Question 1. (10 points)

The rate of uptake of glucose into rat adipocytes is increased by addition of insulin to the medium. The increase reaches a maximum within 30 seconds at 37°C and is energy dependent. The transport of glucose into the cell, however, takes place by facilitated diffusion. The rates of glucose entry as a function of the extracellular glucose concentration in the absence and the presence of insulin are shown in the figure below.

4 pts. A) What is the effect of insulin on the $K_m$ and $V_{max}$ of the transport process?

6 pts. B) State two mechanisms to explain the effect shown in the figure.
Question 2. (24 points)

A muscle biopsy was performed on a person who is incapable of doing prolonged physical work. His mitochondria were isolated and found to be atypical in structure. The respiratory functions of these mitochondria were determined. They are able to carry out electron transport to O$_2$; however, during active O$_2$ consumption, there is no membrane potential across the inner mitochondrial membrane. On the other hand, the matrix pH is 9 when the external pH is 7.

$$RT = 0.6 \text{ kcal/mol}$$
$$F = 23 \text{ kcal/mol volt}$$

6 pts. A) Calculate the protomotive force in these mitochondria.

6 pts. B) $\Delta G$ for the synthesis of ATP in these mitochondria is + 13.5 kcal/mol. What is the minimum number of protons that must be translocated to drive the synthesis of one molecule of ATP?
6 pts. C) In normal mitochondria, the membrane potential across the inner membrane is +0.2 volt, while the ΔpH is 2 during respiration. What is the minimum number of protons required for the synthesis of one molecule of ATP in this case?

6 pts. D) What might cause the atypical mitochondria to retain the ability to form a pH gradient but be unable to develop a membrane potential? Be brief.
**Question 3.** (18 points)

The elongation rate constants for actin filaments are:

<table>
<thead>
<tr>
<th></th>
<th>$k_{on}$(ATP)</th>
<th>$k_{off}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M$^{-1}$sec$^{-1}$)</td>
<td>(sec$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>barbed end (+)</td>
<td>12 x $10^6$</td>
<td>2</td>
</tr>
<tr>
<td>pointed end (-)</td>
<td>1.2 x $10^6$</td>
<td>0.6</td>
</tr>
</tbody>
</table>

An actin filament 0.1 µm in length is placed in a solution of 75 mM KCl, 5 mM Mg$^{2+}$ and 2 mM ATP; actin monomer with bound ATP is added at a concentration of 0.197 µM. The size of the filament is measured as a function of time.

6 pts. A) Determine the value of the critical concentration for the barbed end and for the pointed end.

6 pts. B) What happens to the size of the filament and at the two ends of the filament under these conditions?

6 pts. C) A bacterium binds to the barbed end of the filament in a way that does not prevent binding of actin monomers. Indicate whether or not the bacterium moves; if it does, in which direction and at what speed. Remember that the actin monomer can be represented by a sphere with a diameter of 5 nm.
**Question 4.** (15 points)

Aldehyde dehydrogenase catalyzes the reaction:

$$\begin{align*}
\text{R--CH} & \quad + \quad \text{NAD}^+ & \quad + \quad \text{H}_2\text{O} \\
\rightarrow & \quad \text{R--C}=\text{O}^- & \quad + \quad \text{NADH} & \quad + \quad 2\text{H}^+
\end{align*}$$

Using fast measurements techniques, the following rate profile was obtained for the reaction of 1 pmoles of enzyme with propionaldehyde and NAD$^+$:

Write the **simplest** kinetic scheme (sequence of reactions) to explain the results. Assume that NAD$^+$ binds to the enzyme before the aldehyde, and that either NADH or the carboxylic acid is released first from the enzyme.
Question 5. (20 points)

Influenza hemagglutinin (HAO) is a membrane protein with a single transmembrane domain at the COOH-terminal end. The effect of a reducing environment on the folding of HAO in the endoplasmic reticulum was investigated in these studies. CHO 15B cells, expressing HAO, were pulse-labeled for 2 min with $^{35}$S-methionine. Dithiothreitol (DTT), a reducing agent, was added at 5 mM during the chase with non-radioactive methionine. Then, DTT was washed out of the cells for 2 and 30 min. In one plate, glucose was present so that there was an adequate amount of ATP; in another plate, inhibitors of glycolysis and oxidative phosphorylation were present to deplete the cells of ATP. At various times, the cells were lysed and the extracts were examined by non-reducing SDS-PAGE to determine the mobility of HAO. The results are shown in the figure.

