segment of double stranded DNA. You need to determine which sample is the protein and which is the DNA. How would you distinguish between the two samples?

UV absorption, the protein will show an absorbance maximum at 280 nm, due to the Trp and Tyr residues. DNA will show an absorbance maximum at 260 nm, due to the bases.

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9. (10 pts) Allosteric effects play a critical role in functioning of biological systems. Please answer **both** of the following questions (parts i and ii, you have a choice for part ii).

i) Describe the general framework for allosteric effects. Your answer should include a description of the properties of T and R states and how these properties lead to allosteric effects (6 pts)

- A protein exists in two conformations, T-tense form, which is inactive, R-relaxed form, which is active. (4 pts)
- The two forms are in equilibrium with each other (1 pt)
- Activators push the equilibrium to the R-form, making the protein more active (1/2 pt)
- Inhibitors push the equilibrium to the T-form, making the protein less active. (1/2 pt)

ii) Please do **one** of the following two choices (4 pts)

Choice A: Describe how allosteric effects are important in oxygen delivery (or the adaptation to high altitude)

Choice B: Describe how allosteric effects are important in metabolic regulation (regulation of one pathway is sufficient).

Choice A:

- O_2 delivery. In this case oxygen binding to hemoglobin shows positive cooperativity, the binding of one oxygen increases the affinity for O_2. This allows hemoglobin to become fully saturated with O_2 in the lungs. As oxygen is lost in the tissues the binding becomes worse, so a larger amount of oxygen is released than would be if the binding was non-cooperative.
- Altitude adaptation: Bisphosphoglycerate increases at higher altitudes. BPG is an allosteric inhibitor and although it lowers the affinity of hemoglobin for oxygen it increases the cooperativity. Thus the binding curve is steeper and more oxygen is released when the hemoglobin travels to the tissues.

Choice B:

- Glycogen metabolism is controlled by phosphorylation, which is a covalent allosteric modulation. Glycogen synthase is off when phosphorylated and glycogen phosphorylase is on when phosphorylated.
- Glycolysis/gluconeogenesis is controlled by the allosteric modular F26P. It turns on PFK in glycolysis (enhances R state) and turns off bisphosphatase in gluconeogenesis (enhances the T state).

10. (8 pts) Explain how all enzymes increase the rate of reaction. Support your answer with a brief description of the active site of any enzyme.

- The active site contains a number of functional groups that are pre-ordered due to folding of the enzyme (2 pts)
- e.g. serine proteases (Ser, His, Asp) HIV protease (Asp x 2). (1 pt)
- Because of this, in the enzyme catalyzed reaction, no additional entropy changes occur in going from (ES) to (EX). In the case of the uncatalyzed reaction, these functional groups have to become ordered when going from (S) to (X), which is less favorable than going from (ES) to (EX) (3 pts)
- Consequently the energy difference (activation energy) between (S) to (X) is higher than (ES) to (EX), thus [X]<[EX]. Since there is more transition state in the enzyme catalyzed reaction, the rate will be faster. (2 pts)

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11. (21 pts) You are measuring the binding of a homodimeric protein to the following DNA segment. The protein also binds the amino acid tryptophan (Trp). You measure the binding of DNA to the protein in the absence and presence of tryptophan and RNA polymerase (RNAP) and obtain the binding data shown in the table on the right and plotted in a Hill plot (log (Y/(1-Y)) versus log[L]). Both the Trp binding protein and RNAP are from bacteria.

DNA concentration (Ligand) (nM)	Fraction of DNA bound	Fraction of DNA bound (+ Trp)	Fraction of DNA bound (+ Trp +RNAP)
0.1	0.01	0.10	0.50
1.0	0.10	0.50	0.90
10.0	0.50	0.90	0.99
100.0	0.90	0.99	1.00
K_D	10.0 nM	1.0 nM	0.1 nM

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GAGCGAAATTTCGCACTTGACATTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGAGGAGGAACAGCTATGA
CTCGCTTTAAAGCGTGAACTGTAAATACGAAGGCCGAGCATATTACACACCTTAACACTCTCCTCCTTGTCGATACT
          -35                    -10
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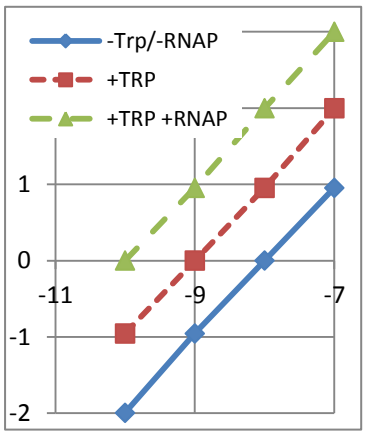
i) What do the -35 and -10 signify on the DNA strand (1 pt)?

