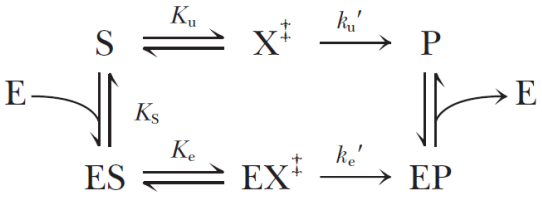
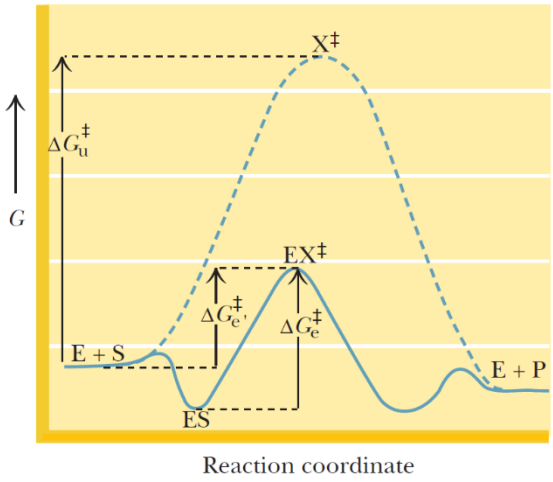
BBL433, Enzyme Science and Engineering
Practise problem set for Minor 1

1. The relationships between the free energy for enzyme catalyzed and un-catalyzed reaction is shown in the below figure. If the energy of the ES complex is 10 kJ/mol lower than the energy of E+S, the value of $\Delta G_e^{\ddagger}$ is 20 kJ/mol, and the value of $\Delta G_u^{\ddagger}$is 90 kJ/mol, what is the rate enhancement achieved by an enzyme in this case?



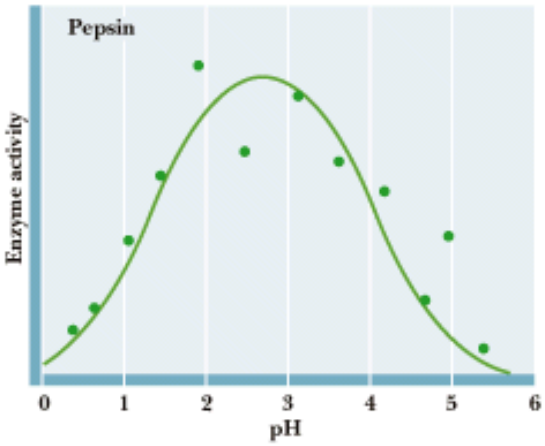


2. A metabolic enzyme generate the amino acid methionine. For a given substrate concentration, an experiment conducted in the presence of high initial concentrations of methionine generates less methionine than an experiment conducted with less initial methionine present. Give a likely explanation for this behavior.
3. The purification of a protein usually requires multiple steps and often involves several type of column chromatorgraphy. A key component of any purification is an assay for the desired protein. The assay can be a band on a gel, a structure in the electron microscope, the ability to bind to another molecule or an enzymatic activity. Enzyme assay allows one to quantify the extent of purification at each step. Consider the purification of enzyme shown in table below.

Procedure	Total volume (mL)	Total protein (mg)	Total activity (units/mg)
Crude extract	2000	15,000	150,000
Ammonium sulfate precipitation	320	4000	140,000
Ion exchange chromatography	100	550	125,000
Gel filtration chromatography	85	120	105,000
Affinity chromatography	8	5	75,000

   a. For each step in the purification procedure, calculate specific acitivity of the enzyme (units of activity per mg of protein). How can you tell that purification has occurred at each step?
   b. Which of the purification steps was most effective? Which was least effective?
   c. If you were to carry the purification though additional steps, how would the specific acitivyt change? How could you tell from specific activity measurement that the enzyme was pure? How might you check on that conclusion?

4. Write short note on aspartic protease mechanism. Explain how the Enzyme activity versus pH is a Gaussian distribution.



5. The enzyme α-glucosidase (EC 3.2.1.20) catalyzes the hydrolysis of maltose into glucose. α -glucosidase is competitively inhibited by the product glucose and inhibited at high maltose concentrations in a partial uncompetitive mode. Determine a kinetic rate expression in terms of the dissociation constants for: the secondary enzyme–substrate complex (K_M), the secondary enzyme–product complex (K_P), the tertiary enzyme–substrate–substrate complex (K_0), and the maximum reaction rates of product formation from the enzyme–substrate active complex (V_{max}) and the enzyme–substrate–substrate partially active complex (V^0_{max}). The molar concentrations of maltose and glucose are [M] and [G], respectively.