Bioengineering Study Program – School of Life Science and Technology – ITB

Third Exam (Final)

BE-2103 Biological Thermodynamics

(Termodinamika Sistem Hayati)

Date: December 6, 2017

Hours: 09.00 – 12.00 (180 minutes)

Closed book and notes. Only text book of Biological Thermodynamics (Donald T. Haynie) is allowed be opened

---

1. When $[L] = K_d$, $F_b = 0.5$. Calculate the concentration of ligand required for 90% saturation and 99% saturation.

2. Consider a homotrimeric protein with three identical and independent binding sites and microscopic association constant of 10^6. Plot the fractional saturation of the protein against the free ligand concentration. Write down equations describing the microscopic binding constant (K_1, K_2, K_3) in terms of the microscopic binding constant.

3. Suppose you are studying the binding of heme to myoglobin using equilibrium dialysis (Chapter 5). The total concentration of myoglobin is 10 μM. The following data were obtained at equilibrium

Experiment	[heme] in chamber without myoglobin (μ*M*)	[heme] in chamber with myoglobin (μ*M*)
1	3	5.3
2	30	7.5

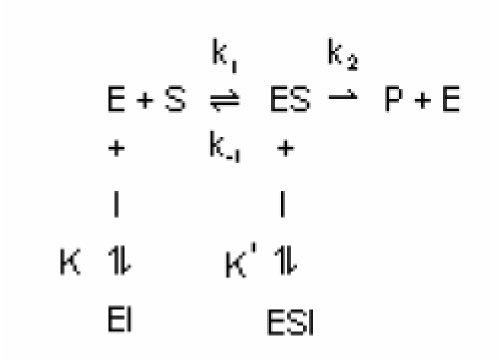
Calculate the concentration of bound and free myoglobin in the chamber where myoglobin is present. Use these values to calculate the fractional occupancy of myoglobin at the two ligand concentrations. Determine the affinity of heme for myoglobin using your favorite method. Can these data be used assess binding cooperativity? Explain.

4. The rate of ATP hydrolysis to ADP and P_i is influenced by the muscle protein of myosin. The following data are tabulated at 25 °C and pH 7.0.

Velocity of reaction in μmoles inorganic phospate produced l^{-1} s^{-1}	[ATP] in μM
0.067	7.5
0.095	12.5
0.119	20.0
0.149	32.5
0.185	62.5
0.191	155.0
0.195	320.0

Find the Michaelis constant of myosin.

5. Derivate the case of mixed inhibition of enzyme to show that $J_p = \frac{k_2[E]_T[S]}{K_M\left(1+\frac{[I]}{K_I}\right)+[S]\left(1+\frac{[I]}{K'_I}\right)}$ (equation 8.67). Use the following scheme figure:



Note: State your assumption clearly and do the derivation with step by step procedure

6. Show that

$$J = \frac{\frac{J^f_{max}[S]}{K^S_M} - \frac{J^r_{max}[P]}{K^P_M}}{1+\frac{[S]}{K^S_M}+\frac{[P]}{K^P_M}}$$

is the rate for a reversible enzymatic reaction. The reaction scheme might look like this $E + S \Leftrightarrow ES \Leftrightarrow P + E$ and $J^{f}_{max} = k_2[E]_T$ $K^{S}_{M} = \frac{k_{-1} + k_2}{k_1}$ $J^{r}_{max} = k_{-1}[E]_T$ $K^{S}_{M} = \frac{k_{-1} + k_2}{k_{-2}}$. You can use the definition of $J = -\Delta[S]/\Delta t = k_1[E][S] - k_{-1}[E \bullet S]$.