Protein determination:
I converted the BSA standard samples to ug/sample (=ul of BSA stock added to each sample since the stock was 1ml/ml=1ug/ul). This allowed me to plot my standard curve with ug as the x-axis (note that I forced the trendline to go through the origin, since we had blanked with a sample containing no protein. I then calculated the ug of AP from the equation of the trendline for each column fraction. This is now ug/10ul sample, since that is the amount I used for the Bradford (BioRad) assay. To get total protein, I would multiply this by 50 (=500ul/10ul), since the column fractions were 500ul total. This factor would be different for your pre-column fractions, which were larger than 500ul, or if you used more or less than 10ul of the fraction in the assay.

Enzyme activity:
To determine enzyme activity in the column fractions, I converted change in absorbance/min to change in concentration (uM/min) using Beer’s law. This represents uM/min/5ul enzyme (the amount used in the assay). I then calculated the total activity represented by the column fraction by multiplying by 500ul/5ul. Once again, this factor will be different for the pre-column fractions (e.g. osmotic shock or cold water wash), because the volume of these is much greater than 500ul, and also should contain the actual volume you used in the assay, which may have been more than 5ul. To get the specific activity, I divided this total activity number by the total protein in the respective fraction, resulting in units of uM/min/ug. To get the % yield for each fraction, you would set the total enzyme activity in the osmotic shock fraction to 100% and determine what % of this activity was still present in each subsequent fraction. The yield of the column fractions can be added up to determine what the total yield off the column was.

Km, Vmax, kcat:
Using the data collected with column fraction #2 and serial dilutions of PNPP, absorbance/min values were again converted to uM/min using Beer’s law. I calculated the reciprocal of these v_0 values and the [S] values in uM to make the double-reciprocal (Lineweaver-Burke) plot. Vmax was taken from the y-intercept of the trendline, and Km calculated from this and the slope (=Km/Vmax) of the line (using the equation in the box, not graphically). Then I converted Vmax from uM/min to umoles/min as above. I then calculated the total activity in column fraction #2 again using Vmax times the aliquot factor, but this time I converted the units to umol/min by multiplying by the volume of the fraction (0.0005L). Now I calculated kcat by dividing by the ug of protein in this column fraction. This value is in umol/min/ug. However, kcat (turnover number) is usually expressed in units of sec$^{-1}$. We can convert to this by first calculating the umol/min/umole enzyme, by multiplying by the MW of AP, which is 94,000g/mol (or 94,000ug/umol). The umoles in the units cancel out, so we now have a turnover number per min, which can be converted to sec$^{-1}$, simply dividing by 60sec/min. This number represents the number of molecules of PNPP converted to PNP by each enzyme molecule in one second.

Of course, I am making the assumption here that the AP enzyme is pure. In reality the turnover number will be higher, because the ug of protein we measured included other contaminating proteins. My values for the wild-type AP differ from those reported by Kantrowitz, but then he even reports different values for both kcat and Km for wild-type in his own papers. We will be comparing our mutant kcat and Km values to our own wild-type values, because we know they were done under the same conditions using protein of similar purity.