The **promoter**, or the region where sigma factor from RNA polymerase binds.

ii) What is the K_D for binding of the protein to the DNA (protein alone, protein + Trp, protein + Trp & RNAP). Write these values in the table above and briefly indicate how you arrived at these values (5 pts).

The Kd value can be obtained from the binding data, when [L]=Kd, Y=0.5. They can also be obtained from the Hill plot, the log Kd is the value of the x-intercept (+3 for justification, +2 for correct values)



iii) Is the binding of the DNA to the protein cooperative? Justify your answer with reference to the Hill plot (3 pts).

No, the slope of the Hill plots are one, indicating non-cooperative binding in all cases.

iv) The protein, when bound to the DNA, greatly *increases* the amount of mRNA production. Based on this information, and the fact that the protein is a homo-dimer, identify the DNA sequence that is the binding site for the protein. *Justify your answer*. [Hint: restriction enzymes are also homodimers, what is the key characteristic of the sequence they recognize?] (4 pts)

If it makes RNA polymerase more active, it likely binds near the promotor sequence.
Since the protein is a homodimer, it probably rcognizes a palindromic seqeunce. There is one to the right of the promoter (boxed in the sequence above).

```
GCGAAATTTCGC
CGCTTTAAAGCG
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v) How would you prove your answer to part iii? Discuss how you would modify the DNA and the type of experiment you would design (4 pts)

Make changes in the DNA sequence and measure the binding affinity. Changes in the palindromic sequence should affect binding.

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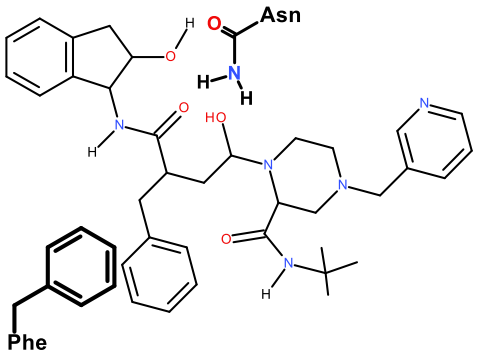
Part vi) of this question is on the following page:

Continuation of Question 11:

vi) Could you use this protein to regulate the expression of proteins from expression vectors? What sequences would you need on the expression vector and how would you turn on production of the protein (4 pts)?

You would want to put the GCGAAATTTCGC sequence up-stream of the promotor (as it is in the sequence on the previous page). The protein will bind there, and increase the activity of RNA polymerase.

12. (20 pts) You are screening a large number of compounds to find new inhibitors of HIV protease. A candidate is shown on the right, interacting with two residues (Asn & Phe) on the protease (in bold).



i) How would you determine whether the inhibitor was competitive or mixed type? What data would you collect and how would you analyze the data (6 pts)?

- Collect initial velocity data with different concentrations of substrates in the absence and presence of a fixed amount of inhibitor.
- Take the reciprocal of the data and plot 1/v versus 1/S.
- If the lines interact on the y-axis, then the Vmax is the same at high [S] and the inhibitor is competitive. (Inhibitor binds to the same site as the substrate, and can be displaced at high [S]).
- If the lines do not interact on the y-axis, then the Vmax is lower at high [S] and the inhibitor is mixed type (it binds elsewhere and causes an allosteric change in the active site). The inhibitor cannot be displaced by high substrate.

ii) After confirming the type of inhibition, you obtain the structure of the protein-drug complex. What technique would you have used to obtain the structure? (1 pt)

X-ray diffraction.

iii) An Asn and a Phe residue in the protease makes several energetically favorable contacts with the bound drug.

a) What thermodynamic interactions between the Asn and the drug are responsible for enhancing the drug binding? (2 pts)

Hydrogen bonding

b) What thermodynamic interactions between the Phe and the drug are responsible for enhancing the drug binding? (2 pts)

van der Waals (1 pt) and the hydrophobic effect (1 pt)

Parts iv) and v) of this question are on the following page.

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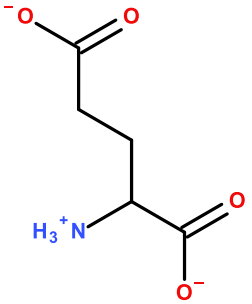
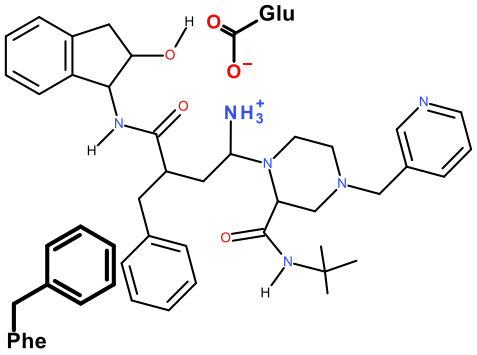
iv) You are using the drug to treat patients infected with HIV. During treatment, a patient develops viruses that are resistant to the drug. You wish to identify the changes in the protease that affect drug binding. You sequence the wild-type and mutant HIV protease genes and obtain the following sequences. What amino acid change has occurred that prevents drug binding? (The entire sequence of the HIV protease gene is on the formula sheet. The primer that was used to generate this sequence data is also indicated on the formula sheet.) Draw the structure of the altered amino acid in the mutant protease (6 pts).

Wild-type sequence:
ACCTACACCTGTCAACATAATT
ProthrProVal**Asn**Ile
Mutant sequence:
ACCTACACCTGTC**GAA**ATAATT
ProthrProVal**Glu**Ile



The codon for Asn (AAC) has been changed to GAA, which codes for the amino acid Glu. the sidechain is –C-C-C(OH)=O.

v) How would you modify the drug so that it would effectively bind to the mutant HIV protease? State whether this modification should increase or decrease K_D. Clearly indicate which group you would change on the **drug**, and how you would change it (3 pts).



The hydrogen bonding Asn has been replaced by a negatively charged sidechain on Glu, placing a positive charge on the drug near the Glu should give a lower K_D, indicating better binding, one possibility is shown on the right.

13. (4 pts) What is specific activity and why is it useful in protein purification?

- It is the ratio of the amount of target protein/total protein.
- It should increase after each purification step since (ideally) the amount of target protein stays the same, but the total protein decreases as impurities are removed.

