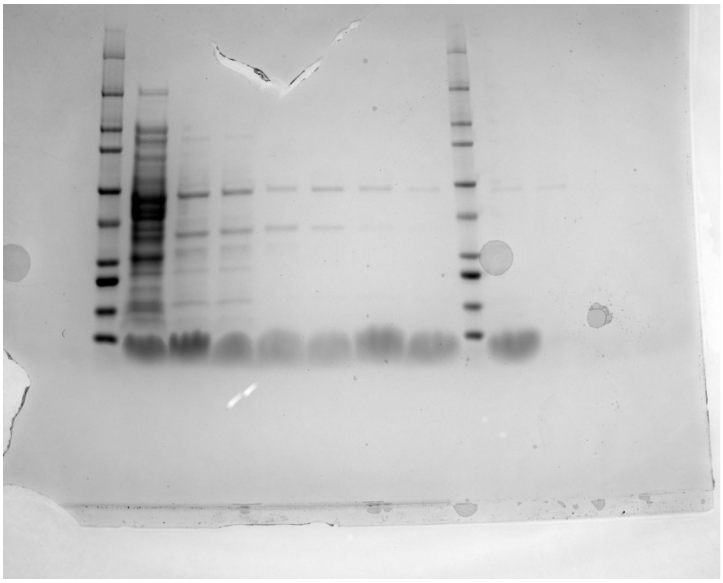
Post lab analysis for Part A:



1) Comment on the purity of your various extracts based on your stained gel.

   Purity increases (bands not ~47 kDa get fainter) from stage 1 to stage 5. However the AP band also gets fainter, telling me we also removed some AP along the way.

2) Do you see a band at your expected AP molecular weight in all lanes? Are there other dominant bands? What proteins do you think they could correspond to?

   Yes, band ~47 kDa in all lanes. No clear bands at the AP dimerized weight of ~94 kDa. There is a clear, consistent band ~35 kDa. This is probably another periplasmic protein with similar properties to AP.

3) Is this proof that there is AP in your samples? What experiment could you do to obtain stronger evidence?

   Probably not absolute proof… You could use a western blot (immunoblot) with specific AP targeting antibodies to obtain stronger evidence.

4) Dialysis was an important step in removing ammonium sulfate from your extract. High salt sample's generally spread out instead of running predictably in a gel. Why does high ionic strength facilitate diffusion during electrophoresis?

   Electrophoresis samples run through the gel via an electric potential so high ionic strength samples will react strongly to the electric field.

Post-Lab analysis questions for part B:

1. What is the purpose of doing a standard curve? Why doesn't the vendor just report standard curve data in their protocols? '

   AKA calibration curve. A standard curve is used to estimate concentration (unknown) from an absorbance measurement (known). We do our own (no vendor reported) because experimental conditions can vary widely and its important to have a precise calibration curve for what we are measuring.

2. Generate standard curves of absorption versus concentration for each concentration assay, and determine the concentration of protein in each of your extracts with error bounds. Do the methods provide similar results? If not, which do you trust most? How do the numbers compare to your previous nano-drop measurements?
   No Bradford assay so can't compare. BCA assay predicts lower concentrations than nanodrop, which is what we expect. Bradford assay is specifically looking at AP, while the nanodrop is a very unspecific method.
3. In this protocol, you were asked to measure your findings in triplicate. What error are you measuring using the directions described in the protocol?
   Error of the triplicate measurements (precision). The error could be attributed to experimenter error, equipment error, etc.

4. What are the shapes of your standard curves? Are they ideal? How does this affect your interpretation of the data?

   Standard curve is linear as expected. The fit isn't great at low absorbances. I would be most confident in the intermediate absorbance predictions of concentrations. I would be careful predicting concentrations from very low or very high absorbance measurements.

5. Compare your results from electrophoresis to your concentration assays. Do you have a band for AP? Does AP remain present throughout the extraction? Was a significant proportion lost, and if so, at what step? Which step removed the most contaminating proteins?

   Qualitative comparison: Results from concentration assay matches band strength. A significant proportion of AP was lost at the first purification step (heat denaturation). This step also removed the most contaminating proteins.

