Equations in Biophysical Chemistry in Year 1[1]

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[1]Available on https://bit.ly/2FTk4Al

To readers

Contents

Preface and Introduction

This book (or booklet) is originally used to supplement the biophysical chemistry module in the first year of MBiochem (oxon). But the contents may largely overlap with many courses in other institutions. Please refer to the table of contents or the list of equations in the appendix on page 21 to find relevant materials.

This book intends to provide (really) clear, (literally) step-by-step derivations of equations in Year 1 biophysical chemistry. Beautifully typeset with LaTeX, this book is aesthetically pleasing to read.

If you are interested in how I was inspired, and/or want to learn more about the technical issues of writing this book in LaTeX, please go to the epilogue on page 22.

Units and symbols conventions in This Book

The units and symbols used strictly follows the IUPAC conventions.[1] Importantly:

1. All symbols of physical quantities have to be written in italics, but *not* the sub-and superscripts. e.g. K_{d}.

2. K is equilibrium constant, K is the unit of temperature (Kelvin) (or lysine, if we're talking about biochemistry), k is the rate constant or Boltzmann constant (also k_{B})

[1] Renner, T. (2007). *Quantities, units and symbols in physical chemistry.* Royal Society of Chemistry. **and** Coghill, A. M., & Garson, L. (2006). *The ACS style guide* (Vol. 3): Oxford University Press, Inc. and The American Chemical Society, New York.

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Part I

Thermodynamics

Chapter 1

Chemical Potential and Multiple Component Systems

1.1 Prerequisite

1.1.1 Equations to be Memorised

These equations forms the basis of this chapter and chapter 2 (Redox and Electrochemistry), and should be memorised firmly.

1. Definition of chemical potential, μ

$$\mu = \frac{G}{n} \tag{1.1}$$

where n is the number of moles. Thus, μ can be seen as "molar Gibbs free energy"

2. Variation of chemical potential in non-standard conditions. The equations have subtle differences when applied to different states, but it can be summarised as

$$\mu_{\mathrm{x}} = \mu_{\mathrm{x}}^{\circ} + RT \ln \left(\frac{\text{concentration of x}}{\text{concentration of x in the standard state}} \right) \tag{1.2}$$

where the standard condition means temperature is 298K (25°C), all gases have a pressure 1 atm, all solutes have a concentration of 1 M. If you are not confident about how to use it, go to section 1.1.2 on page 3.

3. Variation of free energy change.

$$\Delta G = \Delta G + RT \ln \Gamma \tag{1.3}$$

where Γ is the *mass action ratio*. (In many books it's denoted by Q, but in Wormald's book it's Γ.) It can be deduced from the two equations above, but it's very useful (and not terribly hard) to memorise it. If you're unsure about how to use it, go to section 1.1.3 on page 3.

1.1.2 A Closer Look at Variation of μ

- For solute (the most commonly used):

$$\mu_{\mathrm{x}} = \mu_{\mathrm{x}}^{\circ} + RT \ln \left(\frac{[\mathrm{x}]}{[\mathrm{x}]^{\circ}} \right) \tag{1.4}$$

  where [x] is the concentration (you know) and $[\mathrm{x}]^{\circ}$, the "standard concentration", is defined as 1 M in standard conditions, so this often simplifies to:

$$\mu_{\mathrm{x}} = \mu_{\mathrm{x}}^{\circ} + RT \ln [\mathrm{x}] \tag{1.5}$$

  But the original equation can help to understand the *biochemical standard conditions*, as we will see later

- For liquid:

$$\mu_{\mathrm{x}} = \mu_{\mathrm{x}}^{\circ} + RT \ln \left(\frac{[\mathrm{x}]}{[\mathrm{x}]^{\circ}} \right) \tag{1.6}$$

  The equation is similar to that for solute, but the "standard concentration" is the concentration of *pure liquid* at 1 atm and 298K.