14. (6 pts) The following is a list of names. On the right are a number of structures, indicate the correct structure next to the name.

___B___: Ribose

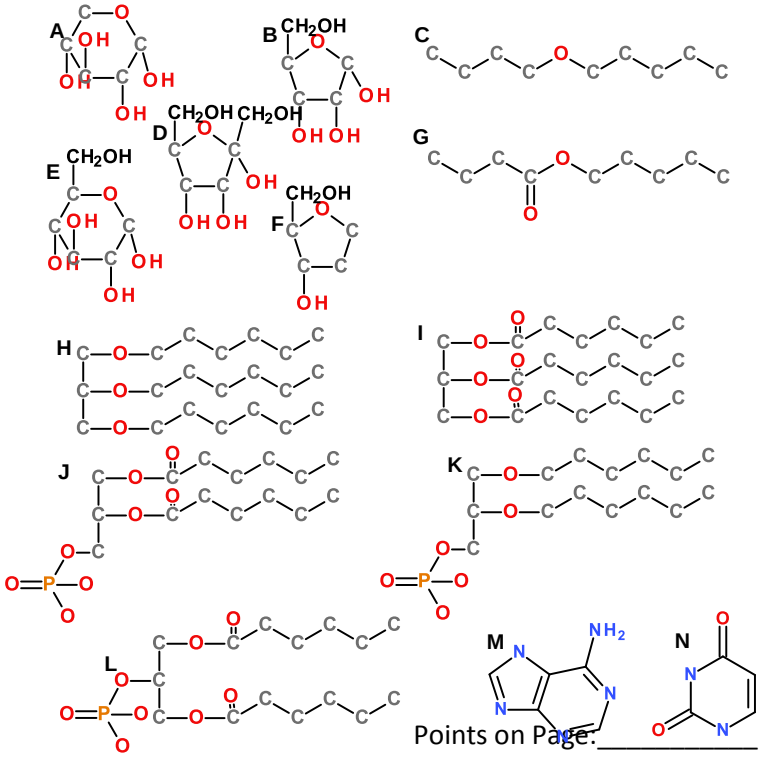
___E___: Glucose

___I___: Triglyceride

___G___: Wax

___J___: Phospholipid

___N___: Pyrimidine



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15. (10 pts) In two weeks I am going on my annual "rim-to-rim" hike across the Grand Canyon where we cover 22 miles (and 2 miles of elevation loss and gain) in about 12 hours.

i) Briefly describe the metabolic pathways that are responsible for energy production during the hike. Which pathways would be operating at the very start of the hike and which would likely become more active later on in the hike (6 pts)

- Initially I would obtain most of my ATP from oxidizing glucose using glycolysis and the TCA cycle.
- After my glycogen stores have dropped (about ½ through) I would rely on fats for energy, fatty acid oxidation, followed by the TCA cycle.

ii) In order to do this hike I carry an ultra-lite pack. What type of food should I carry in my pack, carbohydrates or fats? Which provided more energy/gram and why? (2 pts)

Fats – they are less oxidized than carbohydrates and can provide more energy.

iii) When climbing up to the rim, I consume all of the oxygen in my leg muscles. What other metabolic pathway is occurring so that I can continue to produce ATP. What is the product of that pathway? (2 pts)

In the absence of oxygen, NAD+ is regenerated by reducing pyruvate to lactate (fermentation). This allows glycolysis to continue.

16. (2pts) A base is attached to the sugar of a nucleotide through a ___N-glycosidic____bond, while nucleotides are linked together by __phosphodiester__bonds (a type of covalent bond).

17. (4pt) Both RNA and DNA can form a double helix, describe two similarities and two differences in these structures.

Similar:

- Both helices have bases stacked in the middle and phosphate backbone on the outside.
- Both have Watson-Crick basepairs (G-C and A-U/A-T) that have hydrogen bonds to help bring the two sets of chains together.
- Both have the strands of nucleic acid running antiparallel.
- Both helices are right handed

Different:

- The angle of the basepairs in DNA is perpendicular to the axis, while RNA bases are more inclined.
- DNA bases are stacked in the center axis, while RNA bases are stacked around the center axis producing a cylinder
- The separation of the phosphate chains is not uniform resulting in DNA having a major and minor groove, while the separation of phosphate chains in RNA is uniform so it has deep and shallow grooves.

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18. (2pts) What structural/chemical feature of RNA makes it less stable than DNA in a basic solution and why?

The 2' OH (hydroxyl group) (1pt) that is only present on RNA can be deprotonated at high pH (0.5), forming an active nucleophile. The oxygen attacks the phosphate, cleaving the phosphodiester bond (0.5) and forming a 2'-3' cyclic nucleotide. The cyclic group is subsequently hydrolyzed by water.

19. (4pt) Describe two similarities and two differences of DNA polymerase and RNA polymerase in the synthesis of DNA and RNA, respectively.

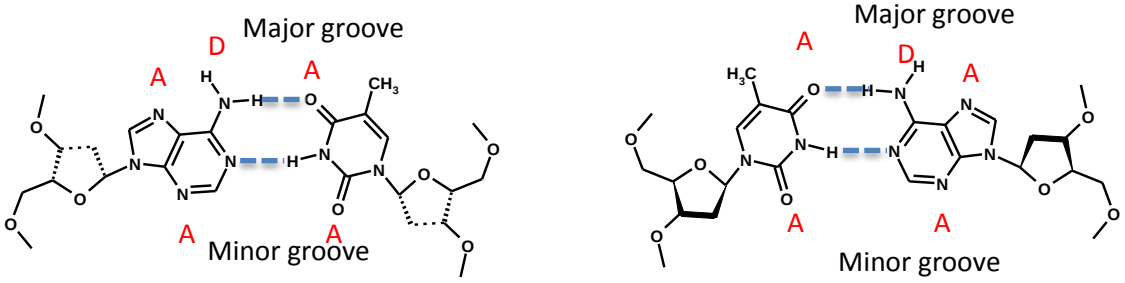
- Similar- both use nucleotide triphosphates as substrates, add these to a growing 3' end, nucleotides are connected by phosphodiester bond. Both use a strand of DNA (in the 3'->5' direction) to serve as template and use Watson-Crick base-pairing interactions to specify the incoming nucleotide. (any two of these are acceptable)
- Different- RNA polymerase uses riboNTPs and uracil, while DNA polymerase uses deoxyriboNTPs and thymidine. DNA polymerase requires a DNA or RNA primer while RNA P is self-priming. Some DNA polymerases have 3'->5' exonuclease/proofreading function, RNAP does not

20.(4 pt) Explain the unique features of restriction site sequences and how a restriction enzyme can cut both strands of the DNA.

2pt-Restriction sites are said to be palindromic, meaning that the sequence that is present is read the same on both DNA strands, for example 5'-GAATTC-3' is basepaired with the identical sequence that runs antiparallel to it.

2pt-Restriction enzymes are homodimers, each half of the enzyme binding to one side of the DNA and each cutting the same recognition sequence resulting the formation of DNA fragments with the same ends (either sticky ends or blunt ends depending on the enzyme).

21.(8pt)Two different base pairs are shown below.



i) Indicate the hydrogen bonds that can occur between the bases and where the major and minor groove would be located. (shown above 1pt for h-bonds, 1 pt for correct labeling of grooves)
ii) Label the hydrogen bond donors and acceptors on the bases in both grooves. (2 pt)
iii) Draw an example of an amino acid side chain that would likely interact with a hydrogen bond acceptor. (This should be a polar or charged amino acid like Ser, Thr, Asn, Gln, Lys, His, Cys, or Tyr- **not** non-polar) 2pt
iv) Explain whether a protein that interacted with the basepair on the right through the minor groove would be capable of a similar interaction with the basepair on the left and why.

Yes, the protein could interact with both basepairs through the minor groove, as both the AT and TA basepairs have hydrogen bond acceptors in a similar configuration. 2pt

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22. (12pt) The sequence below shows a portion of the DNA and protein sequence for the N-terminus and C-terminus of the HIV protease. Your job is to clone this protein into the expression vector shown on the right. (note this vector is referred to in questions 24 & 25 below)

Answer the following questions:

```
5'CGCCTCAGATCACTCTTTGGCAA.......ACTTTAAATTTTCCG3'
3'GCGGAGTCTAGTGAGAAACCGTT.......TGAAATTTAAAAGGC5'
     ProGlnIleThrLeuTrpGln-------ThrLeuAsnPhe
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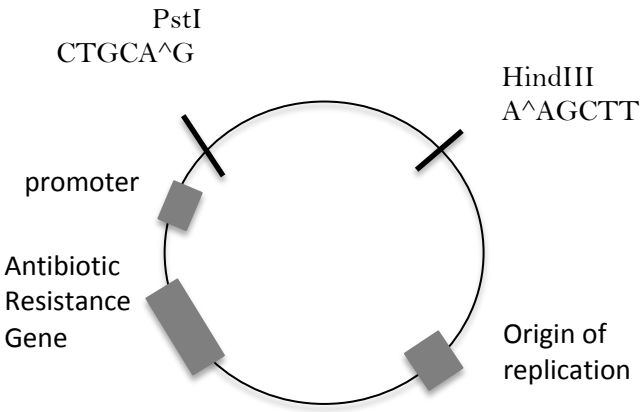
i) If you were going to utilize the start and stop codons present in the vector, write the sequence of both primers (limit 12 base pairs) you would design for PCR (in the 5'->3' direction).



