Exam 1 Review Problems
DO NOT BRING TO EXAM

This packet contains problems from old exams, problems and even stuff from a TA from many years past. Use this as practice only. This is not, by any means, a definitive indication of what will be on your test. With the exception of the problems from the 2001 exam, Professor Yaffe had nothing to do with the course before last year.

Solutions will be provided next week. If you have questions, contact me via email or at the review session.

There is one important note about this packet and the exam. You will **NOT** be allowed into the exam with this packet. If you are caught with this packet, copies of it or even retyped/recopied versions of it, your exam for will voided.

The key is to be familiar with this material. Do not expect to see questions that mimic these problems. Professor Yaffe have indicated that they are working hard to make sure their material is original.

Good Luck!
-Ali

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Exam 1, 2001

1. Consider a human mRNA precursor molecule that is 500 nucleotides long including its single 100nt intron and its 80-nt poly(A) tail. (For this question, disregard its cap.)

   a. How many phosphodiester bonds are there in this pre-mRNA prior to splicing?

   b. How many phosphodiester bonds are there in the mature mRNA?

   c. How many phosphodiester bonds are there in the intron product after this mRNA is spliced by the spliceosome?

2. Chromosomal DNA is isolated from a bacterial species that has 22% dA. What are the percentages of dC, dG and dT?

3. 3'-deoxyadenosine 5'triphosphate (3'-dATP), is the triphosphate form of the antibiotic nucleoside, cordycepin. It is a potent inhibitor of RNA synthesis.

   a. Draw the chemical structure of 3' dATP

   b. On the drawing in part (a) ciricle the atoms that would hydrogen bond with the template strand when 3'-dATP is used as a substrate during RNA synthesis.

   c. Explain why the incorporation of cordycepin (3'-deoxyadenosine) inhibits RNA synthesis.

   d. 3'-dATP does not inhibit DNA synthesis by DNA Pol I or DNA Pol III. Explain why these enzymes would not be expected to use 3'-dATP, whereas *E. coli* RNA Polymerase does use the inhibitor.

   e. Although 3'-dATP does not inhibit DNA synthesis by DNA Pol I or DNA Pol III, this nucleotide is expected to directly inhibit the process of DNA replication in *E. coli*. Explain why.

4. 3 Part question

   a. Write a 15-nucleotide RNA sequence that could code for the peptide PheMetThrCysCal. Indicate the 5' and the 3' end of this sequence.

b. How many other 15-nt RNA sequences could code for the peptide in part (a)? Show how you derived your answer.

c. Comment on the precision of information flow from RNA –> protein –> RNA.

5. Consider the biological synthesis of protein using the amino acid Ala. If the two oxygen atoms of Ala were replaced with heavy isotopes of oxygen so that we could follow the fate of these two oxygens during protein synthesis, in which residue/molecule(s) would these two heavy oxygens reside after translation is complete? Draw the structure of the residue/molecules(s), and circle the heavy oxygen atoms.

6. Consider the use of isoelectric focusing to separate the three polypeptides, A, B, and C.

A = Ala-Gly-His-Pro-Gln-Thr-Val
B = Ile-Leu-Cys-Tyr-Asp-Glu-Ala
C = Lys-Arg-Pro

a. Use table 3.2 from Stryer on page 50 (table 2.2 on page 23 in 4th edition) to write the sequence of these three polypeptides using the one-letter symbols

b. Use table 3.1 from Stryer on page 50 (table 2.1 on page 23 in 4th edition) to predict which residues of these polypeptides will be predominantly charged when these polypeptides are focused at their respective isolelectric points circle these residues in the three sequences that you have written in part (a). For the residues that you circled, indicate whether the charge would be positive or negative.

c. Sketch a simple diagram of the separation of these three polypeptides by isoelectric focusing. Indicate which part of the gel (or paper) is high pH, and which is low pH. Indicate the polarity of the electric field. Indicate the relative positions of the three polypeptides. Please do <u>not</u> calculate the precise pI's of these polypeptides, show only their positions relative to the pH gradient, the electric field and each other.

d. Draw the chemical structure of the predominant species of peptide C when it is focused at its iso electric point.

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Exam 1, 2000

7. Consider the peptide: Ile-Gly-Asp

   a. (A) Draw the chemical structure for the predominant species of the peptide in water at pH 7.0. (pKas: Asp = 4.4, N = 8.0, C = 3.1)

   b. Indicate with asterisks (*) the atom(s) that are chiral centers in the structure that you have drawn in part (a).

   c. What is the net charge of the peptide at pH 2.0, and at pH 12.0?