1.1.3 A Closer Look at $\Delta G = \Delta G^{\circ} + RT \ln \Gamma$

Usage

Derivation

For the reaction

$$a\mathrm{A} + b\mathrm{B} \rightleftharpoons c\mathrm{C} + d\mathrm{D}$$

$$\begin{aligned}
\Delta G &= \sum G(\text{RHS}) - \sum G(\text{LHS}) \\
&= \sum_{\text{RHS}} \mu_{\text{x}} n_{\text{x}} - \sum_{\text{LHS}} \mu_{\text{x}} n_{\text{x}} \\
&= \sum_{\text{RHS}} n_{\text{x}} \left(\mu_{\text{x}}^{\circ} + RT \ln [\text{x}]\right) - n_{\text{x}} \sum_{\text{LHS}} \left(\mu_{\text{x}}^{\circ} + RT \ln [\text{x}]\right) \\
&= \left[c \left(\mu_{\text{C}}^{\circ} + RT \ln [\text{C}]\right) + d \left(\mu_{\text{D}}^{\circ} + RT \ln [\text{D}]\right)\right] - \left[a \left(\mu_{\text{A}}^{\circ} + RT \ln [\text{A}]\right) + b \left(\mu_{\text{B}}^{\circ} + RT \ln [\text{B}]\right)\right] \\
&= \left(c\mu_{\text{C}}^{\circ} + cRT \ln [\text{C}] + d\mu_{\text{D}}^{\circ} + dRT \ln [\text{D}]\right) - \left(a\mu_{\text{A}}^{\circ} + aRT \ln [\text{A}] + b\mu_{\text{B}}^{\circ} + bRT \ln [\text{B}]\right) \\
&= G_{\text{C}}^{\circ} + RT \ln [\text{C}]^{c} + G_{\text{D}}^{\circ} + RT \ln [\text{D}]^{d} - G_{\text{A}}^{\circ} - RT \ln [\text{A}]^{a} - G_{\text{B}}^{\circ} - RT \ln [\text{B}]^{b} \\
&= \left(G_{\text{C}}^{\circ} + G_{\text{D}}^{\circ} - G_{\text{A}}^{\circ} - G_{\text{B}}^{\circ}\right) + RT \left(\ln [\text{C}]^{c} + \ln [\text{D}]^{d} - \ln [\text{A}]^{a} - \ln [\text{B}]^{b}\right) \\
&= \Delta G^{\circ} + RT \ln \left(\frac{[\text{C}]^{c}[\text{D}]^{d}}{[\text{A}]^{a}[\text{B}]^{b}}\right)
\end{aligned} \tag{1.7}$$

which can be generalied to:

$$\Delta G = \Delta G^{\circ} + RT \ln \left(\frac{\prod_{\text{products}} [\text{x}]^{m_{\text{x}}}}{\prod_{\text{reactants}} [\text{x}]^{m_{\text{x}}}}\right) \tag{1.8}$$

where m_{x} is the stiochiometry coefficient of x. For convenience, the ln term is referred to as the *mass action ratio*, Γ:

$$\Gamma = \left(\frac{\prod_{\text{products}} [\text{x}]^{m_{\text{x}}}}{\prod_{\text{reactants}} [\text{x}]^{m_{\text{x}}}}\right) \tag{1.9}$$

and so:

$$\boxed{\Delta G = \Delta G^{\circ} + RT \ln \Gamma} \tag{1.10}$$

Q.E.D

1.1.4 A Closer look at

Whenever you see "at equilibrium" in a question,

1.2 Biochemical Standard State

1.2.1 Conversion Between ΔG° and $\Delta G^{\circ\prime}$

Just treat biochemical standard state as a "non-standard" state in which H^+ is 10^{-7} M and everything else is 1 M. Then you know how to use $\Delta G = \Delta G^{\circ} + RT \ln \Gamma$ to calculate it. Later you'll learn to convert between E° and $E^{\circ\prime}$. It's the same idea! Just treat it as a non-standard state. Let's look at an example:

1.2.2 Calculating $\Delta G^{\circ\prime}$ from Concentrations

Just ignore any concentrations of H^+ (if given, it)

Chapter 2

Redox and Electrochemistry

The plus and minus of redox and electrochemistry often cause frustration. However, if you follow a "stereotypical logic", it can't get wrong.

2.1 Prerequisite

Before we can play with redox, we must be familiar with two equations:

1. Enhanced version (taking electrostatic effects into account) of chemical potential:

$$\boxed{\bar{\mu}_j = \bar{\mu}_j^\circ + RT \ln [j] + z_j F \Phi} \tag{2.1}$$

   where

   - $\bar{\mu}_j$ is the electrochemical potential, with units $\mathrm{kJ\,mol^{-1}}$
   - z_j is the charge on the solute j. It has no units. For example, $z_j(\mathrm{Ca^{2+}}) = 2$, $z_j(\mathrm{Cl^-}) = -1$
   - F is the Faraday constant, i.e. the charge (in coulombs) carried by 1 mole of electrons, which equals to 96485 $\mathrm{C\,mol^{-1}}$
   - Φ is the electric potential, with unit V.

2. Relationship between the redox potential, E, and the Gibbs free energy change, ΔG:

$$\boxed{\Delta G = -nFE}$$
$$(\text{so } \Delta G^\circ = -nFE^\circ \text{ and } \Delta G^{\circ\prime} = -nFE^{\circ\prime}) \tag{2.2}$$

   where n is the electron transferred in the reaction (more on this later).

