PBC Day 3 Interpretation Questions and Answers

1. Interpret your data. Included in your interpretation should be answers to:
What was the total activity of your "AF after PD-10" sample? What was the yield of your purification at each step, from the beginning? Which samples had the highest yield, and are these results what you expected? (Note that your full answer to "interpret your data" should include MORE than just the answers to these three specific questions.)

Answers will vary. However, yield should decrease at every step, because you can only lose protein through a purification step; you can't gain protein, and yield is the percent of total activity at a specific step that is left at that step.

2. Say that one of the TAs responsible for preparing ONPG accidentally added lactose to the ONPG solution to be used for your beta-galactosidase activity assays. Explain how adding lactose to the ONPG solution would affect the results of your assays.

If lactose is added to the assay mix, we would expect to get a faulty measurement of b-galactosidase activity. When lactose is cleaved, galactose and glucose are produced, neither of which causes a change in color. (If the solution consisted of lactose alone, we would be unable to measure any b-galactosidase activity with this assay.) The lactose would act as a competitor for beta-gal away from ONPG. Because of this competition, there will then be less yellow in the solution, leading to an inaccurate measurement of b-galactosidase activity.

3. Fill in the table below that compares the three types of column chromatography that we have used to purify beta-galactosidase.

Column	Chromatography	Resin	Separation	Elution	Order of elution
PD-10	**Gel Filtration**	**Porous beads**	**Size**	**Column Buffer**	**High MW first, low last**
DEAE	**Anion Exchange**	**Positively-charged beads**	**Charge**	**Salt**	**+ first, - last**
APTG	**Affinity**	**Beads linked to APTG**	**Affinity to substrate**	**Sodium Borate (increase pH)**	**Low affinity 1st, b-gal last**

4. So far, we have used the following substrates of beta-galactosidase: APTG, lactose, X-gal, and ONPG. Say that you were trying to use affinity chromatography to purify both wild-type beta-gal, and a mutant form of beta-gal that was mutant in its ability to hydrolyze lactose or lactose analogs (but was able to bind lactose or lactose analogs at wild-type levels). Which of the four substrates listed above could be used as the substrate that would be attached to the beads in the column that you would use to purify wild-type protein? What about for the mutant? Which of the four substrates listed above could be used as the elutant in order to then elute your wild-type protein? What about for the mutant? Explain your answers.

For wild-type, you can only attach APTG to the beads. The other three substrates would get cleaved by beta-gal, and it would thus fall off the column. For the mutant, which can't cleave these substrates, any of the four could be used. To elute either wild-type or mutant, any of the four substrates could be used because both versions of the protein can bind to all of these compounds normally, so all four compounds would compete with the protein's binding to the column and thereby release it from the column.