

Lecture III

Enzyme Kinetics Exercise

PROBLEM 1:

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

[S], mM	V _a , 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

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

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 $R = 8.314 \text{ J mol}^{-1}\text{K}^{-1}$).

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	ΔH° (kcal mol ⁻¹)	ΔS° (e.u.)	ΔG_{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

^aThe 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 ends closed by AT pairs would have a penalty of +0.1 kcal/mol for ΔG_{37}°).

The following equation is used to predict the ΔG_T° at a different temperature, T:

$$\Delta G_T^\circ = \Delta H^\circ - T\Delta S^\circ$$

where T is in Kelvin, ΔH° is in cal/mol, and ΔS° is in units of cal/K · mol (entropy units, e.u.). ΔH° and ΔS° 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 $\Delta G_{Total(37)}^\circ$ is given by:

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

where $\Delta G^\circ(i)$ 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 $\Delta G^\circ(\text{sym})$ 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° and ΔS° , and the total oligonucleotide strand concentration C_T , by using the equation:

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

where R is the gas constant (1.987 cal/K · mol).

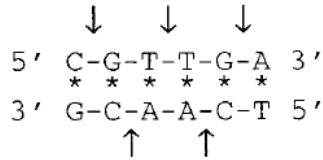
To compute the T_M in Celsius degree:

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

where ΔH^0 is given in cal/mol.

PROBLEM 1

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



PROBLEM 2

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

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

^aWC 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 3

Calculate the $\Delta G_{Total(37)}^{\circ}$ for the sequence GGACTGACG-CCTGGGCTGC (the underlined residues are mismatched) using the NN model.

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:



where

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

Association and dissociation rate constants are defined as follows:

$$\begin{aligned} v_{ass} &= k_{ass}[\text{Antibody}][\text{Antigen}] \\ v_{diss} &= k_{diss}[\text{Complex}] \end{aligned}$$

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	$K_{ass} (M^{-1}s^{-1})$	$K_{diss} (s^{-1})$	$K_a (M^{-1})$	$\Delta G_0 (kcal/molM)$
1	8.68×10^{-5}	1.84×10^{-4}		
2	2.38×10^{-6}	2.21×10^{-4}		
3	3.72×10^{-4}	2.93×10^{-4}		
4	6.15×10^{-5}	2.33×10^{-4}		