Left: 5'-CTGCAGCCTCAG-3', PstI site at 5' end, portion from gene is underlined above.

Right: 5'-AAGCTTAAAATT -3', HindIII site at 5' end, portion from gene is underlined above

(6 pts total: 1 point each for correct restriction sequence at 5' end, 2 point each for correct 6 nt in correct 5'-3' order) -1 if wrong 6 nt or if reversed or complemented sequence

ii) Calculate the Tm for the left primer and state what temperature you would use for the annealing step of PCR.

$T_M = 81.5 + 0.41\times(\%GC) - 625/N$

81.5+ 0.41x(66.7%)-(625/12)= 56.75C (56-57 accepted) (2 pt total: 1 pt correct set up, 1 pt correct answer)

anneal at 51.75 (anything between 51 and 52 accepted) (1 pt)

iii) Describe the steps that you would need to perform to clone the resulting PCR products into the vector that is shown above.

Both the PCR product and the vector should be digested with the HindIII and PstI restriction enzymes (1pt). (Can mention purification of larger fragments from both, but not essential)
Mix the PCR fragment and vector (1pt) with ligase and ATP (1pt) to generate a new vector with the protease gene inserted.

23. (12pt) Describe the three steps of PCR and explain how repeating these steps leads to exponential amplification of a target DNA (be sure to describe relevant features of cycle temperatures, templates, primers). You may draw a figure to support your explanation.