Indicate the structures of the bands in the various lanes. (If you need to write on the back of the page, please indicate)
Question 6. (24 points)

The following studies were done to study the cell cycle of Hela cells. The growth rate of an asynchronously growing culture is shown in Figure 5. Examination of a dish of fixed cells revealed that 10% of the cells are in mitosis. 

$^3$H-Thymidine was added to the medium at time 0; after the given times, the cells were fixed and prepared for autoradiography. Figure 6 shows the fraction of labeled mitotic cells as a function of time after addition of the radioactive thymidine. In Figure 7, the number of silver grains per mitotic cell is plotted as a function of time after addition of $^3$H-thymidine.

Determine the doubling time and indicate the duration of the phases of the cell cycle in Hela cells.
Question 7. (18 points)

AZT (azidodideoxythymidine), a drug used in the treatment of AIDS, acts by inhibiting the viral reverse transcriptase, which is critical for the life cycle of HIV. AZT must be triply phosphorylated to form azTTP (azidodideoxythymidine triphosphate) in order to mimic dTTP (deoxythymidine triphosphate), a substrate for DNA synthesis. When reverse transcriptase mistakes azTPP for dTTP, viral DNA synthesis terminates.

azTTP cannot be given to patients because it can not get into cells. On the other hand, AZT does enter cells and is phosphorylated by the cellular thymidine phosphorylation pathway. The structures of AZT and azTTP are:

5 pts. A) Why can AZT get into a cell while azTTP can not? (Not more than 15 words).

8 pts. B) The values of Km for dTTP with reverse transcriptase were determined at various concentrations of azTTP.

<table>
<thead>
<tr>
<th>azTTP (µM)</th>
<th>0</th>
<th>0.01</th>
<th>0.03</th>
<th>0.04</th>
<th>0.06</th>
<th>0.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km(apparent) (µM)</td>
<td>2.8</td>
<td>3.5</td>
<td>4.9</td>
<td>5.6</td>
<td>7.0</td>
<td>9.1</td>
</tr>
</tbody>
</table>

What is the value of $K_i$ (include units) for azTTP?

5 pts. C) The $K_i$ of azTTP for inhibition of cellular DNA polymerase is ~ 230 µM. Explain why azTTP can be used to inhibit HIV reverse transcriptase without affecting cellular (nonviral) DNA replication?
**Question 8.** (15 points)

A mutant Gαs subunit of the heterotrimeric G protein was found to have decreased GTPase activity although it bound nucleotides. It was noticed that cells expressing the mutant Gαs had increased protein kinase A (PKA) activity compared to cells with wild type Gαs.

5 pts. A) What is the explanation for the increased PKA activity?

10 pts. B) The mutant Gαs was expressed in liver cells. Describe the effect of the mutant on glycogen metabolism (breakdown and synthesis). Be sure to identify the critical enzymes involved in the process.
**Question 9.** (16 points)

Cyclin B is a 48 kD protein involved in the cell cycle. Its levels are known to rise and fall as cells proceed through their development cycle. Xenopus egg extracts are used as an in vitro model for cell cycle studies. Such extracts are effectively “frozen” in late meiosis until \( \text{Ca}^{2+} \) is added, after which cyclin levels drop precipitously and the extract proceeds into interphase.

Radiolabelled cyclin was added to Xenopus egg extract. \( \text{Ca}^{2+} \) was then added and samples were taken at 0, 5, and 10 minutes after the calcium addition. The experiment was also run in the presence of lactacystin. The resultant samples were separated by SDS-PAGE and visualized by autoradiography, giving the following results:

3 pts. A) What is the band in lanes 1 and 4?

