Exam Details

Section 1: Closed book, 1 question, 1 hr (40 points)
- You may not consult any resources for this portion of the exam.
- This portion of the exam must be completed in one sitting with no breaks.
- If you run out of time, continue to finish this section but clearly note what work was done past the time limit. You will receive half credit for this work.
- Unless specifically noted, limit your answers to 5 sentences.
- If your answer contains incorrect information along with correct information, points will be deducted.
Exam Details

Section 2: Open book, 9 questions, 4 hrs (160 points)

- Resources you may use are limited to: your Stryer ‘Biochemistry’ textbook, your lecture notes, problem sets, and any material that has been posted on the course website.
- During this portion of the exam, you may have one break for any amount of time. During the break you may not consult any prohibited resources for exam material and you may not talk to other students about the exam.
- If you run out of time, continue to finish this section but clearly note what work was done past the time limit. You will receive half credit for this work.
- Unless specifically noted, limit your answers to 5 sentences.
- If your answer contains incorrect information along with correct information, points will be deducted.
Exam Details

Section 3: Midterm course survey, 12 questions, unlimited time
- We want to hear about your experiences with the course so far and would appreciate your taking the time to answer these survey questions.
- To protect anonymity, there will be a separate pile to turn in these surveys, and names are not required.
- Please take the survey after you have completed the entire exam.

**Additionally, Brenda Wu is our ombudsperson**

Please write your answers to each question on a separate page(s) with your name on the top of every page.
Practice Problems
How can cold temperatures promote protein denaturation (unfolding)? In your explanation, reference the signs of $\Delta H$ and $\Delta S$ for the unfolding process.
How can cold temperatures promote protein denaturation (unfolding)? In your explanation, reference the signs of $\Delta H$ and $\Delta S$ for the unfolding process.

Water has a defined crystal lattice at low temperatures, and exposure of internal hydrophobics decreases protein-water bonding and increases the water-water lattice structure, which is exothermic (same direction as ice formation). $\Delta H$ is negative.

Hydrophobic residues can “order” water more effectively at lower temperatures. At lower temperatures, hydrophobic cores can form ordered hydration shells to shield from water. Therefore, $\Delta S$ is negative.

The reaction is therefore enthalpy driven.
Cloning: (a) How many mRNA coding possibilities exist for the following pentapeptide? Met-Arg-Lys-Phe-Phe
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From: http://bioinfo.bisr.res.in/project/crat/pictures/codon.jpg

48

1 * 6 * 2 * 2 * 2
Cloning: (b) Below is a page from a researcher's notebook in which the sequence was determined for a 34-nucleotide fragment of viral RNA. It reads: “Depending on the punctuation of the sequence, it would code for one of the following amino acid sequences. No initiation or termination codons are present.” – (F. Sanger’s notebook, ~ 1971)
Cloning: (b con't) Give at least 12 consecutive nucleotides of the 34-nucleotide RNA coding sequence that is consistent with the three peptide sequence possibilities that are proposed.

Tyr-Arg-Glu-Arg-Ser-Gln-Val-Leu-Gln-Arg-Lys
Ile-Glu-Asn-Ala-Arg-Lys-Phe-Phe-Ser-Glu-Ser or Arg
Leu-Ser-Arg-Thr-Leu-Ala-Ser-Ser-Ser-Ala-Lys

From:
http://bioinfo.bisr.res.in/project/crat/pictures/codon.jpg
Cloning: (b con't) Give at least 12 consecutive nucleotides of the 34-nucleotide RNA coding sequence that is consistent with the three peptide sequence possibilities that are proposed.

The entire sequence:

5'-CUAUCGAGAACGCUCGCAAGUUCUUCAGCGAAAG-3'

Tyr-Arg-Glu-Arg-Ser-Gln-Val-Leu-Gln-Arg-Lys
Ile-Glu-Asn-Ala-Arg-Lys-Phe-Phe-Ser-Glu-Ser or Arg
Leu-Ser-Arg-Thr-Leu-Ala-Ser-Ser-Ser-Ala-Lys

From:
http://bioinfo.bisr.res.in/project/crat/pictures/codon.jpg
Which of the following lipids has the lowest melting temperature? Why?

- **A**
  - trans-9-Octadecenoic acid

- **B**
  - cis-9-Octadecenoic acid

- **C**
  - Octadecenoic acid

*Wikipedia* for convenient figure.
Which of the following lipids has the lowest melting temperature? Why?