8. Give two reasons why you would obtain an incorrect molecular weight of a protein using reducing polyacrylamide gel electrophoresis if you forgot to add SDS. Explain briefly.

9. Suppose that you were given a short DNA fragment with the sequence: CAGCAGCAGCAG.

   a. If you were using the Khorana approach to determining the genetic code, what DNA fragment(s) would you need to obtain in order to proceed?

   b. What protein products would you expect to obtain (in the presence of all nucleotides) using these DNA fragments and the Khorana approach?

10. Consider the transfer of information between and within DNA, RNA and protein. At which step(s) does the transfer of information get "proofread" or "double-checked?" Explain with 1-2 sentences for each mechanism of proofreading.

Exam 1, 1999

11. Consider the peptide: Phe-Glu-Thr. Draw the chemical structure for the predominant species of the peptide in water at pH 5.0. Indicate with asterisks the atom(s) that are chiral centers in the structure that you have drawn in. (Use table 3.1 from Stryer on page 50 [table 2.1 on page 23 in 4th edition].)

12. 2 part question

   a. Suppose you performed an SDS-PAGE experiment in which you were able to separate proteins with molecular weights of 100kD, 40kD, and 10kD.

Sketch what the gel might look like, after staining. Indicate the direction of electrophoresis.

b. Now suppose you wanted to repeat the experiment to verify your results, but you made a mistake and added too much methylene-bis-acrylamide when you were preparing the gel. Next to your drawing in part (a), sketch what this second gel might look like. Explain briefly.

13. Draw the chemical structure of UTP. On the drawing in part (a), indicate with an asterisks the nitrogen atom(s) and phosphorus atom(s) that, if radioactive, would lead to incorporation of radioactivity into RNA in an RNA polymerase reaction. Explain briefly.

14. Suppose you were given two strains of E. Coli that were identical except that one strain lacked proofreading activity in DNA polymerase I (the "mutant" strain). Not surprisingly, upon analyzing the DNA in the mutant strain, you find that there are errors that are made during replication. Curiously, you find the errors in the DNA from the mutant strain are found in local regions, separated by much longer regions of low-error DNA that are thousands of nucleotides in length. What is the most likely explanation for these results?

15. 3 part question

a. Draw a line representing a eukaryotic mRNA precursor with two exons and one intron. Indicate the 5' and 3' ends, the intron and the exons, and the locations of the stop and start codons.

b. Sketch the products of the splicing reaction for the mRNA precursor that you sketched in part (a). Again, indicate the 5' and 3' ends, the intron and exons, and the locations of the start and stop codons.

c. In your sketches, indicate with asterisks the location(s) of nucleotide(s) that are connected by three phosphodiester bonds.

16. Propose two short DNA oligonucleotides (12 nt each) that you would synthesize if you wanted to use the Khorana method for making polymers that could be used in a cell-free transcription and translation extract to generate poly-Ile and poly-Asn.

17. 2 part question

a. Consider a peptidyl tRNA in the P-site of a ribosome. Provide a simple sketch of the structure of the peptidyl tRNA, indicating the tRNA simply with "tRNA", and the peptide portion simply with "peptide". Indicate with arrows, and label, the 5' end of the tRNA and the amino terminal end of the peptide.

b. Draw the complete structure of an amino acid residue (Ala) that is directly attached to the tRNA, including the complete structure of the sugar that is directly attached to the peptide.

18. Suppose theat you isolated an HIV strain from a patient's blood that contained a reverse transcriptase that proofread. All else being equal, would you consider this virus more or less of a danger, as compared to the strain that does not proofread? Explain briefly.

Exam 1, 1998

19. Draw the chemical structure for the peptide with the sequence DPF (denoted in one letter abbreviations). Indicate all covalent bonds (except those to hydrogens) in the tripeptide that have free (unhindered) rotation. What is the net charge of the peptide at pH 12?

20. Draw the chemical structure of an RNA fragment with the sequence CUA. What is the sequence of a DNA fragment that is complementary to this RNA fragment?

21. Consider a "charged" tyrosine-tRNA. Draw the chemical structure for the last nucleotide, at the 3' end of the tRNA. Include the entire tyrosine residue.

22. TCA (trichloroacetic acid) has been a particularly useful reagent for biochemical studies of DNA replication, RNA synthesis, and protein synthesis. Explain why.

23. Suppose that you were studying an artificial E. Coli chromosome containing two origins of replication that are far apart from each other. If you were able to isolate the chromosome while it was replicating, and studied it with electron microscopy, how many replication forks would you expect to see? Explain briefly with a sketch.

