Questions from Ed Egelman (36 pts. total)

Each question is worth 3 points. Choose the single best answer.

1) The resolution in a conventional light microscope is mainly set by:
   a) phototoxicity
   b) the wavelength of light
   c) defects in lenses
   d) dehydration
   e) all of the above

2) To do structural biology, we want to choose radiation that minimizes:
   a) ratio of inelastic to elastic scattering
   b) ratio of elastic to inelastic scattering
   c) Rayleigh scattering
   d) Compton scattering
   e) elastic scattering

3) In Cryo-EM of unstained specimens, macromolecules are embedded in:
   a) water
   b) crystalline ice
   c) vitreous or amorphous ice
   d) uranyl acetate
   e) silicone

4) The first 3D reconstruction of a biological assembly by EM involved:
   a) an icosahedral virus
   b) an asymmetric ribosome
   c) a helical polymer
   d) a 2D crystal
   e) a 3D crystal

5) The simplest icosahedral virus will have how many copies of the capsid protein:
   a) 3
   b) 20
   c) 40
   d) 60
   e) 180
6) The dihedral point group symmetry D7 will generate an assembly having how many identical subunits:
   a) 1  
b) 7  
c) 14  
d) 21  
e) 28

7) When bound water is excluded from an interface:
   a) the entropy of the water decreases, making this unfavorable  
b) the entropy of the water decreases, making this favorable  
c) the entropy of the water increases, making this unfavorable  
d) the entropy of the water increases, making this favorable  
e) there is no change in the entropy of the water

8) The strength of a protein-protein interface is largely proportional to:
   a) the volume of each protein  
b) the radius of each protein  
c) the exposed surface area of each protein  
d) the buried surface area  
e) all of the above

9) The linear increase in length of existing flagellar filaments with number of added monomers implied:
   a) that nucleation is very favorable  
b) that nucleation is suppressed  
c) that the nucleus is a dimer  
d) that the nucleus is a trimer  
e) none of the above

10) In the absence of an energy source:
    a) the rates of addition of monomers must be the same at both ends of a polymer  
b) the rates of removal of protomers must be the same at both ends of a polymer  
c) both (a) and (b)  
d) the ratio of rates of addition/removal must be the same at both ends  
e) all of the above

11) Treadmilling
    a) can occur at equilibrium if there is an energy source  
b) cannot occur at equilibrium if there is an energy source  
c) can occur at steady-state if there is an energy source  
d) can occur at steady state in the absence of an energy source  
e) both (a) and (c)
12) The unusual sequence conservation of actin may be due to:
   a) the large number of actin-binding proteins
   b) the unusual structure of the actin monomer
   c) the helical polymer formed by actin
   d) allosteric relations within the actin subunit
   e) conservation of an ATPase activity
Questions from Bill Pearson (20 pts. total)

1) Why is percent identity not as useful as the expectation (E()-value) when considering whether two proteins are likely to be homologous? (5 pts)

2) When might an alignment have relatively high-identity but a poor E()-value? (2 pts)

3) When might an alignment have low identity (< 20%) but still have a statistically significant E()-value indicating homology? (3 pts)
4a) Draw a schematic diagram of a 600 residue protein that contains 3 homologous copies of a 100 residue domain in the N-terminal half, and 4 homologous copies of a 50 residue domain in the C-terminal third of the protein. (3 pts)

4b) Plot the local alignment graph of the protein compared with itself below: (7 pts)
Question 1

(1A) A 'typical' biological lipid is dioleoylphosphatidylcholine DOPC (PC headgroup with two 16-carbon single C9-C10 unsaturated acyl chains). Removal of one of the acyl chains yields the corresponding lyso (1-chain) PC. What structures do each of these lipids form when dispersed in aqueous solution, and why?

(1B) You now measure the permeability of DOPC to six ions, described below (note that these ionic radii are approximate values):

<table>
<thead>
<tr>
<th>ion</th>
<th>radius (Å)</th>
<th>charge state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>0.6</td>
<td>+1</td>
</tr>
<tr>
<td>Mg</td>
<td>0.6</td>
<td>+2</td>
</tr>
<tr>
<td>Ag</td>
<td>1.2</td>
<td>+1</td>
</tr>
<tr>
<td>Sr</td>
<td>1.2</td>
<td>+2</td>
</tr>
<tr>
<td>Cs</td>
<td>1.8</td>
<td>+1</td>
</tr>
<tr>
<td>Ba</td>
<td>1.8</td>
<td>+2</td>
</tr>
</tbody>
</table>

Rank these six ions from MOST permeable to LEAST permeable, and explain/justify your reasoning

(1C) Because of the presence of double-bonds in the acyl chains of unsaturated lipids, bilayers formed from highly unsaturated lipids are slightly more polar than those comprised of saturated lipids. Therefore, compared to a saturated-lipid bilayer, does it take more or less energy to partition an ion into the interior of a polyunsaturated lipid bilayer? Please justify your answer.
Question 2

(2A) What secondary structure, if any, is typically found in the membrane-embedded portions of integral membrane proteins? Please justify/explain your answer.

(2B) Please describe (briefly) an integral membrane protein that we did not discuss in class.

(2C) What is this? Explain (briefly) how it is obtained. State and explain any structural features that you observe. Note that the black rectangle (unit cell) is 71Å x 81Å in dimension (width by height).
Question 3

Refer to the diagram below. The protein crystal is located at position A. X-rays of wavelength $\lambda$ are incident upon the crystal. X-rays are diffracted at an angle of $2\theta$.

$\lambda = 1.54\text{Å}$

AO = 50 cm

BO = 20 cm

Reminders: $(AB)^2 = (AO)^2 + (BO)^2$

$\tan(2\theta) = \frac{BO}{AO}$

What is the resolution $d$ corresponding to the diffracted beam AB?

[Assume $n=1$ in the Bragg Equation]
Question 4

You have solved two crystal structures of a G-protein coupled receptor (GPCR), one in the absence and one in the presence of a potent agonist (ligand that binds with high affinity). In the absence of agonist, the atoms in the residues lining the binding pocket have an average B factor of $80\text{Å}^2$. In the presence of agonist, the average B factor of these atoms drops to $30\text{Å}^2$.

(4A) What is your interpretation of this result?

(4B) What is the difference in root-mean-square amplitude of movement between these two sets of atoms in the two structures?