Interpretation questions, PBC Day 3

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.)

2. Say that one of the TAs responsible for preparing ONPG accidentally added some 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.

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

Column	Type of Chromatography	Make-up of Resin	Property that drives separation	What is used for elution?	What elutes first; what elutes last?
PD-10					
DEAE					
APTG					

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 from the column? What about for the mutant? Explain your answers.