As a community committed to the Augustinian ideals of truth, unity and love, Villanova University prides itself on maintaining the highest standards of academic integrity and does not tolerate any forms of academic dishonesty or misconduct. Accordingly, each student who takes an examination is expected to sign the following statement:

I, (sign your name)

have not had any unsanctioned prior access to this examination and will conduct myself in an honest manner in regard to all aspects of this examination. Unless authorized, I will not discuss the contents of this examination with other students.

You may use your calculator for the exam, but no other materials. The exam will end promptly at 5:50 PM.

Equations:

\[ r = \frac{0.61 \lambda}{(n \sin \alpha)} \]
\[ \lambda = \frac{h}{E} \]
\[ F = \frac{k(q_1q_2)}{r^2} \]
\[ F = \frac{k(q_1q_2)}{\varepsilon r^2} \]
\[ K_a = \frac{[H^+][A^-]}{[HA]} \]
\[ pK_a = pK_a + \log ([A^-]/[HA]) \]
\[ \Delta G = \Delta G^{\circ} + RT \ln ([B]/[A]) \] (assuming stoichiometry of 1A --> 1B)
\[ \Delta G^{\circ} = -RT \ln K_{eq} \]

Constants:

\[ R = 8.31415 \text{ J mol}^{-1} \text{ K}^{-1} \]
\[ k = 1.38044 \times 10^{-23} \text{ J K}^{-1} \] (Boltzmann’s constant)
\[ k = 8.99 \times 10^9 \text{ Nm}^2/\text{C}^2 \] (Coulomb’s constant)
\[ N \text{ (Avagadro’s number)} = 6.022 \times 10^{23} \text{ mole}^{-1} \]
\[ h = 6.626 \times 10^{-34} \text{ J sec} \]
\[ \text{deg Kelvin} = \text{deg Centigrade} + 273.15 \]
1. (15 pts.) What is the pH of a mixture of 0.2 M glucuronic acid and 0.05 M sodium glucuronate in an aqueous solution? The pKa of glucuronic acid is 3.82.

\[
pH = pK_a + \log \frac{[B]}{[A]}
\]

\[
pH = 3.82 + \log \frac{0.05 \text{ M}}{0.2 \text{ M}}
\]

\[
pH = 3.22
\]
2. (15 pts.) When applied to the center of a paper electrophoresis apparatus, will the following molecules migrate towards the anode (+), cathode (-), or remain stationary when separated by paper electrophoresis at pH 7.0?

<table>
<thead>
<tr>
<th>Molecule</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronic acid (see problem 1 for structure)</td>
<td>3.82</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>9.80</td>
</tr>
<tr>
<td>Serine</td>
<td>2.2, 9.2</td>
</tr>
</tbody>
</table>

When the pH of the solution is above the pK for the ionizable group, it will be deprotonated. Glucuronic acid will be deprotonated and hence will have a -1 charge at pH 7. It will migrate towards the anode.

At pH 7.0, the solution pH is below the pK for ethanolamine (you should recognize that the amine is the only ionizable group here). Thus, the amine will be protonated and have a +1 charge, and the ethanolamine will migrate towards the cathode.

There are two ionizable groups on serine – the amine and the carboxylic acid. The pH is above the pK for the carboxylic acid (it will be deprotonated and have a -1 charge) and below the pK for the amine (it will be protonated and have a +1 charge). Serine will be neutral and not migrate.

Also acceptable for serine – the pI is the average of the two pKs, or 5.7. Since 7 is above the pI, the molecule will have a net (albeit very slight) negative charge, and will move towards the anode.
3. (15 pts.) In glycolysis, dihydroxyacetone phosphate (DHAP) is isomerized into glyceraldehyde-3-phosphate (G3Pi). The $\Delta G^\circ$ (37 degrees) for this reaction is +7.5 kJ/mole. Calculate the equilibrium constant for this reaction, and the percentage of DHAP and G3Pi at equilibrium.

\[
\begin{align*}
\text{DHAP} & \quad \rightleftharpoons \quad \text{G3Pi} \\
\text{CH}_2\text{OH} & \quad \text{HO} \\
\text{CH}_2\text{OPO}_3^{-2} & \quad \text{OH} \\
\text{CH}_2\text{OPO}_3^{-2} &
\end{align*}
\]

\[\Delta G^\circ = -RT \ln K\]

\[+7.5 \text{ kJ/mole} = -(8.31415 \text{ J/mol deg})(310 \text{ deg}) \ln K\]

\[K = 0.054\]

