Lecture III

Enzyme Kinetics Exercise

PROBLEM 1:
An enzymatic assay was carried under two different sets of conditions out using a pure substrate S. The results are tabulated below.

[S], mM	**Va, mM/s**	**V_b, mM/s**
1.5	0.21	0.08
2.0	0.25	0.1
3.0	0.28	0.12
4.0	0.33	0.13
8.0	0.44	0.16
16.0	0.40	0.18

a. Plot the data using the Lineweaver-Burke plot
b. Calculate the values of V_{max} and K_m for both sets of conditions, starting from the Michaelis-Menten equation.
c. Suggest possible reasons why the two sets of results might be different.

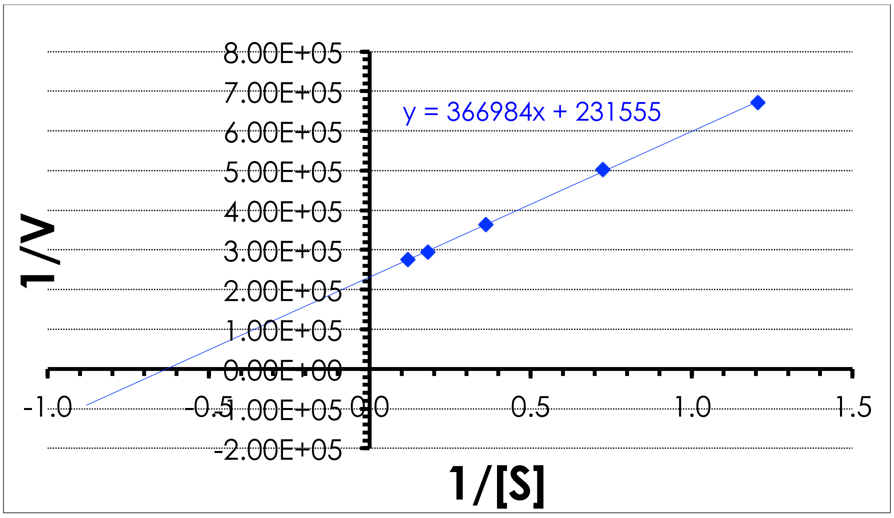
Solution:
From the Michealis-Menten equation

$$v = \frac{v_{max}[s]}{K_m + [s]}$$

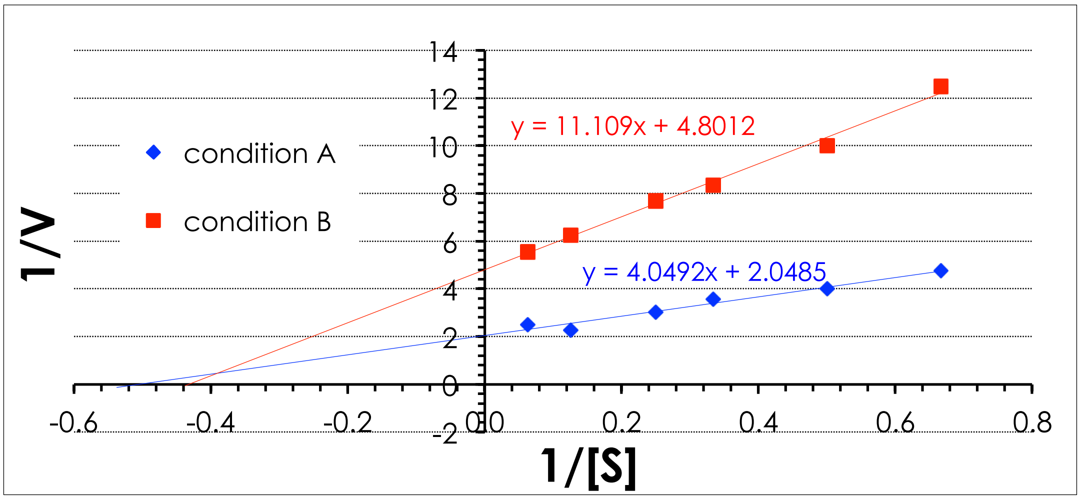
we can obtain:

$$\frac{1}{v} = \frac{K_m}{v_{max}} \cdot \frac{1}{[s]} + \frac{1}{v_{max}}$$

By plotting 1/v *vs* 1/[s] we have the Lineweaver-Burk Plot.