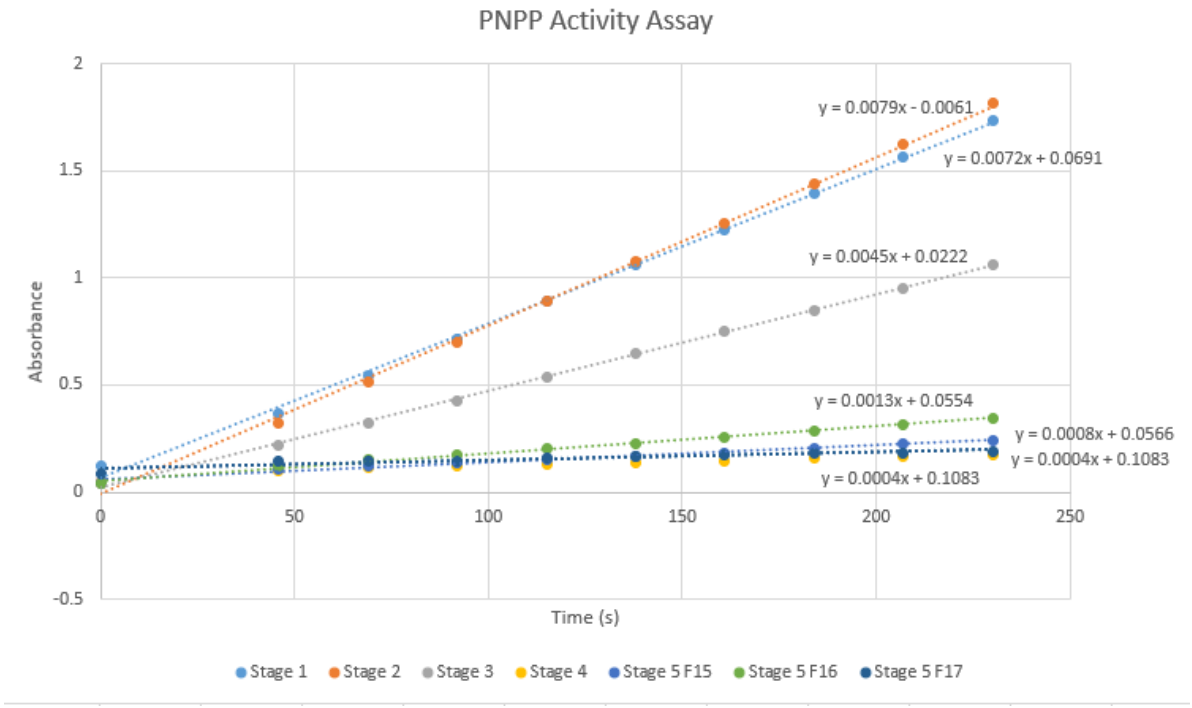
6. Estimate what proportion of protein in each Stage extract is AP. Was extraction successful?

	Stage 1	Stage 2	Stage 3	Stage 5 F1	Stage 5 F2	Stage 5 F3
	Periplasmic extraction	Heat denaturation	Precipitation and Dialysis	Ion Exchange Chromatography		
Nanodrop (mg/ml)	21.13	19.02	2.48	N/A	N/A	N/A
Absorbance - BCA	3.318	1.153	0.842	0.3607	0.4123	0.296

Conc. - BCA (mg/ml)	5.605396	1.711511	1.152758	0.286451	0.379376	0.170144
Proportion AP	26.5%	9.0%	46.5%	N/A	N/A	N/A

Post Lab analysis for part C:

1. Plot the absorbance (y-axis) vs time (x-axis). Fit the initial (linear) points to a line and calculate the slope of that line and estimate error bounds. If the initial points are not linear, you may want to acquire more data or devise (and justify) another method to determine this slope.

