# PBC Day 4 Interpretation questions (with answers)

1. Interpret your Bradford assay data. Calculate S.A. for each of your samples.

**Answers will vary, but the S.A. should increase throughout the purification.**

2. Which samples did you expect to have the highest S.A.? Did your calculations support those expectations? Explain.

**We expect to have the highest S.A. in the D Total and/or AF after PD-10.**

**S.A.= T.A./T.P.**

**So, provided that the student could measure total protein for their latest purification steps, the T.A. should increase and the T.P. should decrease dramatically. If they couldn't measure the T.P., then people may not realize that this is a "good thing" (particularly for the final steps of the purification), indicating that the majority of the non-Betagal protein in the samples is gone.**

2. In each of the following cases how did your experiment go "wrong"?

    a. After turning on the power for your gel to run, you see many bubbles and are assured by your TA that everything is fine. Five minutes later you look at your gel again and despite the fact that the bubbles are still going, you cannot find the blue 2x sample dye anywhere in your gel. Why?

**The student probably plugged the leads into the power supply in the opposite manner: black into red and red into black. Therefore, with the samples being negatively charged due to the SDS, they have run into the top buffer chamber.**

    b. After the Commassie-stained gels have been dried and passed back, you notice that the majority of your bands are at the top of the gel but the groups around you have their bands spread out throughout the gel. Someone suggests you might not have run your gel all the way to the bottom, but you show them that the dye front is 1 cm from the bottom. What happened?

**Although the student used the 2x Sample Buffer, they probably omitted the heating step, so the tertiary and quaternary structures of the proteins weren't fully denatured, thereby resulting in many larger molecules running through the gel.**