24. You are trying to purify the reverse transcriptase ("**RT**") from a new retrovirus. Analysis of a partially purified enzyme preparation by two-dimensional get electrophoresis shows that it is contaminated with other proteins. One contaminant ("**A**") has a molecular weight of 45 kD (similar to that of **RT**). The

pI of RT is 6.6, which is 4 units lower than that of **A**. The other contaminant ("**B**") is a large protein with a molecular weight of 120 kD and a pI that is similar to that of **RT**.

a. Draw a sketch of the 2D-gel, indicating the locations of **RT**, **A**, and **B**. Label the axes.

b. Propose an efficient way to purify the **RT** to homogeneity in large amounts. Assume that you do not have access to preparative IEF or HPLC.

25. Would you expect increased or decreased efficiency of translation from an mRNA if you inserted 6 nucleotides immediately upstream (i.e., toward the 5'-end) of the initiator AUG codon? Explain.

26. Suppose that you developed an imaging technique that allowed you to observe, within living cells, the processes of transcription and translation. With bacteria, you observe that DNA is sometimes connected via a mRNA and a ribosome, to a growing protein chain. However, you never see such structures with human cells. Why not?

Exam 1, 1996

27. Draw the chemical structure of the branch-site A residue of an intron after splicing has occurred. Indicate the locations of any phosphodiester bonds to this nucleotide.

28. tRNA synthetases "charge" tRNA molecules in a two-step reaction. Suppose that you carried out both steps of this reaction in the presence of radioactive ATP (^{32}P incorporated at all phosphorus atoms in ATP). Where would you expect the radioactivity to be located after charging was complete? Explain briefly.

29. In working out the genetic code, why was it important to add DNAse to the cell-free protein synthesis system?

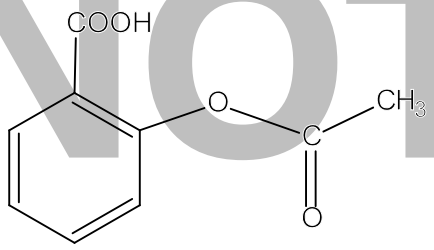
30. Protein engineering refers to an area of structural biology in which scientists try to alter the properties of proteins. Suppose you were able to engineer a DNA polymerase that contained a modified 3' to 5' exonuclease activity with the following property: the exonuclease activity required that the 3'-nucleotide <u>was</u> part of a double helix. What would you expect the properties of this engineered enzyme to be, in terms of fidelity and overall speed of replication, as compared

to the otherwise identical DNA polymerase that completely lacks 3' to 5' exonuclease activity? Explain.

31. Previously, it was thought that peptidyl transferase activity was contained within several of the proteins in the 50S ribosomal particle. Recently, it was discovered that this dogma is almost certainly wrong: the peptidyl transferase activity appears to be contained in the 23S rRNA instead. What is the significance, in terms of evolution, of this new discovery?

Exam 1, 1995

32. Aspirin (acetyl salicylic acid) has a carboxylate with a pKa of 3.5. In order to enter the bloodstream, aspirin must pass through the membrane lining the stomach (pH approx. 1) or the small intestine (pH approx. 6). In general, electrically neutral molecules pass through a membrane more easily than charged molecules. Would you expect more aspirin to be absorbed in the stomach or small intestine? Why?



aspirin (acetyl salicylic acid)

33. Draw the chemical structure of Ser-Pro-Asp. Indicate all covalent bonds (except those to hydrogens) in the tripeptide that have free (unhindered) rotation.

34. Why would it be a mistake to add SDS to an isoelectric focusing gel?

35. Draw the structure of dATP. Indicate which of the phosphorous atom(s), if radioactive, would allow you to obtain radioactive DNA in a DNA polymerase system. Explain briefly.

36. What would be the primary significance of a discovery that a processivity factor for DNA polymerase forms a circular, “ring”-shaped assembly, in which the hole in the middle of the “ring” has a diameter of ~35Å?

37. None of the mechanisms for DNA replication that were discussed for E. Coli can

account for the complete replication of linear, double-stranded DNA molecules. Why?

38. Why do oligonucleotides (short DNA molecules) containing Shine-Dalgarno sequences inhibit translation in prokaryotes? Why don't they do so in eukaryotes?

39. Assuming random translation starts, what products would you expect upon addition of poly-(CGA) RNA to a cell-free protein-synthesis system? Propose a column-based strategy to separate these products.

Exam 1, 1994

40. Consider the peptide: Ala-Glu-His-Lys. (Use table 3.1 from Stryer on page 50 [table 2.1 on page 23 in 4th edition].)

    a. Draw the structures of this peptide at pH 2 and a pH 12. Sketch a sodium hydroxide titration curve for this peptide, starting at pH 2 and ending at pH 12.

    b. Suppose you wanted to separate, by cation-exchange chromatography, this peptide from another peptide containing the sequence Ala-Ala-His-Lys. Would you get a better separation of the two peptides on a CM column at pH 2 or at pH 12? Why?