To get percentage, the equilibrium constant \(K = \frac{\text{[Product]}}{\text{[Reactant]}}\). The solution indicates that there is 1.0 DHAP for every 0.054 molecules of G3Pi. To get percentage of G3Pi

\[\% = \frac{0.054}{1.00 + 0.054} = 5.1\%\]

Percentage of DHAP = 100\% - 5.1\% = 94.9\%
4. (15 pts.) The intracellular potassium ion concentration is 100 mM while the extracellular potassium ion concentration is 10 mM. Calculate the energy required to transport $10^{-6}$ moles of potassium out of the cell against the potassium concentration gradient. Assume that the cells are at 37°C.

As mentioned in class, since you are moving the same ion from one compartment to another, equilibrium will be reached when the intracellular and extracellular concentration of potassium ions are equal. When this occurs, the $\Delta G^\circ$ (37 degrees) for this process is calculated to be 0. The free energy change for the situation when the two concentrations are different can be described mathematically as:

$$\Delta G = \Delta G^\circ + RT \ln \left\{ \frac{100 \text{ mM}}{10 \text{ mM}} \right\}$$

$$= 0 + (8.31415 \text{ J/mol deg})(310 \text{ deg}) \ln (10)$$

$$\Delta G = 5935 \text{ J/mole}$$

You need to determine the amount of energy (in Joules) required to move $10^{-6}$ moles of potassium against that gradient, so multiply the $\Delta G$ by $10^{-6}$ moles to get

$$5.93 \times 10^{-3} \text{ Joules or } 5.93 \times 10^{-6} \text{ kiloJoules}$$

NOTE – I made an error in writing the problem – I should have said “into” the cell rather than “out of” the cell as shown above. This would affect only the sign on the answer, and not the numbers. As a result, I did not deduct points if the sign on the answer was incorrect.
5. (15 pts.) Methylation of guanine at the C6 keto group yields O\textsuperscript{6}-methylguanine (see picture below). O\textsuperscript{6}-methylguanine is mutagenic in that it can form improper base pairs with thymine. Draw the structure of the O\textsuperscript{6}-methylguanine base paired to a thymine base.

I will accept any thymine orientation where a ketone oxygen is hydrogen bonded to the NH\textsubscript{2} and where the amine proton is hydrogen bonded to the 6-O-methyl G ring nitrogen.
6. (15 pts.) Given the piece of DNA shown below, give the sequence of its complementary DNA strand and the sequence of the mRNA strand synthesized using the original strand as a template:

5’-GACCGTTTCGCACTCCGTATCT-3’

Complementary DNA strand:

3’-CTGGCAAGCGTGAGGCATAGA-5’

OR

5’-AGATACGGAGTGCAACGGTC-3’

The mRNA strand is the same as the complementary DNA strand, with U replacing T:

5’-AGAUACGGAGUGCAACGGUC-3’
7. (10 pts.) The gene for the protein lactate dehydrogenase was isolated from both humans and carp. The length of the double stranded DNA from both species was 1.3 kilobases. DNA melting analysis indicated that the human DNA had a Tm of 73°C while the carp DNA had a Tm of 83°C. (a) Which DNA has a higher GC content? (b) Checking the DNA solutions after the experiment, the human DNA was found to be suspended in 0.5 M NaCl while the carp DNA was suspended in 0.2 M NaCl. Does this change your opinion about the relative GC content in the two DNAs? Explain.

(a) The higher the melting point, the higher the GC content in the DNA (all other factors the same). Therefore, the carp DNA had a higher GC content than the human DNA.

(b) At higher ionic strengths, the structure of ds DNA is stabilized due to removing some of the charge repulsion at the surface of the DNA. As a result, DNA in high ionic strength will have a higher melting temperature than DNA at a lower ionic strength. Since the human DNA was measured at a higher ionic strength than the carp DNA, the human DNA sample would have a higher than expected melting temperature. Since its melting temperature is still lower than the carp, we can still conclude that the carp DNA had a higher GC content.