You may ignore the following two subsections and jump directly to their applications starting at section 2.2 on page 6

2.1.1 A Closer Look at Equation 2.1

Let's first check the units are correct:

2.1.2 A Closer Look at Equation 2.2

2.2 Membrane Potential

Membrane potential is denoted by $\Delta\Phi$, and by convention, it is the electric potential inside the cell compared to outside the cell:

$$\Delta\Phi = \Phi(\text{in}) - \Phi(\text{out}) \tag{2.3}$$

$\Delta\Phi$ is the electric potential across the membrane at which the movement of a charged ion (with different concentrations on each side) into the membrane is exactly balanced by its movement out of the membrane (i.e. at equilibrium). For example, if you have 100 mM K^+ inside and 10 mM K^+ outside. Apparently K^+ wants to go outside, but in doing so, it lefts a negative charge behind (if its motion is not balanced by the m

For a clear understanding of membrane potential, I recommend reading Chapter 6 of *Principles of Neural Science*[1] If you prefer videos, visit Harvard's Fundamentals of Neuroscience (mcb80x)[2] (I know it's biased because I am (will be) a neuroscientist, but the concept of membrane potential is so central so neuroscience so that books and courses of neuroscience are all dedicated to explain this clearly)

2.3 Redox Reactions

2.3.1 Simple Rules

To play with redox reactions, we just need to learn some simple new rules (plus rules and equations that have been learnt previously).

1. Positive (or less negative) E° means the reaction wants to go forward, negative (or more positive) E° means the reaction wants to go backward. (Also applies to E and $E^{\circ\prime}$; same for other rules)

2. Reversing the reaction changes the plus-minus sign of E°

3. Adding two redox equations gives a new equation with E° that equals to the sum of the two original E°s

4. Multiplying the reaction by a constant does *not* change E°

[1] Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. J. (2013). *Principles of Neural Science* (5 ed.): McGraw-Hill.

[2] https://www.mcb80x.org/map#!/staging/electrical_properties/resting_potential

Let's look at one example:

$$\begin{aligned} \mathrm{Cr_2O_7^{2-}} + 14\,\mathrm{H^+} + 5\,\mathrm{e^-} &\longrightarrow 2\,\mathrm{Cr^{3+}} + 7\,\mathrm{H_2O} \quad E^\circ = 1.33\,\mathrm{V} \\ \mathrm{Cu^{2+}} + 2\,\mathrm{e^-} &\longrightarrow \mathrm{Cu} \qquad\qquad\qquad\quad E^\circ = 0.34\,\mathrm{V} \end{aligned} \tag{2.4}$$

For these two half-equations, which one will go forward and which one backward? $\mathrm{Cu^{2+}/Cu}$ will go backward because it's less positive (rule 1). So let's reverse it:

$$\begin{aligned} \mathrm{Cr_2O_7^{2-}} + 14\,\mathrm{H^+} + 5\,\mathrm{e^-} &\longrightarrow 2\,\mathrm{Cr^{3+}} + 7\,\mathrm{H_2O} \quad E^\circ = 1.33\,\mathrm{V} \\ \mathrm{Cu} &\longrightarrow \mathrm{Cu^{2+}} + 2\,\mathrm{e^-} \quad E^\circ = -0.34\,\mathrm{V} \end{aligned} \tag{2.5}$$

Note the change of sign (rule 2). Before we add them together, we need to balance them, using number of electrons as a reference:

$$\begin{aligned} 2\,\mathrm{Cr_2O_7^{2-}} + 28\,\mathrm{H^+} + 10\,\mathrm{e^-} &\longrightarrow 4\,\mathrm{Cr^{3+}} + 14\,\mathrm{H_2O} \quad E^\circ = 1.33\,\mathrm{V} \\ 5\,\mathrm{Cu} &\longrightarrow 5\,\mathrm{Cu^{2+}} + 10\,\mathrm{e^-} \quad E^\circ = -0.34\,\mathrm{V} \end{aligned} \tag{2.6}$$

Note that the values of E° is unchanged (rule 4). Now we can add them together:

$$2\,\mathrm{Cr_2O_7^{2-}} + 28\,\mathrm{H^+} + 5\,\mathrm{Cu} \longrightarrow 4\,\mathrm{Cr^{3+}} + 5\,\mathrm{Cu^{2+}} + 14\,\mathrm{H_2O} \quad \Delta E^\circ = 0.99\,\mathrm{V} \tag{2.7}$$

where $0.99 = 1.33 + (-0.34)$ (rule 3).