41. Suppose that you isolate an infectious agent that causes disease. You find that the infectious agent is inactivated by RNAse. In contrast, DNAse treatment has no effect on the infectious agent. Which of the following proteins would you predict is most likely to be encoded by the infectious agent:

    * DNA-dependent DNA polymerase
    * DNA-dependent RNA polymerase
    * RNA-dependent DNA polymerase
    * RNA-dependent RNA polymerase

    Explain briefly.

42. You isolate a DNA Polymerase I from a mutant strain of E. Coli. The enzyme contains a 3' to 5' exonuclease activity that is only active when the 3' nucleotide is part of a double helix. You compare the properties of this enzyme to normal (wild-type) DNA polymerase I.

a. Define processivity in two sentences or less. Would you expect there to be a difference in the processivity of the mutant and wild-type enzymes? Explain.

b. Define proofreading in two sentences or less. Would you expect there to be a difference in the proofreading abilities of the mutant and wild-type enzymes? Explain.

43. Suppose that you are trying to express the gene for human growth hormone in a cell-free E. Coli transcription system. You add a DNA fragment from a human cell line that includes the complete gene for growth hormone as well as the 5' and 3' regions that usually surround this gene. You find that there is no RNA synthesis when you add the human growth hormone gene. A control experiment shows that an E. Coli gene is transcribed very efficiently by the cell-free E. Coli transcription system you are using. What changes would you make in the human DNA fragment to try and get transcription of the human gene in the E. Coli system?

44. You add poly(ACCA) RNA to a cell-free E. Coli protein synthesis system. Although inefficient, you are able to monitor some protein synthesis. What polypeptide product(s) would you expect to isolate?

PSET 1, 2001

45. 5 part question

a. Draw the peptide Glu-Trp-Gln-Arg at pH 7.2 using the pKa values from Stryer.

b. What is the overall charge of the polypeptide at this pH? At pH 1? At pH 13?

c. Indicate which bonds are free/are not free to rotate.

d. Label all chiral carbons with an *.

PSET 2, 2001

46. Consider the following experiment: Incubate single stranded DNA, a small appropriate RNA primer, specifically radio labeled dNTP's, and E. coli DNA Pol I

under conditions that favor DNA polymerization. Quench the reaction by trichloroacetic acid precipitation and separate products by filtration through a dine-pored sieve.

a. Draw the structure of dATP Number the atoms of the base and sugar, and label phosphates as α, β, or γ.

b. Circle the atoms that make hydrogen-bonding contact with thymidine in a Watson-Crick base-pair.

c. After filtration in the experiment above, where would you be able to detect radioactivity if the γ phosphate were radiolabeled with ^{32}P?

d. The β phosphate?

e. The α phosphate?

f. If C1' were radiolabeled with ^{14}C?

g. If N1 were radiolabeled with ^{15}N?

PSET 3, 2001

47. A prokaryotic organism named *Kerobero* is recovered from samples collected during a Mars expedition. Your preliminary observations indicate that the organism appears to be exactly like *E. coli*, except that its RNA undergoes splicing. You wish to find out more about transcription and translation in this organism by studying one of its genes, named *sakura*.

a. A partial sequence of the **coding strand** DNA of the *sakura* gene is given below:

16 nt 7 nt 20 nt 18 nt
5'~~TTGACA~~~~~TATAAT~~~~~AGCAGGAGGTTTGACCTATGACTTACAGGTAAGT~~~~~CTGAC~~~~~TTTCCACAGGGGA~~3'
+1

Based on this sequence:

i. What is the expected sequence for the pre-mRNA (primary transcript)?

ii. What is the expected sequence for the mature mRNA (after splicing)?

iii. What are the first five amino acids of the protein that would be synthesized?

b. Now, suppose that you transfer the *sakura* gene to ***E. coli*.**

i. What is the sequence of the mRNA that would be transcribed (**hint:** think about the difference between *Kerobero* and *E. coli*) ?

ii. What is the amino acid sequence of the peptide that would be synthesized?

iii. Draw the chemical structure for the predominant species of the peptide in water at pH 11 (use the pK_a values on p. 23 of Stryer's *Biochemistry* 4th edition).
(1) Label each atom that is a chiral center with an asterisk (*).
(2) Indicate all covalent bonds (except those to hydrogens) that have free (unhindered) rotation with curved arrows.

c. Suppose that there are mutations in the active site of the *E. coli* tyrosyl-tRNA synthetase. These mutations substitute hydrophobic residues for hydrophilic residues in the pocket that binds the tyrosine side chain. What effect might this mutated tRNA have on the peptide sequence? Explain.

d. Propose an efficient way to purify the peptide in part b from the peptide in part c.