In the exercise we have:



$V_{max,a}$=0.488 mM/s
$K_{m,a}$=1.977

$V_{max,b}$=0.208 mM/s
$K_{m,b}$=2.314

1) intercept with y axis (x=1/[S]=0) => 1/Vmax
2) intercept with x axis (y=1/V=0) => -1/Km
3) Slope = Kmax/Vmax
You chose two of these 3 equation and you find the two incognita Km and Vmax.

PROBLEM 2:
An enzyme catalyzed reaction of the form $S \leftrightarrow P$
Has a ΔG° value of 3.4 kJ mol^{-1}. Calculate the equilibrium constant for the enzymatic process at 298 K (Universal Gas constant R = 8.314 J $mol^{-1}K^{-1}$).

Solution:

ΔG° = -RT ln Ka
exp(-ΔG°/RT) = Ka

if:
T = 298 K
R = 8.314 J/mol K
ΔG°=3.4 **k**J/mol = 3400 J/mol
—> Ka = 0.254

Exercises on DNA/DNA pairing and bond energy

This table presents the thermodynamic nearest neighbor (NN) parameters for Watson-Crick base pairs in 1 M NaCl.

TABLE 1 Nearest-neighbor thermodynamic parameters for DNA Watson-Crick pairs in 1 M NaCl[a]

Propagation sequence	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (e.u.)	$\Delta G°_{37}$ (kcal mol^{-1})
AA/TT	−7.6	−21.3	−1.00
AT/TA	−7.2	−20.4	−0.88
TA/AT	−7.2	−21.3	−0.58
CA/GT	−8.5	−22.7	−1.45
GT/CA	−8.4	−22.4	−1.44
CT/GA	−7.8	−21.0	−1.28
GA/CT	−8.2	−22.2	−1.30
CG/GC	−10.6	−27.2	−2.17
GC/CG	−9.8	−24.4	−2.24
GG/CC	−8.0	−19.9	−1.84
Initiation	+0.2	−5.7	+1.96
Terminal AT penalty	+2.2	+6.9	+0.05
Symmetry correction	0.0	−1.4	+0.43

[a]The slash indicates the sequences are given in antiparallel orientation. (e.g., AC/TG means 5′-AC-3′ is Watson-Crick base paired with 3′-TG-5′). The symmetry correction applies to only self-complementary duplexes. The terminal AT penalty is applied for each end of a duplex that has a terminal AT (a duplex with both end closed by AT pairs would have a penalty of +0.1 kcal/mol for $\Delta G°_{37}$).

The following equation is used to predict the ΔG^0_T at a different temperature, T:

$$\Delta G^0_T = \Delta H^0 - T\Delta S^0$$

where T is in Kelvin, ΔH^0 is in cal/mol, and ΔS^0 is in units of $cal/K \cdot mol$ (entropy units, e.u.). ΔH^0 and ΔS^0 are assumed to be temperature independent; this is an excellent approximation for nucleic acids.

The NN Model.

The NN model for nucleic acids assumes that the stability of a given base pair depends on the identity and orientation of neighboring base pairs.

According to this model, the total $\Delta G^0_{Total(37)}$ is given by:

$$\Delta G^0_{Total(37)} = \sum_i n_i \Delta G^0(i) + \Delta G^0(init \quad /term\ G*C) + \Delta G^0(init \quad /term\ A*T) + \Delta G^0(sym)$$

where $\Delta G^0(i)$ are the standard free-energy changes for the 10 possible Watson–Crick NNs (Table 1), n_i is the number of occurrences of each nearest neighbor (*i.e.* the number of pairs), *i*, and $\Delta G^0(sym)$ equals +0.43 kcal/mol (1cal =4.184J) if the duplex is self-complementary and zero if it is non-self-complementary.

Prediction of the Melting Temperature T_M.