Of course, in reality you won't do this step-by-step. This is just to show the logic you should have in your brain when approaching this kind of questions.

2.3.2 Nernst Equation in Redox Reactions

The Nernst equation is used to solve for E and ΔE (and $E^{\circ\prime}$ and $\Delta E^{\circ\prime}$), i.e. E in non-standard conditions. If you don't like it, don't remember it. It'll be fine. It is just combining $G = -nFE$ with You may not like to use it because

Chapter 3

Solution Thermodynamics

Part II

Kinetics

Chapter 4

Chemical Kinetics

Chapter 5

Enzyme Kinetics

5.1 The Michaelis–Menten equation

5.1.1 Two Approximation Methods

Two approximation methods, namely steady state approximation and equilibrium approximation, can be used to derive the M-M equation. Both are actually based on the same scheme: (but with different interpretation)

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

where E, S, ES, and P represents free enzyme, substrate, enzyme-substrate complex, and the product, respectively. (k_2, the turnover number, is also known as k_{cat}) In both methods:

- The **rate** (of product formation), v, is equal to $k_2[\mathrm{ES}]$ **(Remember this!)**

$$v = k_2[\mathrm{ES}] \tag{5.1}$$

- The Michaelis-Menten constant, K_{m}, is equal to the **rate constant of [ES] breakdown divided by rate constant of [ES] formation**. But the exact calculation is slightly different, as we will see shortly.

- In deducing the Michaelis-Menten equation, both methods relate [ES] to $[\mathrm{E}]_{\mathrm{total}}$, and thus relate v to $V_{\max}$ using the fact that

$$V_{\max} = k_2[\mathrm{E}]_{\mathrm{total}} \tag{5.2}$$

  (Because the theoretical maximum rate is achieved when all enzymes ($[\mathrm{E}]_{\mathrm{total}}$) are in [ES] state.)

The difference is that:

- **Equilibrium approximation** treats the first part (red) as an independent process of the second part, so it first calculates [ES] based on the equilibrium (red) part, then use this value to calculate the second (black) part. It thus assumes that breakdown of ES to products (the black part) has negligible perturbation on the equilibrium (the red part).

  If we only look at the red part, what is the rate constant of [ES] formation and breakdown? It is k_1 and k_{-1}. So what is the K_m? It is:

  $$K_\mathrm{m} = \frac{k_{-1}}{k_1}$$

  You might have noticed that this is in fact equivalent to the dissociation constant of [ES] in the equilibrium $\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES}$! If you don't see this, don't worry:

  $$\begin{gathered}
  \mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \\
  \text{Rate of ES formation} = \text{rate of forward reaction} = k_1[\mathrm{E}][\mathrm{S}] \\
  \text{Rate of ES formation} = \text{rate of backward reaction} = k_{-1}[\mathrm{ES}] \\
  \textbf{At equilibrium:} \\
  \text{Rate of forward reaction} = \text{rate of backward reaction, so:} \\
  k_1[\mathrm{E}][\mathrm{S}] = k_{-1}[\mathrm{ES}] \\
  \text{Rearrange:} \\
  \frac{k_{-1}}{k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}
  \end{gathered} \tag{5.3}$$

  which is the dissociation constant of [ES] (i.e. the equilibrium constant of the backward reaction).

- **Steady state approximation** takes into account that breakdown of [ES] to P will increase the rate of disappearance of [ES], so it is a more accurate approach.

  $$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

  If we look at both parts of the equation, what is the rate constant of [ES] formation? It is still k_1. But what is the rate constant of [ES] breakdown? This time it is $(k_{-1} + k_2)$. So what is the K_m? It is:

  $$K_\mathrm{m} = \frac{k_{-1} + k_2}{k_1}$$

A simple way to remember the assumptions made by the two methods and subsequent steps in deriving the M-M equation is to remember that the definition of K_m in the equilibrium approximation is the same as in the steady state

approximation but ignoring k_2.

$$\begin{aligned} K_\mathrm{m} &= \frac{k_{-1}+k_2}{k_1} && \text{Steady state approximation} \\ K_\mathrm{m} &= \frac{k_{-1}}{k_1}\left(= \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} = K_\mathrm{d}^{\mathrm{S/ES}}\right) && \text{Equilibrium approximation} \end{aligned} \tag{5.4}$$

5.1.2 Equilibrium Approximation

This is a less vigorous approach, but it's simpler. It also give rise to the equations of enzyme inhibition in section 5.2 on page 15. It is based on the following model in which E + S are in equilibrium (red) with ES, independent of the formation of product (black), then a little [ES] give rise to P (see 11):

$$\mathrm{E} + \mathrm{S} \underset{}{\overset{K_\mathrm{m}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

