PBC Day 2 Interpretation Questions

1. Calculate the total activity for each of your samples (DEAE/AF Load, DEAE-Total, AS-S and AS-P). Are your results consistent with your expectations? Why or why not?

2. Oops! You didn't read your laboratory protocol very well, and forgot to run the PD-10 column today. Instead, you took your resuspended AS-P and loaded that sample directly onto the equilibrated DEAE column, then followed that protocol exactly.
   a) What is the purpose of running the PD-10 column today?
   b) In what fraction from **your** DEAE column (FT, 0.1M, 0.4M #1, 0.4M #2, 1.0M) will you expect to find Bgal? Explain your answer.

3. You have a solution containing ammonium sulfate (salt), B-galactosidase and a positively charged protein called SPA (Super Protein Andy); the pH of the solution is 7.5.

   Using the techniques you have learned thus far in the PBC module, design a purification scheme that will allow you to separate B-galactosidase away from the salt and SPA.

   Be sure to be specific about:
   a) What columns you'd run
   b) In what order you'd run them
   c) Where you'd expect to find Bgal, salt and SPA after running each column (e.g. in the flowthrough, stuck on the column until eluted with (buffer name), etc.) and why.