Tips/Guide for getting started with Post-Lab Analysis for Lab 1

1. You will need to prepare your standard curve (plotting A405 against [pNP]). You will be using this curve to help calculate the Molar Extinction coefficient (εM) in question 2. Therefore, to make this calculation as easily as possible you should plot your standard curve **using [pNP] in Moles/L**.
2. Calculate the Molar Extinction coefficient (εM) using the formula A= εM \* c \* l (where l = pathlength) as shown in your lab manual. Your standard curve shows the relationship between Absorbance (A) and Concentration (c) 🡪 Standard curve slope = ΔA/Δc. Therefore the equation given in question 2 can be simplified to **Slope = εM \* l**. (Rearrange and solve for εM).
3. Consult your standard curve and R2 value to help answer this question.
4. Be mindful of the units used in the calculation of εM in question 2.
5. Construct your A405 against Time graphs – make sure to **plot the time in minutes** to simplify your next set of calculations. Follow the equations provided in your lab manual but **USE YOUR OWN εM VALUE** **CALCULATED IN QUESTION 2**. (Ignore the εM value given in the lab manual in question 5). Be sure to convert your final answers for **Vo into μmol/L-min**.
6. Make sure you are calculating the effective [pNPP] used in your reactions (last column of Table 1.2). **Convert these [pNPP] into μmol/L**.
7. Make your Michaelis-Menten plots of **Vo (μmol/L-min) against [pNPP] (μmol/L)** for each concentration of Alkaline phosphatase. Follow these same units when making your subsequent plots (Lineweaver-Burke, Eadie-Hofstee, Hanes). Calculate KM and VMAX as stated in the lab manual.