First step is denaturation-1pt (94-98 C- 1pt), that converts double stranded DNA to single stranded DNA -1pt

Second step is annealing-1pt (50-60 C or 5C below primer Tm-1pt)- this allows the left and right primers to bind to complementary sequence in the opposite strands of the template (1pt), so that it can be recognized as a substrate for Taq polymerase-1pt

Third step is elongation-1pt (72-78C-1pt), Taq polymerase adds nucleotides to the 3' end of the DNA that are complementary to the template strand-1pt.

Amplification is exponential because by repeating these three cycles the PCR product from one cycle becomes template for the subsequent cycles (1pt), increasing the total amount of DNA by 2^N, where N equals the number of reaction cycles (1pt).

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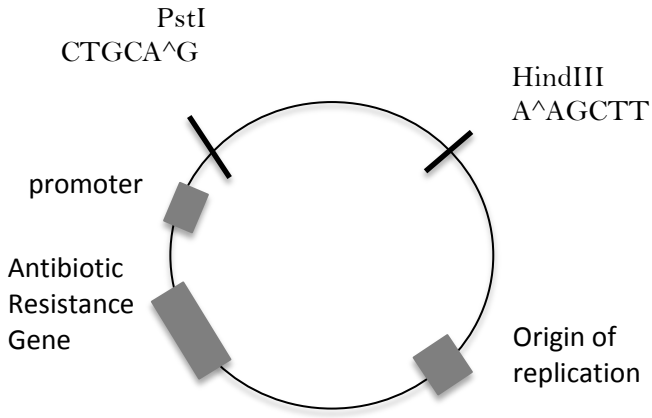
24. (7pts) The expression vector shown in problem 22 (shown again on the right) is missing several of the regulatory transcription and translation sequences that are listed below. Place these in the correct order (5' to 3') by numbering 1 through 7 (fill in the blanks), including the placement of the inserted PCR product.



i) mRNA terminator _____7____
ii) PCR product _____5____
iii) Start codon _____4____
iv) Lac operator _____2____
v) Stop codon _____6____
vi) Ribosome binding site _____3____
vii) Promoter _____1____

(1pt each, subtract 1 pt per time a number is out of relative order)

25. (4 pts) Answer one of the following two choices:

Choice A. In lecture we discussed two additional sequences that can be added to expression vectors to aid in protein purification. Describe one of these two sequences in terms of where they would be placed in the vector shown in question 24 and how the sequence can aid in purification.

Choice B. There are two sequences in the expression vector shown in question 24 that are needed for maintenance in bacteria. Describe one of these two sequences and explain why they are needed for the plasmid to be maintained.

Choice A: a leader peptide sequence (1pt) could be added to the construct just after the ATG start codon (1pt) that can signal to the cell to excrete the protein outside (1pt) and is cleaved from the final protein after exit from the cell (1pt) or a His6-tag (1pt) could be added to the end of the protease coding sequence (1pt), but just before the stop codon that can allow purification of the protein over a Ni column (2pt).

Choice B: the antibiotic resistance gene allows for selection of only those bacteria that have taken up the plasmid during transformation (2pt) and that maintain the plasmid (1pt) during subsequent growth of the culture. Treatment with antibiotic kills all cells that do not express the resistance gene. (1pt) The origin of replication is necessary to have the cells replicate the plasmid (2pt) so that upon cell division each of the daughter cells will receive a copy of the plasmid (2pt) .

26. (4 pt) DNA sequencing requires the incorporation of a modified nucleotide called a dideoxyNTP. Describe the modification and explain how this is utilized in Sanger sequencing.

A dideoxy nucleotide has a hydrogen only on the 3' carbon of ribose, unlike both dNTPs and NTP's that have a 3' OH group. (1pt) The dideoxy NTPs still have a triphosphate group and can be incorporated onto the growing 3' end of a DNA chain, but since they lack the 3' OH, the chain terminates after the addition of the ddNTP. (1pt) Each ddNTP base can be conjugated to a different fluorescent dye, leading to different sized fragments being made during DNA synthesis and each one having a specific fluorescent tag corresponding to the 3' nucleotide that was inserted. (1pt) By using a combination of dNTPs and a smaller amount of ddNTPs (roughly a 100:1 ratio), each potential DNA fragment can be made and after separation of fragments by size the sequence of the DNA deduced after detection of each fluorescent tag as the fragments pass a detector during gel electrophoresis. (1pt)

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27. (8 pts) Describe the steps involved in "charging" of tRNAs, include all enzymes and molecules needed for these reactions.

