Prelab #7.

1. In this Purification protocol we are using 2 different reagents for the purpose of lysing the bacterial cells.
   1. Please name them and describe their mechanism of action? (2pts)
   2. There are alternative methods of lysing cells, please name at lease 2 of them and explain how they work? (2 pts)
   3. Why is EDTA used in our buffer? Please name some alternative reagents you could use in place of the EDTA. 2pts
2. In this experiment we are purifying β galactosidase with an MBP affinity tag.
   1. What property of MBP makes it advantageous for separating the fusion protein from the thousands of proteins that are present in the bacterial cell lysate. (2 pts)
   2. There are quite a few affinity tags that are available in the protein biochemist tool kit. Please name 3 and describe their properties that are important for carrying out affinity purification, (ie what kind of resin do they bind) and what reagent is needed for Eluting the protein off the affinity column. (3 pts)
3. You are G1 graduate student who is given the task in studying a novel protein kinase known as PKZ which has been implicated as an oncogene responsible for the loss of cell cycle control, and therefore an attractive drug target for a cancer therapy. You want to ultimately crystalize this protein on its own and in complex with its downstream binding partners so that you can rationally design a highly specific inhibitor. This requires high quantities of ultra-pure protein and you need to work out the purification scheme for this protein which has not been recombinantly expressed before.
   1. You want to design an affinity tagged construct of the protein, but are unsure of where to engineer the tag (whether N-term or C-term). Knowing that the kinase active site is at the N-term, logically where do you think the site tag should be attached and why? (2pts)
   2. Ultimately for crystallographic studies you want the tag removed so that it does not interfere with the crystallization studies. Please describe how you would go about removing the tag. (2pts)
   3. You carry out the affinity purification of PKX with your tag of choice and want to analyze the purity throughout the entire procedure, what analytical method would you use to ensure the purity? Please describe an additional method to validate that what you are looking at is your protein of interest (hint you have antibodies against this kinase and its tag at your disposal) (2pts)
   4. Based on the results in c. you realize that there are several contaminant proteins in the final purification step. Why do you think there are contaminant proteins that elute out with the tagged PKX? (2 pts)
   5. What methods would you use in removing these contaminant protein species from your protein of interest, and how do they work. (Hint: they have different sizes and charges with comparison your protein of interest). (4pts)
   6. Given that your methods in e) worked and you have a super pure protein sample in the final stages of the purification, how would you verify that your recombinant PKX is fully functional and its use is therefore valid in structural studies. (2pts)