T_M is defined as the temperature at which half of the strands are in the double-helical state and half are in the "random-coil" state. The T_M is calculated from the predicted ΔH^0 and ΔS^0, and the total oligonucleotide strand concentration C_T, by using the equation:

$$T_M = \frac{\Delta H^0}{\Delta S^0 + R \ln C_T}$$

where R is the gas constant (1.987 $cal/K \cdot mol$).

R = 8.314 J/(mol K) = 1.987 cal/(mol K)

1 cal = 4.184 J

To compute the T_M in Celsius degree:

$$T_M = \frac{\Delta H^0 \cdot 1000}{\Delta S^0 + R \ln C_T} - 273.15$$

since 0 K = -273.15 °C

where ΔH^0 is given in cal/mol.

PROBLEM 1

Calculate the $\Delta G^0_{Total(37)}$ and T_M for the sequence CGTTGA-TCAACG using the NN model. Consider a total strand concentration of 0.4mM.

```
    ↓   ↓   ↓
5′ C-G-T-T-G-A 3′
   * * * * * *
3′ G-C-A-A-C-T 5′
      ↑   ↑
```

Solution

To compute the $\Delta G^0_{Total(37)}$ we use the equation:

$$\Delta G^0_{Total(37)} = \sum_i n_i \Delta G^0(i) + \Delta G^0(init \quad /term\ G \cdot C) + \Delta G^0(init \quad /term\ A \cdot T) + \Delta G^0(sym)$$

From table 1 we have:

$$\Delta G^0(init \quad /term\ G \cdot C) = +1.96$$
$$\Delta G^0(init \quad /term\ A \cdot T) = +0.05$$
$$\Delta G^0(sym) = 0$$
$$\sum_i n_i \Delta G^0(i) = \Delta G_{CG-GC} + \Delta G_{GT-CA} + \Delta G_{TT-AA} + \Delta G_{TG-AC} + \Delta G_{GA-CT} = -2.17 - 1.44 - 1.00 - 1.45 - 1.30$$

$$\Delta G^0_{Total(37)} = -5.35 kcal/mol$$

To compute the TM we use the equation

$$T_M = \frac{\Delta H^0 \cdot 1000}{\Delta S^0 + R \ln C_T} - 273.15$$

where

$$\Delta H^0_{Total(37)} = +0.2 + 2.2 - 10.6 - 8.4 - 8.5 - 7.6 - 8.2 = -40.9 kcal/mol$$
$$\Delta S^0_{Total(37)} = -5.7 + 6.9 - 27.2 - 22.4 - 22.7 - 21.3 - 22.2 = -114.6 cal/K \cdot mol$$

So

$$T_M = \frac{-40.9 cal/mol \cdot 1000}{-114.6 cal/K \cdot mol + 1.987\ cal/K \cdot mol \ln(0.0004)} - 273.15 = 41.11°C$$

PROBLEM 2

Calculate the TM for a non self-complementary duplex with $\Delta H^0_{Total(37)} = -45.5 kcal/mol$, $\Delta S^0_{Total(37)} = -132.5\ e.u.$, and a strand concentration of 0.2mM for each strand.

Solution

$$T_M = \frac{-45.5 cal/mol \cdot 1000}{-132.5 cal/K \cdot mol + 1.987\ cal/K \cdot mol \ln(0.0002)} - 273.15 = 31.35°C$$

e.u. = non-SI entropy unit = 1 cal/(mol K) = 4.184 J/(mol K)

Internal Single Mismatches

The nearest-neighbor model can be extended beyond the Watson-Crick pairs to include parameters for interactions between mismatches and neighboring base pairs. Table 2 provides the complete thermodynamic database for internal single mismatches.