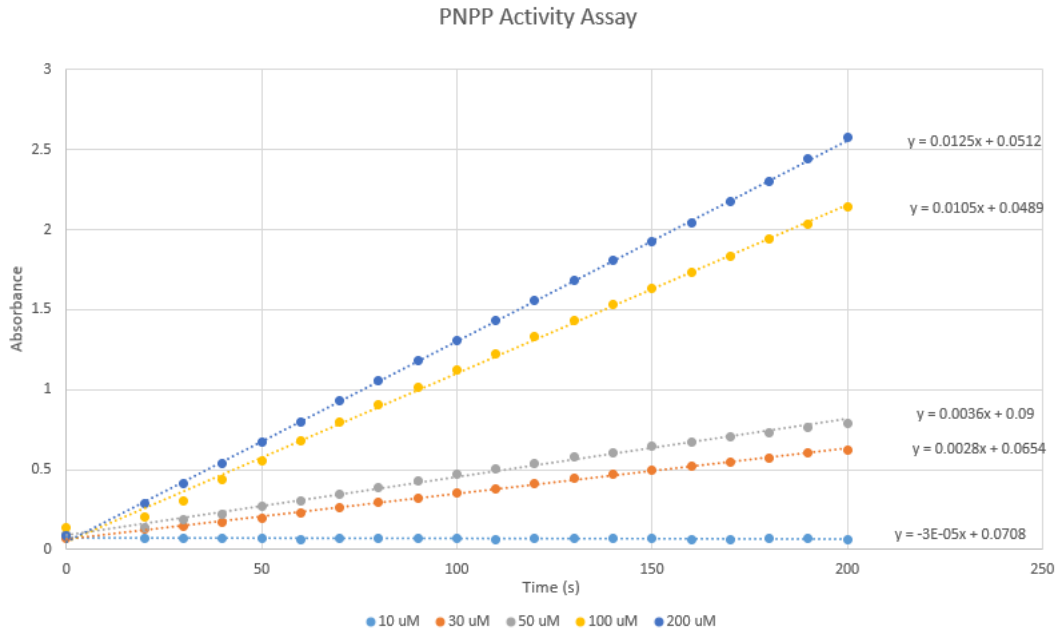


2. Convert the ΔA/time to millimolar/time using the millimolar absorptivity that you measured. *This is the rate of formation of PNP.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5 F15	Stage 5 F16	Stage 5 F17
Absorbance/sec	0.0072	0.0079	0.0045	0.0004	0.0008	0.0013	0.0004
Formation rate of PNP (M/sec)	3.6E-07	3.95E-07	2.25E-07	2E-08	4E-08	6.5E-08	2E-08

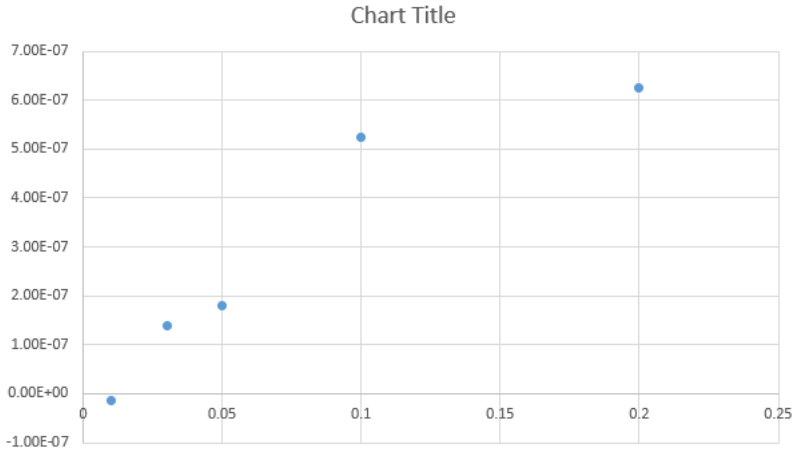
Post Lab analysis for Part D:

1. Determine VO for each substrate concentration [S] with error bounds by plotting the ΔA/time, and using the absorptivity of 50µM PNPP, to determine VO in units (millimolar PNPP converted/time).

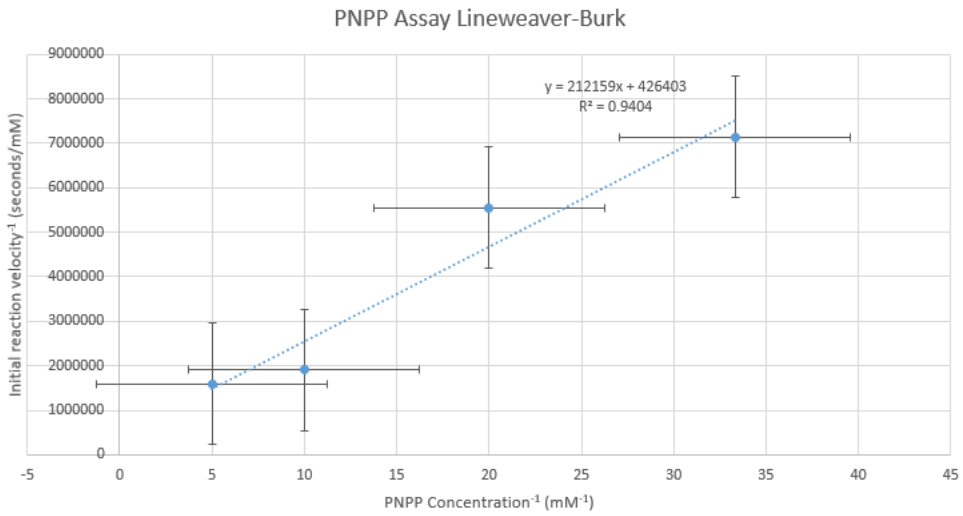


	10 uM	30 uM	50 uM	100 uM	200 uM
Abs. vel. (1/sec)	-3.00E-04	2.80E-03	3.60E-03	1.05E-02	1.25E-02
Conc. Vel. (mM/sec)	-1.50E-08	1.40E-07	1.80E-07	5.25E-07	6.25E-07

2. Plot VO (with error bars) versus [S].



3. Plot 1/VO (with error bounds) versus 1/[S], (Lineweaver-Burk plot) and calculate the values of Km and Vmax with error bounds from the intercepts.



4. Is your trend linear? Why or why not? How do your findings compare to literature values? What are potential sources of error?

The trend is relatively linear