Answers to Problem Set 4

1. 10-5
A. The difference in rate of loss of the ESR signals is due to the location of the nitroxide radical in the two phospholipids. The nitroxide radical in phospholipid 1 is on the head group and therefore is in direct contact with the external medium. Thus, it can react quickly with ascorbate. However, the nitroxide radical in phospholipid 2 is attached to a fatty acid chain and therefore is partially buried in the interior of the membrane. As a consequence, it is less accessible to ascorbate and is reduced more slowly.

B. The key observation is that the extent of loss of ESR signal in the presence and absence of ascorbate is the same in experiments with resealed red cell ghosts but different in experiments with intact red cells. These results suggest that there is an undefined reducing agent in the cytoplasm of red cells that can reduce the more exposed phospholipid 1 but cannot reduce the less exposed phospholipid 2. Thus, in intact red cells phospholipid 2 is stable in the absence of ascorbate; in the presence of ascorbate the spin-labeled phospholipids in the outer monolayer are reduced, causing loss of half the ESR signal. Phospholipid 1, on the other hand, is not stable in intact red cells in the absence of ascorbate because the phospholipids in the inner monolayer are accessible to the cytoplasmic reducing agent, which destroys half the ESR signal. When ascorbate is added, labeled phospholipids in the outer monolayer are also reduced, causing loss of the remaining ESR signal.

C. The results in Figure 10-2 indicate that the labeled phospholipids were introduced equally into the two monolayers of the red cell plasma membrane. Phospholipid 2 was 50% sensitive to ascorbate, indicating that half the label was present in the outer monolayer, and 50% insensitive to ascorbate, indicating that half was present in the inner monolayer. Phospholipid 1 was 50% sensitive to the cytoplasmic reducing agent, indicating that half the label was in the inner monolayer, and 50% sensitive to ascorbate, indicating that half the label was in the outer monolayer.

2. 10-12
A. spectrin = $3 \times 10^5$ molecules/cell
   band 3 = $9 \times 10^5$ molecules/cell
   glycophorin = $2.3 \times 10^5$ molecules/cell

The calculation for the number of spectrin molecules per red blood cell is shown in detail below. In essence, one first calculates the fraction of total protein that is spectrin and then converts that
number into the number of spectrin molecules using the molecular weight of spectrin and Avogadro’s number.

\[
\text{spectrin} \times \frac{5 \text{ mg protein}}{1 \text{ cell}} \times \frac{0.25 \text{ spectrin}}{1 \text{ mmol spectrin}} \times \frac{250,000 \text{ mg}}{1 \text{ mmol spectrin}} \times 6 \times 10^{23} \text{ molecules} = 3 \times 10^5 \text{ molecules}
\]

The calculated number of glycophorin molecules per cell is too low by a factor of 2.5 because about 60% of the molecular weight of glycophorin is carbohydrate, which is not stained by Coomassie blue.

B. The fraction of the plasma membrane that is occupied by band 3 is the area of the face of a single band 3 molecule (\(\pi r^2\)) times the total number of band 3 molecules per cell \((9 \times 10^5)\) divided by the total area of the red blood cell \((10^8 \text{ nm}^2)\). Note that the height of the molecule is irrelevant to the calculation.

\[
\frac{\text{band 3}}{\text{plasma membrane}} = \frac{3.14 \times (3 \text{ nm})^2}{\text{molecule}} \times \frac{9 \times 10^5 \text{ molecules}}{\text{cell}} \times \frac{1 \text{ cell}}{10^8 \text{ nm}^2} = 0.25
\]

Thus band 3 occupies about 25% of the surface area of a red blood cell. This somewhat surprising result is consistent with freeze-fracture electron micrographs of red blood cells, which show a high density of intramembranous particles that are thought to be dimers of band 3 (see MBOC Figure 10-29).

3. 11-7
A. These data indicate that insulin stimulation of glucose uptake results from a redistribution of preexisting glucose transporters from an internal pool to the plasma membrane. The fivefold increase in rate of glucose uptake in insulin-treated cells is accompanied by a fivefold increase in the number of glucose transporters in the plasma membrane. Furthermore, the increase in glucose transporters in the plasma membrane is accompanied by a corresponding decrease in the number of glucose transporters present in the internal membrane fraction.

B. \(K_m\) and \(V_{max}\) do not change. The \(K_m\) for glucose transport in the untreated cells and in the insulin-treated cells is about 2 mM, which is the concentration of glucose under both conditions at which the rate of transport is half-maximal (Figure 11-2). The fivefold increase in the rate of
glucose transport can be fully accounted for by the fivefold increase in the number of transporters in the plasma membrane. It may seem confusing that this fivefold increase in rate of transport does not mean that the Vmax has increased; however, Vmax refers to the maximum rate for a specified quantity of enzyme. When the observed experimental rates are adjusted for the fivefold difference in number of transporters, the maximum rates of transport are identical; therefore, Vmax has not changed.