K_m is defined as the dissociation constant of ES:

$$K_\mathrm{m} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} \text{ so } [\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{m}} \tag{5.5}$$

Define θ as the proportion of [ES], and note that $[\mathrm{E}]_\mathrm{total}$ is equal to the sum of concentration the two forms of E:

$$\theta = \frac{[\mathrm{ES}]}{[\mathrm{E}]_\mathrm{total}} = \frac{[\mathrm{ES}]}{[\mathrm{E}]+[\mathrm{ES}]} = \frac{\frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{m}}}{[\mathrm{E}]+\frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{m}}} \tag{5.6}$$

Multiplying by K_m and dividing by [E]:

$$\boxed{\theta = \frac{[\mathrm{S}]}{K_\mathrm{m}+[\mathrm{S}]}} \tag{5.7}$$

(Note how this is reminiscent of the ligand binding equation.) Recall that $v = k_2[\mathrm{ES}]$, and $V_\mathrm{max} = k_2[\mathrm{E}]_\mathrm{total}$: (equations 5.1 and 5.2 on page 11)

$$v = k_2\theta[\mathrm{E}]_\mathrm{total} = V_\mathrm{max}\theta \tag{5.8}$$

Substituting the result in equation 5.17 for θ, we arrive at the Michaelis–Menten equation:

$$\boxed{v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m}+[\mathrm{S}]}} \tag{5.9}$$

Q.E.D

5.1.3 Steady State Approximation

The steady state takes a more holistic approach to analyse the kinetic scheme

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

At steady state:

$$\underbrace{k_1[\mathrm{E}][\mathrm{S}]}_{\text{rate of [ES] formation}} = \underbrace{(k_{-1} + k_2)[\mathrm{ES}]}_{\text{rate of [ES] breakdown}} \tag{5.10}$$

Again we relate [ES] to $[\mathrm{E}]_{\text{total}}$, but this time we use subtraction:

$$[\mathrm{E}] = [\mathrm{E}]_{\text{total}} - [\mathrm{ES}] \tag{5.11}$$

Plug into equation 5.10:

$$k_1[\mathrm{S}]([\mathrm{E}]_{\text{total}} - [\mathrm{ES}]) = (k_{-1} + k_2)[\mathrm{ES}] \tag{5.12}$$

Work up to find an expression for [ES]:

$$\begin{aligned}
k_1[\mathrm{S}][\mathrm{E}]_{\text{total}} - k_1[\mathrm{S}][\mathrm{ES}] &= (k_{-1} + k_2)[\mathrm{ES}] \\
(k_{-1} + k_2)[\mathrm{ES}] + k_1[\mathrm{S}][\mathrm{ES}] &= k_1[\mathrm{S}][\mathrm{E}]_{\text{total}} \\
(k_{-1} + k_2 + k_1[\mathrm{S}])[\mathrm{ES}] &= k_1[\mathrm{S}][\mathrm{E}]_{\text{total}} \\
[\mathrm{ES}] &= \frac{k_1[\mathrm{S}][\mathrm{E}]_{\text{total}}}{(k_{-1} + k_2) + k_1[\mathrm{S}]} \\
\text{dividing by } k_1\text{: } [\mathrm{ES}] &= \frac{[\mathrm{S}][\mathrm{E}]_{\text{total}}}{\frac{k_{-1} + k_2}{k_1} + [\mathrm{S}]}
\end{aligned} \tag{5.13}$$

K_{m} is defined as: (see 11)

$$K_{\mathrm{m}} = \frac{k_{-1} + k_2}{k_1} \tag{5.14}$$

Plugging into equation 5.13:

$$[\mathrm{ES}] = \frac{[\mathrm{S}][\mathrm{E}]_{\text{total}}}{K_{\mathrm{m}} + [\mathrm{S}]} = [\mathrm{E}]_{\text{total}} \left(\frac{[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]} \right) \tag{5.15}$$

So rate equals: (if you don't see this, review equations 5.1 and 5.2 on page 11)

$$v = k_2[\mathrm{ES}] = \underbrace{k_2[\mathrm{E}]_{\text{total}}}_{V_{\max}} \left(\frac{[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]} \right) \tag{5.16}$$

Substitute for $V_{\max}$, giving the Michaelis-Menten equation:

$$\boxed{v = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}} \tag{5.17}$$

Q.E.D

5.2 Inhibition

5.2.1 The General Equation

Behaviours of all types of inhibition can be explained by the general model and quantified by the variants of the general equation.

