Bi10 Prelab #9

Any questions regarding this prelab should be sent to Peiwei (pcchen@caltech.edu).

1. You are performing a β-galactosidase activity assay on both crude protein extract and purified β-galactosidase by measuring the absorbance of o-nitrophenol (ONP), the yellow cleavage product of ONPG in this reaction. You know that 0.0045 absorbance units at 420 nm equals 1 nmol of ONP/mL, and you also know that the specific activity of pure β-galactosidase is 3×10^5 U/mg. See p.53 of your lab manual for the definition of a unit. You prepare reactions according to the instructions on p.52 in your lab manual. You use 10 µL of 10-fold diluted crude extract for one reaction and 10 µL of 10-fold diluted pure protein for another reaction. The absorbance you measure after 10 minutes are given as below.

	Total protein concentration (mg/mL)	Total volume of sample (mL)	Absorbance at 420 nm after 10 min
Crude extract	9.71	10.5	0.48
Pure protein	0.42	4.4	0.69

   a) Calculate the concentration in µM of ONP in each of your samples after the 10 min period. (2 point)

   b) Calculate the rate of each reaction in terms of µM/min. (1 point)

   c) Calculate the total activity (in U) of each reaction. (2 points)

d) Calculate the specific activity (in U/mg) of the enzyme in each reaction, and then calculate the fold-purification of the enzyme, as determined by specific activity. (4 points)

e) You finished the experiment early and was happy. However, your best friend who did the experiment right next to you obtained a **higher** specific activity of the enzyme from crude extract than purified protein. Do you think the result is reliable? If so, explain why this is what we shall expect; if not, suggest at least 3 possible reasons why your friend might have got this result. (3 points)

f) After the experiment, you are explaining what you have done to your roommate, who is a math major senior and has forgotten all the chemistry/biology learned in the freshman year. He asked, how did you stop the reaction after 10 min? What is the mechanism? Could you have used sodium **bicarbonate**, which is more common in laboratories? Please answer and explain why. (4 points)

2. The Michaelis-Menten kinetics
   a) Write the Michaelis-Menten kinetics equation, and sketch a graph showing the relationship between the reaction rate vs. substrate concentration. (3 points)

b) Explain in one sentence what K_m means in the Michaelis-Menten kinetics equation. Why is K_m important/useful? (2 points)
Hint: can we, and when can we, obtain V_{max}, the maximal reaction rate?

c) How would the addition of a competitive or a non-competitive inhibitor for the enzyme alter the K_m and V_{max}, respectively? Why? (4 points)