- **A**: trans-9-Octadecenoic acid
  - Formula: \( \text{CH}_{27}\text{CH}(_2\text{CH})_{17}\text{CH}(_2\text{CH})\text{CH}=\text{CH}-\text{CH}_{17}\text{CH}_{2}\text{COOH} \)
  - Melting temperature: 45°C

- **B**: cis-9-Octadecenoic acid
  - Formula: \( \text{CH}_{27}\text{CH}(_2\text{CH})_{17}\text{CH}(_2\text{CH})\text{CH}=\text{CH}-\text{COOH} \)
  - Melting temperature: 14°C

- **C**: Octadecenoic acid
  - Formula: \( \text{CH}_{27}\text{CH}_{2}\text{COOH} \)
  - Melting temperature: 70°C

*Wikipedia* for convenient figure.
### TABLE 1. Characterization of important fatty acids in foods (CL = chain length, DB = double bonds, MP = melting point (°C))

<table>
<thead>
<tr>
<th>Common Name</th>
<th>CL</th>
<th>DB</th>
<th>MP</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyric acid</td>
<td>4</td>
<td>0</td>
<td>-8</td>
<td>Milkfat</td>
</tr>
<tr>
<td>Caproic acid</td>
<td>6</td>
<td>0</td>
<td>-2</td>
<td>Milkfat</td>
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<tr>
<td>Caprylic acid</td>
<td>8</td>
<td>0</td>
<td>16</td>
<td>Milkfat</td>
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<tr>
<td>Capric acid</td>
<td>10</td>
<td>0</td>
<td>31</td>
<td>Milkfat</td>
</tr>
<tr>
<td>Lauric acid</td>
<td>12</td>
<td>0</td>
<td>44</td>
<td>Cocos fat</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>14</td>
<td>0</td>
<td>54</td>
<td>Animal fat</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16</td>
<td>0</td>
<td>63</td>
<td>Animal fat</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>18</td>
<td>1&lt;sub&gt;cis&lt;/sub&gt;</td>
<td>1</td>
<td>Animal fat, fish oil</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18</td>
<td>0</td>
<td>70</td>
<td>Animal fat</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18</td>
<td>1&lt;sub&gt;trans&lt;/sub&gt;</td>
<td>13</td>
<td>Fats and oils</td>
</tr>
<tr>
<td>Vaccenic acid</td>
<td>18</td>
<td>1&lt;sub&gt;cis&lt;/sub&gt;</td>
<td>40</td>
<td>Butter</td>
</tr>
<tr>
<td>Linoleic acid</td>
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<td>2&lt;sub&gt;cis 9,12&lt;/sub&gt;</td>
<td>6</td>
<td>Phosphatides</td>
</tr>
<tr>
<td>α-Linoleic acid</td>
<td>18</td>
<td>3&lt;sub&gt;cis 9,12,15&lt;/sub&gt;</td>
<td>14</td>
<td></td>
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<tr>
<td>Arachidic acid</td>
<td>20</td>
<td>4&lt;sub&gt;cis 5,8,11,14&lt;/sub&gt;</td>
<td>76</td>
<td>Animal fats</td>
</tr>
<tr>
<td>Timnodonic acid</td>
<td>20</td>
<td>5&lt;sub&gt;cis 5,8,11,14,17&lt;/sub&gt;</td>
<td>50</td>
<td>Phosphatides</td>
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<tr>
<td>Erucic acid</td>
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<td>1&lt;sub&gt;cis&lt;/sub&gt;</td>
<td>80</td>
<td>Cerebrosides</td>
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<tr>
<td>Clupandonic acid</td>
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<td>5&lt;sub&gt;cis 7,10,13,16,19&lt;/sub&gt;</td>
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<td></td>
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<td>Nervonic acid</td>
<td>24</td>
<td>1&lt;sub&gt;cis&lt;/sub&gt;</td>
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<td>Cerebronic acid</td>
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<td>0</td>
<td>100</td>
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<tr>
<td>Hydroxynervonic acid</td>
<td>24</td>
<td>1&lt;sub&gt;cis&lt;/sub&gt;</td>
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### Addendum:

**TABLE 1. Characterization of important fatty acids in foods (CL = chain length, DB = double bonds, MP = melting point (°C))**

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Boskou and Elmadfa, “Frying of Food”
Which of the following hydropathy plots corresponds to a membrane protein?
Which of the following hydropathy plots corresponds to a membrane protein?

(A)

(B)

(C)

(C) Corresponds to a membrane protein. Interestingly, (A) is also a membrane protein, which is made up of B-strands.
Which of the following hydropathy plots corresponds to a membrane protein?

Ex. Sucrose porin
A hallmark of Alzheimer's disease is the formation of intracellular deposits made of the protein β amyloid. In the figure below, supersaturated solutions of model β amyloid peptides were prepared, and the formation of β amyloid aggregates was measured by the turbidity (cloudiness) of the solution. What conclusion can you draw about the requirements of β amyloid for aggregate formation? Explain using the data in the figure.

▲ β26-39 (residues 26-39)
◯ β26-40 (residues 26-40)
◻ β26-42 (residues 26-42)
⚫ β26-43 (residues 26-43)
A hallmark of Alzheimer’s disease is the formation of intracellular deposits made of the protein β amyloid. In the figure below, supersaturated solutions of model β amyloid peptides were prepared, and the formation of β amyloid aggregates was measured by the turbidity (cloudiness) of the solution. What conclusion can you draw about the requirements of β amyloid for aggregate formation? Explain using the data in the figure.