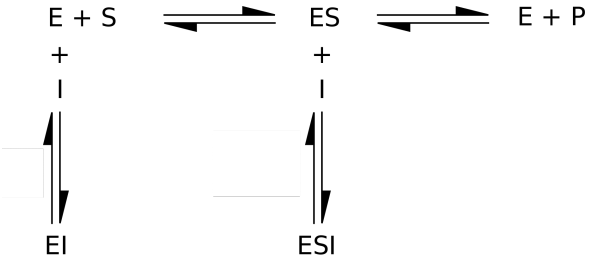

Figure 5.1: The general scheme of enzyme inhibition

E, S, and I associate to form ES, EI, and ESI, and only ES will give the product (Fig. 5.1). Earlier we deduced the rate equation for *uninhibited* reactions by relating [ES] to $[\mathrm{E}]_{\mathrm{total}}$ (see section 5.1.2 on page 13). Here we use a very similar approach.

Define θ as the proportion of [ES], and note that $[\mathrm{E}]_{\mathrm{total}}$ is equal to the sum of concentration of all four forms of E:

$$\theta = \frac{[\mathrm{ES}]}{[\mathrm{E}]_{\mathrm{total}}} = \frac{[\mathrm{ES}]}{[\mathrm{E}] + [\mathrm{EI}] + [\mathrm{ES}] + [\mathrm{ESI}]} \tag{5.18}$$

Recall that $v = k_2[\mathrm{ES}]$, and $V_{\max} = k_2[\mathrm{E}]_{\mathrm{total}}$:

$$v = k_2\theta[\mathrm{E}]_{\mathrm{total}} = V_{\max}\theta \tag{5.19}$$

Next let's work out θ. We need some dissociation constants. For $\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES}$, $\mathrm{E} + \mathrm{I} \rightleftharpoons \mathrm{EI}$ and $\mathrm{ES} + \mathrm{I} \rightleftharpoons \mathrm{ESI}$ we have:

$$K_{\mathrm{d}}^{\mathrm{S/ES}} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} \text{ so } [\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_{\mathrm{d}}^{\mathrm{S/ES}}}$$

$$K_{\mathrm{d}}^{\mathrm{I/EI}} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{EI}]} \text{ so } [\mathrm{EI}] = \frac{[\mathrm{E}][\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/EI}}}$$

$$K_{\mathrm{d}}^{\mathrm{I/ESI}} = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]} = \frac{[\mathrm{ES}][\mathrm{I}]}{[\mathrm{ESI}]} \text{ so } [\mathrm{ESI}] = \frac{[\mathrm{ES}][\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/ESI}}} = \frac{\frac{[\mathrm{E}][\mathrm{S}]}{K_{\mathrm{d}}^{\mathrm{S/ES}}}[\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/ESI}}} = \frac{[\mathrm{E}][\mathrm{S}][\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/ESI}} K_{\mathrm{d}}^{\mathrm{S/ES}}} \tag{5.20}$$

Combining equations 5.18 and 5.20:

$$\theta = \frac{\frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{d}^{\mathrm{S/ES}}}}{[\mathrm{E}] + \frac{[\mathrm{E}][\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}} + \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{d}^{\mathrm{S/ES}}} + \frac{[\mathrm{E}][\mathrm{S}][\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}} K_\mathrm{d}^{\mathrm{S/ES}}}} \tag{5.21}$$

Dividing by [E] and multiplying by $K_\mathrm{d}^{\mathrm{S/ES}}$:

$$\theta = \frac{[\mathrm{S}]}{K_\mathrm{d}^{\mathrm{S/ES}} + \frac{[\mathrm{I}] K_\mathrm{d}^{\mathrm{S/ES}}}{K_\mathrm{d}^{\mathrm{I/EI}}} + [\mathrm{S}] + \frac{[\mathrm{S}][\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}} \tag{5.22}$$

Rearrange:

$$\theta = \frac{[\mathrm{S}]}{K_\mathrm{d}^{\mathrm{S/ES}} \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}}\right) + [\mathrm{S}] \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)} \tag{5.23}$$

Combining equations 5.19 and 5.23:

$$v = V_\mathrm{max}\theta = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{d}^{\mathrm{S/ES}} \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}}\right) + [\mathrm{S}] \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)} \tag{5.24}$$

Note that $K_\mathrm{d}^{\mathrm{S/ES}}$ is actually equal to K_m, so the equation is also written as

$$\boxed{v = V_\mathrm{max}\theta = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m} \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}}\right) + [\mathrm{S}] \left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)}} \tag{5.25}$$

Q.E.D

This is the general equation.