TABLE 2 Nearest-neighbor ΔG°_{37} increments (kcal mol^{-1}) for internal single mismatches next to Watson-Crick pairs in 1 M NaCl[a]

Propagation sequence	X	Y: A	C	G	T
GX/CY	A	0.17	0.81	−0.25	WC
	C	0.47	0.79	WC	0.62
	G	−0.52	WC	−1.11	0.08
	T	WC	0.98	−0.59	0.45
CX/GY	A	0.43	0.75	0.03	WC
	C	0.79	0.70	WC	0.62
	G	0.11	WC	−0.11	−0.47
	T	WC	0.40	−0.32	−0.12
AX/TY	A	0.61	0.88	0.14	WC
	C	0.77	1.33	WC	0.64
	G	0.02	WC	−0.13	0.71
	T	WC	0.73	0.07	0.69
TX/AY	A	0.69	0.92	0.42	WC
	C	1.33	1.05	WC	0.97
	G	0.74	WC	0.44	0.43
	T	WC	0.75	0.34	0.68

[a]WC indicates a Watson-Crick pair, which is given in Table 1. Error bars and ΔH° and ΔS° parameters are provided in the original references.

PROBLEM 4

Calculate the $\Delta G^0_{Total(37)}$ for the sequence GGACTGACG-CCTGGCTGC (the underlined residues are mismatched) using the NN model.

Solution

To compute the $\Delta G^0_{Total(37)}$ we use the equation:

$$\Delta G^0_{Total(37)} = \sum_i n_i \Delta G^0(i) + \Delta G^0(init\ /term\ G\cdot C) + \Delta G^0(init\ /term\ A\cdot T) + \Delta G^0(sym)$$

From table 1 we have:

$$\Delta G^0(init\ /term\ G\cdot C) = +1.96$$
$$\Delta G^0(init\ /term\ A\cdot T) = 0$$
$$\Delta G^0(sym) = 0$$

$$\sum_i n_i \Delta G^0(i) = \Delta G_{GG-CC} + \Delta G_{GA-CT} + \Delta G_{AC-TG} + \Delta G_{CT-GG} + \Delta G_{CG-GT} + \Delta G_{GA-CT} + \Delta G_{AC-TG} + \Delta G_{CG-GC}$$

$$= -1.84 - 1.30 - 1.44 - 0.32 - 0.47 - 1.30 - 1.44 - 2.17$$

$$\Delta G^0_{Total(37)} = -8.32 kcal/mol$$

Exercises on Antibody/Antigen affinity and bond energy

If a monovalent antibody fragment is used for analysis, the equilibrium of antigen–antibody binding is defined as:

$$Antibody + Antigen \overset{K_a}{\Leftrightarrow} Complex$$

where

$$K_a = \frac{[Complex]}{[Antibody][Antigen]}$$

Association and dissociation rate constants are defined as follows:

$$v_{ass} = k_{ass}[Antibody][Antigen]$$
$$v_{diss} = k_{diss}[Complex]$$

where v_{ass} and v_{diss} represent the rates of association and dissociation, respectively, and k_{ass} and k_{diss} represent the rate constants of association and dissociation, respectively. At equilibrium v_{ass} is equal to v_{diss} and the following equation is obtained:

$$K_a = \frac{k_{ass}}{k_{diss}}$$

The Gibbs' energy of formation (ΔG_0) of an antigen–antibody complex is given by:

$$\Delta G_0 = -RT \ln K_a$$

where R is the gas constant (1.987 cal/molK) and T is temperature.

The free energy of complex formation represents a balance between enthalpic (ΔH_0) and entropic (ΔS_0) forces as defined by the equation:

$$\Delta G_0 = \Delta H_0 - T\Delta S_0$$

In general, antigens and antibodies in solution have to overcome large entropic barriers before they can form a tight binding.

PROBLEM 1

For the following Antigen/antibody couples, calculate the K_a and the Gibbs' energy of formation (at 298K).

Ab/Ag pair	K_{ass} ($M^{-1}s^{-1}$)	K_{diss} (s^{-1})	K_a (M^{-1})	ΔG_0(kcal/molM)
1	8.68 $\times 10^{-5}$	1.84 $\times 10^{-4}$		
2	2.38 $\times 10^{-6}$	2.21 $\times 10^{-4}$		
3	3.72 $\times 10^{-4}$	2.93 $\times 10^{-4}$		
4	6.15 $\times 10^{-5}$	2.33 $\times 10^{-4}$		