There is a dependence on sequence for amyloid formation. Including residue 40 speeds up amyloid formation compared to a peptide that stops at 39, and inclusion of residues 42/3 speeds up the formation even more. While all peptides are capable of forming aggregates, the length of the C-terminus determines the speed of aggregation.
A supersaturated solution of β26-40 (Δ), a solution of β26-43 below its solubility threshold (□), and a mixture of those two solutions (●) were incubated together, and the formation of aggregates was determined by measuring the turbidity of the solution. Based on the data below, which step in aggregate formation is most dependent upon peptide composition? Explain how you came to this conclusion. (Hint: think about our discussion of the kinetics of aggregate formation during recitation)
A supersaturated solution of β26-40 (△), a solution of β26-43 below its solubility threshold (□), and a mixture of those two solutions (●) were incubated together, and the formation of aggregates was determined by measuring the turbidity of the solution. Based on the data below, which step in aggregate formation is most dependent upon peptide composition? Explain how you came to this conclusion. (Hint: think about our discussion of the kinetics of aggregate formation during recitation)

The β26-43 peptide can be used to “seed” the formation of β26-40 aggregates, speeding up this process much more than β26-40 peptides alone. This suggests that initial formation of aggregates -- nucleation -- is the rate-determining step, with β26-40 having a very slow nucleation and β26-43 having a fast nucleation. Therefore, the C-terminus of the protein is critical for aggregate nucleation and therefore aggregate formation.

In 1998, Rod MacKinnon’s group solved an X-ray crystal structure of the K+ channel. The structure provided several clues as to how the K+ channel maintains selectivity.

(a) Provide labels for elements A, B and C. Pictured below is one subunit of the transporter, explain which have a diameter of 3 Å in the intact channel? 10 Å?
In 1998, Rod MacKinnon’s group solved an X-ray crystal structure of the K+ channel. The structure provided several clues as to how the K+ channel maintains selectivity.

(a) Provide labels for elements A, B and C. Pictured below is one subunit of the transporter, explain which have a diameter of 3 Å in the intact channel? 10 Å?

A = selectivity filter, B = ion (K+), C = exit channel. The selectivity filter has a diameter of 3 Å, and the exit channel has a diameter of 10 Å.
Early experiments to test selectivity used nonyltriethylammonium (NTA, pictured below) to block the channel. Given the crystal structure, what was the reasoning for using NTA, and why does it block the channel?
(b) Early experiments to test selectivity used nonyltriethylammonium (NTA, pictured below) to block the channel. Given the crystal structure, what was the reasoning for using NTA, and why does it block the channel?

NTA is used because the charged group is about the size of a hydrated K+ ion. The K+ ions must desolvate to pass through the selectivity filter. The ethyl arms of NTA prevent if from passing through.
(c) The K+ channel is so selective, it can differentiate K+ (1.33 Å radius) and Na+ (0.95 Å). How does the channel achieve this?
(c) The K+ channel is so selective, it can differentiate K+ (1.33 Å radius) and Na+ (0.95 Å). How does the channel achieve this?

The K+ ion binds perfectly with the carbonyl groups in the selectivity channel to dehydrate the ion.

The Na+ ion is too small, so it does not bind properly and has a much higher energy bound to the channel than when free in solvent.

The K+ enters the channel because of the low energy barrier, but the Na+ ion cannot.
Below is a graph of an amino acid's placement in alpha helices, averaged over many helices. Dotted lines indicate the start and ends of a helix, and a value of 1.0 indicates average abundance. Propose the identity of the amino acid and explain your choice.
Below is a graph of an amino acid's placement in alpha helices, averaged over many helices. Dotted lines indicate the start and ends of a helix, and a value of 1.0 indicates average abundance. Propose the identity of the amino acid and explain your choice.

Proline: extra bond between side chain and amine N limits conformational flexibility and disrupts hydrogen bonding.

Glycine: H side chain increases conformational flexibility and destabilizes helix structure.

Asparagine, etc: side chain hydrogen bond donors/acceptors compete with backbone for hydrogen bonds in the helix.

Isoleucine, etc: beta branched amino acids have steric clashes in helices.

Figure from: Richardson & Richardson (1988) Science 240: 1648-1652.
(T/F) For the following questions, indicate whether the statement is True (T) or False (F). If false, please explain and correct the erroneous statement.

a) Restriction endonucleases use disulfide linkages in order to create a closed loop around the substrate that is to be cleaved.

b) Bacterial Restriction Endonucleases cleave double-stranded DNA by attack of water on the phosphate linkage and release the 5' hydroxyl group.
(T/F) For the following questions, indicate whether the statement is True (T) or False (F). If false, please explain and correct the erroneous statement.

a) Restriction endonucleases use disulfide linkages in order to create a closed loop around the substrate that is to be cleaved.

False. These enzymes need to cleave dsDNA regardless of how long the DNA is. If it were a closed loop, the DNA would have to thread through. Additionally some don’t use disulfide linkages (eg. EcoRV)

b) Bacterial Restriction Endonucleases cleave double-stranded DNA by attack of water on the phosphate linkage and release the 5’ hydroxyl group.

False. The attack by water on the dsDNA phosphate linkage releases the 3’ hydroxyl group. The 5’ retains the phosphate.