5.2.2 Specific Types of Inhibition

Mixed Inhibition

Mixed inhibition means both EI and ESI will form (the inhibitor will bind to free enzymes as well as ES complexes). This means $K_\mathrm{d}^{\mathrm{I/EI}} \neq \infty$ and $K_\mathrm{d}^{\mathrm{I/ESI}} \neq \infty$. Plug in values for $K_\mathrm{d}^{\mathrm{I/EI}}$ and $K_\mathrm{d}^{\mathrm{I/ESI}}$, [I], [S], and V_max and the rate can be calculated. Other types of inhibition really are "special cases" of mixed inhibition. **Don't memorise the equations!** Just think about the definitions of these types of inhibition and how the $K_\mathrm{d}^{\mathrm{I/EI}}$ and $K_\mathrm{d}^{\mathrm{I/ESI}}$ values are different. But for completeness, I'll include the rationales here.

Competitive Inhibition

In competitive inhibition, only EI, but not ESI, forms. This means $K_\mathrm{d}^{\mathrm{I/ESI}} = \infty$. So the general equation (5.25) reduces to:

$$v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m}\left(1 + \dfrac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}}\right) + [\mathrm{S}]} \tag{5.26}$$

Compare it to the rate equation of the uninhibited reaction (the Michaelis–Menten equation):

$$v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

We note that only K_m changed, and this new value is called "apparent" K_m, or $K_\mathrm{m}^\mathrm{app}$:

$$K_\mathrm{m}^\mathrm{app} = K_\mathrm{m}\left(1 + \frac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/EI}}}\right) \tag{5.27}$$

Uncompetitive Inhibition

Uncompetitive is the inverse of competitive inhibition. In uncompetitive inhibition, only ESI, but not EI, forms. This means $K_\mathrm{d}^{\mathrm{I/EI}} = \infty$. So the general equation (5.25) reduces to:

$$v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]\left(1 + \dfrac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)} \tag{5.28}$$

Be aware, uncompetitive inhibition reduces both K_m and V_max by the same factor. We can see this first by rearranging:

$$v = \frac{\dfrac{V_\mathrm{max}}{\left(1 + \dfrac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)}[\mathrm{S}]}{\dfrac{K_\mathrm{m}}{\left(1 + \dfrac{[\mathrm{I}]}{K_\mathrm{d}^{\mathrm{I/ESI}}}\right)} + [\mathrm{S}]} \tag{5.29}$$

and comparing it to the uninhibited reaction:

$$v = \frac{V_\mathrm{max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

This is not quite important in exams, though.

Non-Competitive Inhibition

The non-competitive inhibition is yet another special case in which both ES and ESI form but their dissociation constants are equal. ($K_{\mathrm{d}}^{\mathrm{I/EI}} = K_{\mathrm{d}}^{\mathrm{I/ESI}} \neq \infty$) So the general equation (5.25) becomes:

$$v = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}}\left(1 + \frac{[\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/EI}}}\right) + [\mathrm{S}]\left(1 + \frac{[\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/EI}}}\right)} \tag{5.30}$$

Dividing by the red part:

$$v = \frac{\frac{V_{\max}}{\left(1 + \frac{[\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/EI}}}\right)}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]} \tag{5.31}$$

and comparing to the uninhibited reaction:

$$v = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

We note that only $V_{\max}$ is reduced, and this "apparent" $V_{\max}$ is equal to:

$$V_{\max}^{\mathrm{app}} = \frac{V_{\max}}{\left(1 + \frac{[\mathrm{I}]}{K_{\mathrm{d}}^{\mathrm{I/EI}}}\right)} \tag{5.32}$$

5.2.3 Why Specificity Constant Specifies Specificity

All textbooks says:

$$\text{Specificity Constant} = \frac{k_{\mathrm{cat}}}{K_{\mathrm{m}}} \tag{5.33}$$

But none of them explain it!

$$\mathrm{P}_1 \xleftarrow{v_1} \mathrm{S}_1 + \mathrm{E} + \mathrm{S}_2 \xrightarrow{v_2} \mathrm{P}_2$$

Specificity constant is used to calculate the relative rate of two reactions catalysed by the same reaction. For the reactions shown above:

$$\frac{v_1}{v_2} = \frac{\text{specificity constant 1} \times [\mathrm{S}_1]}{\text{specificity constant 2} \times [\mathrm{S}_2]} = \frac{\frac{k_{\mathrm{cat1}}}{K_{\mathrm{m1}}} \times [\mathrm{S}_1]}{\frac{k_{\mathrm{cat2}}}{K_{\mathrm{m2}}} \times [\mathrm{S}_2]} \tag{5.34}$$

Now you know how to *use* specificity constant (many textbooks even omit this!), but, *why*?