PSET 2, 1998

48. Although DNA polymerase I requires both a template and a primer, the following single-stranded polynucleotide was found to serve as a substrate for DNA polymerase I in the absence of any additional DNA or RNA.

3'OH-TGAGCCCATAGCCGGGGCTCTAACCGTAGACCACGAATAGCATTACC-p5'

Propose a mechanism for how this polynucleotide may be a substrate for DNA polymerase I. (Hint: think about the mechanisms of transcription termination.) Give the structure of the product of this reaction mechanism, excluding newly added base pairs.

49. During DNA replication 3' to 5' exonuclease proofreading activity removes

nucleotides incorrectly incorporated into a growing DNA strand. There is no analogous proofreading activity during translation. A proofreading step that hydrolysed the last peptide bond formed when an incorrect amino acid was inserted into a growing polypeptide would be chemically impractical. Why?

50. A single nucleotide addition followed by a single nucleotide deletion approximately 20 base pairs apart in the DNA causes a change in the protein sequence from

    His-Thr-Glu-Asp-Trp-Leu-His-Gln-Asp to
    His-Asp-Arg-Gly-Leu-Ala-Thr-Ser-Asp.

    Which nucleotide has been added and which nucleotide has been deleted? What are the original and the new mRNA sequences?

Problems from 7.05 TA Jane Woo

51. 3 Part Question

    a. Draw the chemical structure of the tetrapeptide at pH 7.0: ala-glu-val-lys. Over what range of pH would the net charge of this peptide be 0?

    b. Draw the chemical structure and calculate the net charge on each of the following amino acids: aspartic acid, lysine, and cysteine; at each of the following pHs: 3.1, 7.0, and 10.0.

    c. Calculate the net charge on the following small peptide, for pH=6 and pH=8: NH_2-his-glu-ala-cys-COOH.

Exam 1, 1999

52. Propose two short DNA oligonucleotides (12 nucleotides each) that you would synthesize if you wanted to use the Khorana method for making polymers that could be used in a cell-free transcription and translation extract to generate poly-Ile and poly-Asn.

53. Two part question

    a. Consider a peptidyl tRNA in the P-site of a ribosome. Provide a simple sketch of the structure of the peptidyl tRNA, indicating the tRNA simply with “tRNA,” and the peptide portion simply with “peptide.” Indicate with

arrows, and label, the 5' end of the tRNA and the amino terminal of the peptide.

b. Draw the complete structure of an amino acid residue (Ala) that is directly attached to the tRNA, including the complete structure of the sugar that is directly attached to the peptide.

Exam 1, 1998

54. Consider a "charged" tyrosine-tRNA. Draw the chemical structure for the last nucleotide, at the 3' end of the tRNA. Include the entire tyrosine residue.

Exam 1, 1996

55. Draw the chemical structure of the branch-site A residue of an intron after splicing has occurred. Indicate the locations of any phosphodiester bonds to this nucleotide.

56. tRNA synthetases "charge" tRNA molecules in a two-step reaction. Suppose that you carried out both steps of this reaction in the presence of radioactive ATP (^{32}P incorporated at all phosphorus atoms in ATP). Where would you expect the radioactivity to be located after charging was complete? Explain briefly.

57. In working out the genetic code, why was it important to add DNAse to the cell-free protein synthesis system?

58. Previously, it was thought that peptidyl transferase activity was contained within several of the proteins in the 50S ribosomal particle. Recently, it was discovered that this dogma is almost certainly wrong: the peptidyl transferase activity appears to be contained in the 23S rRNA instead. What is the significance, in terms of evolution, of this new discovery?

59. Suppose that you isolate an infectious agent that causes disease. You find that the infectious agent is inactivated by RNAase. In contrast, DNAase treatment has no effect on the infectious agent. Which of the following proteins would you predict is most likely to be encoded by the infectious agent:

(a) DNA-dependent DNA polymerase
(b) DNA-dependent RNA polymerase
(c) RNA-dependent DNA polymerase
(d) RNA-dependent RNA polymerase

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60. What was the significance of demonstrating a lariat structure in a splicing intermediate? Draw the complete chemical structure at the branch site of a lariat intermediate. Include the central adenine residue and the three nucleotides to which it is connected. You don't need to draw out the bases of the three nucleotides.