In-Class Problems

1. Our body can recognize an outside microbe or small foreign molecule and produce antibodies to attack the foreign substance. The antibody produced is such that it does not harm the molecules present naturally in our body such as glucose. What is the property of antibodies that can afford this?

2. In what ways can the specific binding interactions observed in biomolecules such as antibodies be useful for analytical/bioanalytical chemistry?

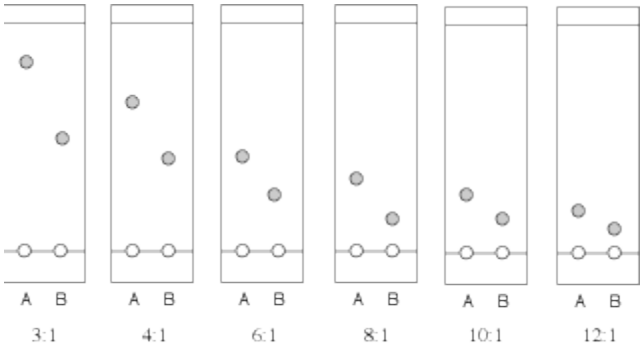
3. A biochemist in the food industry is asked to obtain an enzyme A that will be useful in keeping bread fresh. The biochemist has been able to obtain the gene for the enzyme A and produce it in a fermentation broth. However, the biochemist is faced with the difficulty of purifying this enzyme. One property of this enzyme that may be useful to the biochemist is that it has a binding affinity to maltose. What do you think would be an easy approach to purify the enzyme A?

4. What problems would you run into if there are other biomolecules present in the sample that bind to maltose but much more weakly than enzyme A? What could be done to ensure that these contaminants do not interfere with the purification of enzyme A?

5. What could be done if you would want to purify a biomolecule of interest using affinity chromatography but there is no biospecific ligand available that can be bound to the column?

6. What is the effect of the number of wash steps on the purity obtained for the biomolecule of interest?

7. Your task is to separate protein A from protein B by affinity chromatography.
    a. The following are the results of eluting these two proteins in different elution buffer systems. Which is the best elution system to elute protein A? protein B?



    b. The following two buffer systems were found to separate proteins A and B by affinity chromatography:
        i. A: NaCl/SDS 150 mM:1.0 %
        ii. B: NaCl/SDS 150 mM:1.2 %
    c. Which protein binds strongly to the affinity resin?
    d. In order to separate a mixture of A and B as in problem (b) by affinity chromatography:
    e. Which buffer system must be used first?
    f. Which compound will elute from the column first?
    g. A student loaded a protein mixture on a affinity chromatography column that was denatured, then proceeded to elute with NaCl/SDS 150 mM:1.0 %. He/she found that all of the mixture came off in the first column volume. Why did this happen?
    h. You see only one protein coming off the column when you suspect protein A and B. Where might the other protein be? How can you recover this protein?

8. What would happen if you have EDTA in your sample buffer while using metal-affinity chromatography?

9. A gradient of pH is employed for the elution of protein of interest from an antibody-based affinity column. If you are working with a protein that is active between pH 6-8 can you use pH gradient to elute the protein from an affinity column?

10. While performing metal affinity purification you accidently added elution buffer containing high concentration of imidazole before the binding wash to remove impurities. This caused your protein to elute along with impurities. How could you purify the same protein sample again?
11. You are working with a protein in a HEPES buffer containing 100 mM calcium and 10 mM NaCl pH 7.0 as the binding buffer. After the binding step you add wash buffer which is 100 mM sodium phosphate pH 7.0 containing 150 mM NaCl. What would be the effect of this buffer change?
12. How would you prevent contamination from one purification cycle to another?