In fact the math behind is very simple. Let's first write the kinetic scheme:

$$\mathrm{P}_1 \xleftarrow{k_{\mathrm{cat1}}} [\mathrm{ES}_1] \underset{\text{i.e. } K_{\mathrm{m1}}}{\overset{K_{\mathrm{d}}^{\mathrm{S}_1/\mathrm{ES}_1}}{\rightleftharpoons}} \mathrm{S}_1 + \mathrm{E} + \mathrm{S}_2 \underset{\text{i.e. } K_{\mathrm{m2}}}{\overset{K_{\mathrm{d}}^{\mathrm{S}_2/\mathrm{ES}_2}}{\rightleftharpoons}} [\mathrm{ES}_2] \xrightarrow{k_{\mathrm{cat2}}} \mathrm{P}_2$$

Then, using equilibrium approximation (c.f. section 5.1.2 on page 13):

$$\begin{aligned} \frac{v_1}{v_2} &= \frac{k_{\mathrm{cat1}}[\mathrm{ES}_1]}{k_{\mathrm{cat2}}[\mathrm{ES}_2]} \\ &= \frac{k_{\mathrm{cat1}} \times \dfrac{[\mathrm{E}][\mathrm{S}_1]}{K_{\mathrm{m1}}}}{k_{\mathrm{cat2}} \times \dfrac{[\mathrm{E}][\mathrm{S}_2]}{K_{\mathrm{m2}}}} \end{aligned} \tag{5.35}$$

Because two reactions are in the same environment and use the same enzyme, [E] is the same so can be eliminated. So this reduces to:

$$\boxed{\frac{v_1}{v_2} = \frac{\dfrac{k_{\mathrm{cat1}}}{K_{\mathrm{m1}}} \times [\mathrm{S}_1]}{\dfrac{k_{\mathrm{cat2}}}{K_{\mathrm{m2}}} \times [\mathrm{S}_2]}} \tag{5.36}$$

Q.E.D

Simple! (And not very long!)

Part III

Quantum Physics

Appendix A

List of Equations

Epilogue

Why I Wrote This Book

I wrote this book because I am *neither* good at physics *nor* math. I usually do not directly see the math behind many equations, which the authors/lecturers might thought apparent (because they don't write them in the books/lecture notes). So I had to do them myself—on scratch paper and hoping I'll remember them—and forgot completely after one week. So I realised the importance of making notes. I decided to use LaTeX to make by notes, and the effect was just amazing, so I wanted to share it—and here comes the book.

The Beauty of Small Books

I was not a fan of small books—until I met Dickens' *Carbonyl Group*[1] and Sompayrac's *Immune System*[2]. I used to believe that only thick textbooks teach. Sure, they are incredibly accurate and comprehensive, and if you manage to master every bit of such a book, you'll certainly become an expert in that subject. But it's too time-consuming. Also, often, the *relationships* between different topics within a subject are more important than the comprehensive details of every single topic, and the organisation of "thick textbooks" makes them incompetent in producing such "big pictures". Big textbooks are also not optimised for your understanding—they just describe the facts. I was particularly impressed by such a clear understanding I gained in Sompayrac's *Immune system*—a 138-page book! I might be able to describe what I learnt from this little book more clearly than that from Alberts' *Molecular Biology of The Cell* (I finished reading both).

The Beauty of LaTeX

This is how I would describe LaTeX in one sentence: LaTeX allows you to create professional-looking documents (be they reports, articles or books etc.) with

[1] Dickens, T. K. & Warren S.(2018). *Chemistry of the Carbonyl Group: a step-by-step approach to understanding organic reaction mechanisms* (2 ed): Wiley

[2] Sompayrac, L. (2012). *How The Immune System Works* (5 ed.): Wiley Blackwell.

great ease, and makes it difficult to produce ugly documents. For example:

- If you type two spaces between two words in Microsoft Word, it will result in two spaces; in LaTeX, it's still one.
- In Microsoft Word, after you manually add hyphenations, if you add words before any hyphenated words, they will move to their corresponding next lines, retaining their hyphens and you'll need to remove them manually. In LaTeX, hyphenations are added whenever necessary—automatically!

Also, importantly, it's free! For more motivations, google "why LaTeX".

Dexter Chua's Cambridge Notes[3], written with LaTeX, were an important motivation for me to use LaTeX to make my notes—and to share them. (Although I never ever understand the contents—and Chua is much stronger than me; he's been at Harvard since 2018)

[3] http://dec41.user.srcf.net