3 pts. B) The ladder in lanes 2, 5 and 6 appear to occur at regular increments of \(~7\text{kD}\). What is the source of this pattern?
5 pts. C) Propose a mechanism responsible for the decrease in cyclin levels observed in Xenopus extracts upon Ca^{++} addition.

5 pts. D) What features might you find on cyclin B that is important for this mechanism? How would you test this?
Question 10. (16 points)

6 pts. A) The velocity of an enzymatic reaction was measured as a function of substrate concentration at three concentrations of enzyme: 0.5 nM, 5 nM and 25 nM. On one set of axes, draw the Lineweaver-Burk plots of the results with the enzyme concentrations.

10 pts. B) Aspartate transcarbamoylase (ATCase) catalyzes the reaction:

![Chemical reaction diagram]

The initial velocity of ATCase as a function of aspartate concentration is shown in Figure A. Succinate binds to the same site on ATCase as aspartate. Using the aspartate concentration corresponding to C in Figure A, the initial velocity of the reaction is measured as a function of succinate concentration; the results are shown in Figure B.
Which of the following statements are true and which are false?

_____ a) Succinate acts as a competitive inhibitor at low concentrations and as a noncompetitive inhibitor at high concentrations.

_____ b) There are cooperative interactions between the binding site for aspartate.

_____ c) Succinate binds preferentially to the T state of ATCase , which has low affinity for aspartate.

_____ d) Succinate binds preferentially to the R state of ATCase, which has a high affinity for aspartate.

_____ e) Succinate is an allosteric inhibitor of ATCase.
Question 11. (24 points)

Refer to the colored photocopy for figures.

The most prominent divergent region in the 130 kDa motor domain (S1 fragment) of myosin occurs at the 50K/20K junction, a region of the molecule involved in actin binding. In the Figure, the junctional region is at the 50kDa cleft.

Myosin chimeras have been constructed by substituting a 9-amino-acid junctional region of Dictyostelium myosin with those from myosins of other species. The purified wild-type and chimeric myosins were assayed for ATPase activity under three conditions, all at saturating ATP concentration. The motile activity of the myosins was measured in a sliding filament in vitro motility assay (last column). In this assay, the myosin was applied to a glass slide so as to coat its surface, then a solution of fluorescent F-actin was introduced and movement initiated by the addition of ATP. In this system multiple myosin molecules interact with an actin filament. Movement at 30°C was recorded and analyzed. The results are shown in the Table.

<table>
<thead>
<tr>
<th></th>
<th>High salt Ca(^{2+})-ATPase (Pi/head/sec)</th>
<th>Mg(^{2+})-ATPase (Pi/head/sec)</th>
<th>Actin-activated Mg(^{2+})-ATPase (Pi/head/sec)</th>
<th>Sliding velocity (µm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type myosin</td>
<td>2.9</td>
<td>0.07</td>
<td>0.78</td>
<td>3.1</td>
</tr>
<tr>
<td>(Dictyostelium)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chimera with</td>
<td>4.4</td>
<td>0.12</td>
<td>4.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Rabbit skeletal myosin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chimera with</td>
<td>4.8</td>
<td>0.11</td>
<td>0.44</td>
<td>2.2</td>
</tr>
<tr>
<td>chicken smooth muscle</td>
<td></td>
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</tbody>
</table>

It is evident that the \( V_{\text{max}} \) values for the actin-activated ATPase activities of the chimeras differ by a factor of 9; however, the velocities measured in the in vitro motility assays are within a factor of 2.

The actin-activated myosin ATPase cycle is shown in the Figure.
8 pts. A) Why is there a difference between the values of the Mg\(^{2+}\)-ATPase and the actin-activated Mg\(^{2+}\)-ATPase activities for each myosin? What is the effect of actin on the mechanism of the reaction?

8 pts. B) What is the explanation for the Ca\(^{2+}\)-ATPase activities compared to those for the Mg\(^{2+}\)-ATPase activities of the various myosins.

8 pts. C) How is it possible for the actin-activated ATPase activities not to be correlated with the sliding velocities? Explain in terms of the cycle shown in the figure.