More Problems

1. In the text, you should consider the problems at the end of chapter 13. There's lots of good ones, but minimally, you should be happy with your answers to...

- 13.1
- 13.3
- 13.5
- 13.17

13.18 is also worth giving some serious thought.

2. In the paper by Doyle et al on the structure of potassium channel, consider the following:

a) In the first paragraph of the last column on p. 69, the authors claim that "there are two closely related varieties of K+ channels, those containing two membrane-spanning segments per subunit and those containing six"

1) This conclusion was arrived at before there were any structures. Describe the analysis that gave rise to this conclusion.

Concluded from hydrophobicity analysis of channel protein sequences

2) Explain why this analysis apparently failed to predict the existence of the pore helix.

A helix that short is not uniquely identifiable as a membrane helix.

3) On p. 70, beginning at the bottom of the second column, the authors note that sequence conservation is strongest for the amino acids corresponding to the pore region, and, interestingly, the inner helix. Inspection of Figure 1 shows that in the inner helix, residues 93, 97, 99, and 110 are particularly conserved. Bring up this structure in Protein Explorer, and make those amino acids visible (for example by displaying the whole molecule as a backbone representation, and then selecting the individual amino acids - [Enter: **select 93** in the command window to select residue 93] - and displaying them as ball and stick structures so you can see them.

1) Are the side chains of these amino acids all located on the same side of the inner helix?

No

2) The location of these side chains suggests that these sequences are not conserved in order to preserve their interactions with potassium ions passing through the channel. Explain.

They don't face the channel, vestibule, or any other aqueous space.

3) The location of these side chains does suggest a reason for their conservation through evolution. What is that reason?

Subunit/Subunit interactions

c) In the first column on p. 74, the authors explain the hydrophobicity of the pore lining by suggesting that "it would be counterproductive to achieving a high throughput of K+ ions were the lining of the channel to interact strongly with ions outside of the selectivity filter".

1) Why would it be counterproductive?

It would slow the movement of ions through the channel.

2) This argument should apply to the selectivity filter as well. Explain what makes the selectivity filter different.

Several factors. One is that slowing the movement is the price paid for selectivity. The second is that the carbonyls of the selectivity filter are not energetically preferable to interactions with water, and thus will not form an energy well compared to either the vestibule or the extracellular solvent. In addition, providing a sequence of equivalent carbonyls means that there is not energetic incentive to stay in one place in the filter.

d) In the authors' discussion of Figure 8c (which actually appears in the second column on p. 75), the network of Val, Tyr, and Trp side chains is described as "like a layer of springs stretched radially outward to hold the pore open". This language makes it sound as if keeping the pore diameter from becoming smaller is at least as, if not more, important than keeping the pore diameter from becoming larger. Explain why keeping the pore from becoming smaller is so important.

To exclude sodium, the pore must be kept too large to encourage dehydration of the Na ion.

3) A bright-eyed molecular biologist had an idea for a project. Having noted that the GTPase of transducin molecule is so very slow, she thought she would improve the efficiency of the response of the visual system to light by engineering a new transducin with a much more active (faster) GTPase activity. Is this change likely to produce a better system for seeing in the dark?

No. Increasing the rate of GTP hydrolysis increases the rate of deactivation of the alpha subunit, and would result in a reduction in the activation of phosphodiesterase activity, and thus the persistence of the Na leak, with concomitant reduction in the hyperpolarization response to photon absorption.

4) Speaking of seeing in the dark, somebody, somewhere, noted that in rhodopsin, mutation of a glu residue to an ala close to the Schiff base linkage with retinal results in night blindness in humans. Why might this be so?

The glu residue will stabilize the protonated Schiff base which is the product of the reattachment of the cis-retinal to the rhodopsin. A reduction in the stability of that protein/chromophore adducut will result in a reduction in the number of rhodopsins which have reacquired the cis-retinal, and thus a reduction in the number of rhodopsins able to respond to a photon of light.