Charging of tRNAs is performed by amino acyl tRNA synthetases (1pt) which are each specific for a given tRNA and amino acid and so these enzymes attach the correct amino acid to the correct tRNA (1 pt) . The reaction occurs in two steps. First the amino acid is activated by the attachment of an AMP to the carboxylate group (2 pt). This reaction requires ATP and releases pyrophosphate, which is subsequently cleaved to inorganic phosphate, making the reaction favorable through indirect coupling (2pt). In the second step, the amino acid is transferred to the 3'-OH of the tRNA (1pt), releasing AMP (1pt).

28. (10 pts) Answer one of the following three choices (for each feel free to include well-labeled diagrams with an explanation):

Choice A: Describe the steps involved in the initiation, elongation and termination of transcription in bacteria. Be sure to include information regarding the sequences needed and factors involved in these reactions.

Choice B: Describe the lac operon and how it can be used to regulate transcription in bacteria. Be sure to include information regarding the DNA sequences involved and the molecules needed to turn on and off transcription.

Choice C: Describe the steps involved in the initiation, elongation and termination of translation in bacteria. Be sure to include information regarding sequences needed for these steps and factors involved in the reactions.

Choice A: First holoenzyme (Polymerase and sigma) binds to promoter (3pt). Second, an open complex forms where about 12 bp of DNA is melted in the (-9-+2 region) (1pt). Third, the polymerase starts RNA synthesis in the 5'->3' direction (no primer needed) (2pt) and when the chain is about 10 nucleotides long, σ-subunit dissociates,(1pt) leaving core enzyme to transcribe the RNA.(1pt) Chain termination occurs at specific DNA sequences and can either involve Rho factor or an RNA hairpin structure (1pt) that result in dissociation of the polymerase from the RNA and DNA template. (1pt)

Choice B: The lac operon contains a DNA sequence called the lac operator that is located 3' of the binding site for sigma and overlaps the start site for transcription. (2pt) When the lac repressor is bound it blocks transcription by preventing RNA polymerase from associating with the promoter (2pt), while when it is released transcription can occur. (2pt) The lac repressor exists in two states, a relaxed form that binds to the lac operator and a tense form that does not bind operator. (2pt) The tense form has a binding site for IPTG (lactose); when IPTG is present the tense, or non-DNA binding form is favored. (1pt) In the absence of IPTG the lac repressor binds to the lac operator, (1pt) preventing RNA polymerase (RNAP) from binding. The addition of IPTG causes the lac repressor to leave the DNA, allowing RNAP to bind and initiate the synthesis of mRNA.

Choice C:

- In the initiation of translation, mRNA first associates with the 30S subunit – the ribosome binding site (RBS) in the mRNA (also called the Shine-Dalgarno sequence) forms Watson-Crick hydrogen bonds to a region at the 3' end of 16S rRNA in the 30S subunit of the ribosome. (2pt) Next, fMet-tRNA^{fMet} and the 50S subunit join to form the 70S initiation complex (2pt) .
- In elongation, the fMet-tRNA occupies the P site and the next aminoacyl-tRNA-AA is brought to the A site (1pt) . This step requires the hydrolysis of GTP. Peptidyl transfer then occurs - the amino group of the A-site amino acid attacks the carbonyl of the amino acid (or peptide) attached to P-site tRNA (1pt) . Next, translocation of the ribosome downstream on the RNA occurs in a step that requires the hydrolysis of GTP, moving the uncharged tRNA to the E-site where it leaves the complex and the peptide to the P-site (2pt) . Steps 4-6 repeat until a stop codon is encountered
- At the stop codon protein release factor (which looks like a tRNA) binds and triggers the hydrolysis of peptide from the tRNA in the P-site. The complex disassembles (2pt).

29. (6 pt) Explain why there are 20 amino acids and 64 codons, but only 25-35 types of tRNAs on average per cell.

There can be fewer tRNAs than codons because of the allowance for wobble base pairs in the 3rd position of a codon allowing for G-U, A-I, C-I and U-I non-Watson-Crick base pairs in addition to the standard base pairs. Due to this, the anticodons of some tRNAs can interact with 2-3 different codons, all of which will encode the same amino acid.

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