Assignment 1: Enzyme Kinetics assignment

Due in your next lab session (week 5) before the start of the prac work for the day.

You must hand in:

1. **A one page summary of your results from the enzyme kinetics experiment** carried out in week 4. (2 marks)
   
   It must contain:
   
   i) the four graphs discussed in the lab notes: the Michaelis Menten plot, the Lineweaver-Burk plot, the Eadie-Hofstee plot and the Hanes-Wolfe plot
   
   ii) your estimates of $K_M$ and $V_{max}$ from each plot
   
   iii) your comment on which estimate is, in your opinion, the most accurate (based on the spread and weighting of the data). This is easiest done in Excel.

2. **Similar analysis of the assigned data. (3 marks)**
   
   You will be assigned a series of data (I-V) as an Excel file which you will download from the WebCT link. This is data obtained from performing similar experiments to those you performed in class with an isoenzyme of alkaline phosphatase, isolated from arctic shrimp.
   
   Refer to the next page for more information concerning the assay and the results.
   
   It must contain:
   
   i) the Michaelis-Menten plot showing both sets of data (control and experimental) from your assigned data
   
   ii) one of the other plots you did on your own results (the one that in your opinion gave the most reliable estimates of $K_M$ and $V_{max}$) showing both sets of data
   
   iii) your answers to the questions/tasks on the following pages
Enzyme Kinetics Assignment: Assigned Data (I-V)

Your research team has been investigating an isoenzyme (a different (but very similar) enzyme which catalyses the same reaction) of alkaline phosphatase, known as arctic shrimp alkaline phosphatase (SAP). It has potential uses in molecular biology to remove the terminal 5’ phosphate from DNA and dNTPs. This is particularly useful to remove dNTPs after PCR, before the amplified product is sequenced.

SAP has been isolated from arctic shrimp, Pandanus borealis and has the unusual property of being active at 4°C although its temperature optimum remains at 37°C. Unlike the liver, calf thymus or E. coli alkaline phosphatase (ALP), it is more heat labile, being readily heat inactivated at 60°C. This enables the researcher to heat inactivate the phosphatase before the next reaction begins.

Members of your team have performed a number of enzyme assays on this arctic enzyme, with unknown inhibitors and altered enzyme concentrations. There are five different conditions (I-V) under which the enzyme has been assayed. Each series of results represents one such condition and also has the control set included.

You will be assigned by your demonstrator one of these series of results. It is your task to work out, from the data provided in the Excel spreadsheet, what is the variation in conditions of the experimental arctic enzyme assay. The five possible variations are:

- An enzyme preparation with a higher concentration of enzyme was used
- A competitive inhibitor was added
- A non-competitive inhibitor was added
- An uncompetitive inhibitor was added
- A mixed inhibitor was added

The control assay enzyme conditions were as follows:

“Arctic shrimp alkaline phosphatase (SAP) activity (1 µg/mL) was assayed at 25°C in 0.1 M glycine buffer, pH 10.4, containing 1 mM MgCl₂ and the substrate 4-NitroPhenol Phosphate (4-NPP) at concentrations ranging from 0.1 to 1.5 mM. The rate of appearance of the product, 4-Nitrophenol (4-NP) was monitored over a 2 min time period spectrophotometrically at 420 nm (ε = 15 mM⁻¹cm⁻¹). The SAP isoenzyme is a homodimer with a subunit molecular weight of 54,000.”

You have performed all your assays in a 1 mL assay. As per the notes the molecular weight of the “regular” ALP is ~50,000, however this is the subunit molecular weight. All alkaline phosphatases are dimers.
Questions/Tasks to be handed in with the report:

a. Compare the SAP $k_{\text{cat}}$ and $K_M$ with the $k_{\text{cat}}$ and $K_M$ you found for standard ALP (E. coli) performed in class. Which enzyme, SAP or ALP, has a higher affinity for the substrate? Which enzyme is the more efficient? In each case justify your answer. [The $k_{\text{cat}}/K_M$ is the measure of catalytic efficiency. The higher this ratio the more efficient the enzyme (see lecture U2L3 for an explanation). To calculate the $k_{\text{cat}}/K_M$ you must first convert your $K_M$ to M as the units of $k_{\text{cat}}/K_M$ are $M^{-1}s^{-1}$.]

b. Determine which of the five sets of conditions (described previously) corresponds to your assigned series of data.

c. If your data set corresponds to a preparation with an altered enzyme concentration, work out how much enzyme was added to the assays.

d. If your data set indicates an inhibitor was added, determine the type of inhibitor and comment briefly (1 to 2 sentences) on how the inhibitor might act, i.e., does it bind to E alone or to ES? does it resemble the substrate?

e. Considering the control enzyme assay presented in the data set given, what would be the absorbance at 420 nm of the assay at 1.5 min in the 1.5 mM assay?

f. What changes could you make to the enzyme assay described on the previous page to distinguish between thermophilic (high temperature adapted), mesophilic (mid range temperature preferring) and psychrophilic (cold adapted) alkaline phosphatases? Show how this modified assay would distinguish the three classes of alkaline phosphatase.