4. Glucose is transported by a specific transporter, the glucose transporter, which becomes saturated at high concentrations of glucose. Ethylene glycol appears to diffuse across the membrane; it is not transported by a saturable carrier.

5. A. Na$^+$ moves from the extracellular solution into the cytoplasm, down its concentration gradient.

\[
\Delta G = \Sigma v_i \mu_i \quad \mu = \mu^* + RT \ln(C) + zF \psi \\
\Delta G = RT \ln \left(\frac{[Na^+_{in}]}{[Na^+_{out}]}\right) + zF \left(\psi_i - \psi_o\right) \\
= (600 \text{ cal/mol}) \ln \left(\frac{50}{460}\right) + (1) (23 \text{ kcal V}^{-1} \text{ mol}^{-1}) (-0.06 - 0)V \\
= -1.33 \text{ kcal/mol} + (-1.38 \text{ kcal/mol}) \\
= -2.71 \text{ kcal/mol} \\
\Delta G < 0, \text{ so Na}^+ \text{ moves into the cytoplasm as indicated.}
\]

B. K$^+$ moves from the cytoplasm into the extracellular solution, down its concentration gradient.

\[
\Delta G = RT \ln \left(\frac{[K^+_{out}]}{[K^+_{in}]}\right) + zF \left(\psi_o - \psi_i\right) \\
= (600 \text{ cal/mol}) \ln \left(\frac{10}{400}\right) + (1) (23 \text{ kcal V}^{-1} \text{ mol}^{-1}) (0 - (-0.06))V \\
= -2.21 \text{ kcal/mol} + 1.38 \text{ kcal/mol} \\
= -0.83 \text{ kcal/mol} \\
\Delta G < 0, \text{ so K}^+ \text{ moves out of the cytoplasm as indicated.}
C. Cl\textsuperscript{-} is at electrochemical equilibrium and there is no net movement of the ion.

\[
\Delta G = RT \ln \left( \frac{[\text{Cl}^{-}\text{in}]}{[\text{Cl}^{-}\text{out}]} \right) + zF (\psi_i - \psi_o) \\
= (600 \text{ cal/mol}) \ln \left( \frac{54}{540} \right) + (-1) (23 \text{ kcal V}^{-1} \text{ mol}^{-1}) (-0.06 - 0) \text{V} \\
= -1.38 \text{ kcal/mol} + 1.38 \text{ kcal/mol} \\
= 0 \text{ kcal/mol}
\]

\(\Delta G = 0\), so system is at Cl\textsuperscript{-} equilibrium.

D. The existence of a membrane potential ordinarily indicates that the membrane is selectively permeable to ions. If the membrane were only permeable to K\textsuperscript{+}, movement of K\textsuperscript{+} out of the cell would create a membrane potential negative inside (-96 mV). Movement only of Na\textsuperscript{+} into the cell through a Na\textsuperscript{+} channel would create a membrane potential positive inside (+58 mV). And movement of Cl\textsuperscript{-} alone down its concentration gradient would create a membrane potential negative inside (-60 mV). Since the actual membrane potential is negative inside (-60 mV), one can deduce that the permeabilities of the membrane for K\textsuperscript{+} and for Cl\textsuperscript{-} make the principal contributions to the membrane potential. In fact, the permeabilities of K\textsuperscript{+}, Na\textsuperscript{+}, and Cl\textsuperscript{-} are in the ratio of 1: 0.05: 0.4.

E. \(\Delta G'_{\text{ATP}} = \Delta G'^{\circ} + RT \ln ([\text{ADP}][\text{Pi}]/[\text{ATP}]) \)

\[
= -7.5 \text{ kcal/mol} + (600 \text{ cal/mol}) \ln((0.5 \times 0.1)/7) \\
= -7.5 \text{ kcal/mol} + (-7.1 \text{ kcal/mol}) \\
= -14.6 \text{ kcal/mol}
\]

\[
\Delta G'_{\text{Na}} = 3 \times 2.71 \text{ kcal/mol} = 8.13 \text{ kcal/3 mol} \quad (\text{from part A}) \\
\Delta G'_{\text{K}} = 2 \times 0.83 \text{ kcal/mol} = 1.66 \text{ kcal/2 mol} \quad (\text{from part B})
\]

\[
\Delta G'_{\text{Na}} + \Delta G'_{\text{K}} = 8.13 + 1.66 = 9.79 \text{ kcal/5 mol}
\]

Efficiency: \(\frac{\Delta G'_{\text{Na}} + \Delta G'_{\text{K}}}{\Delta G'_{\text{ATP}}} = \frac{9.79}{14.6} = 0.67\)