PART I:

SYSTEMS MICROBIOLOGY

‘A living cell as a well-stirred bioreactor’

<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Michaelis-Menten kinetics</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>Equilibrium binding and cooperativity</td>
<td>8</td>
</tr>
<tr>
<td>III</td>
<td>A Genetic Switch in Lambda Phage</td>
<td>16</td>
</tr>
<tr>
<td>IV</td>
<td>Synthetic genetic switches</td>
<td>26</td>
</tr>
<tr>
<td>V</td>
<td>Stability analysis</td>
<td>29</td>
</tr>
<tr>
<td>VI</td>
<td>Biological Oscillators</td>
<td>32</td>
</tr>
<tr>
<td>VII</td>
<td>Modeling <em>Escherichia coli</em> chemotaxis</td>
<td>41</td>
</tr>
<tr>
<td>VIII</td>
<td>Stochastic Chemical Kinetics</td>
<td>49</td>
</tr>
</tbody>
</table>
Michaelis-Menten kinetics

The goal of this chapter is to develop the mathematical techniques to quantitatively model biochemical reactions. Biochemical reactions in living cells are often catalyzed by enzymes. These enzymes are proteins that bind and subsequently react specifically with other molecules (other proteins, DNA, RNA, or small molecules) defined as substrates. A few examples:

1. The conversion of glucose (substrate) into glucose-6-phosphate (product) by the protein hexokinase (enzyme).
2. Transcription: binding of the RNA polymerase (enzyme) to the promoter region of the DNA (substrate) results in transcription of the mRNA (product).
3. The phosphorylation of a protein: the unphosphorylated protein CheY (substrate, regulating the direction of rotation of the bacterial flagella) is phosphorylated by a phosphate CheZ (enzyme) resulting in CheY-p (product).

All these reactions involve a substrate $S$ reacting with an enzyme $E$ to form a complex $ES$ which then in turn is converted into product $P$ and the enzyme:

\[ E + S \begin{array}{c} \leftrightarrow \\ k_1 \end{array} ES \begin{array}{c} \rightarrow \\ k_2 \\ k_1 \end{array} E + P \]  \[ \text{[1.1]} \]

In this scheme there are two fundamental different reactions. The first reaction depicted with the double arrow is a reversible reaction reflecting the reversible binding and unbinding of the enzyme and the substrate. The second reaction is an irreversible reaction in which the enzyme-substrate complex is irreversibly converted into product and enzyme symbolized by the single arrow. The rate of a reaction is proportional to the product of the concentrations of the reactants. The kinetics of the chemical equations above is described by the following set of coupled differential equations:
\[
\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]
\]
\[
\frac{d[E]}{dt} = -k_1[E][S] + (k_{-1} + k_2)[ES]
\]
\[
\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_2)[ES]
\]
\[
\frac{d[P]}{dt} = k_2[ES] \equiv v
\]

Note that \( k_1 \) and \( k_{-1} \) have different units, \( 1/(\text{Ms}) \) and \( 1/\text{s} \) respectively. The turnover rate \( v \) is defined as the increase (or decrease) in product over time, which is directly proportional to the concentration of enzyme-substrate complex \([ES]\). For the analysis below we will assume initial conditions: \([S]_{t=0} = S_0; [E]_{t=0} = E_0; [ES]_{t=0} = 0; [P]_{t=0} = 0\).

Since the enzyme is a catalyst that facilitates the reaction but does not react itself, the total concentration of enzyme (free + bound) should be constant:
\[
E_0 = [E] + [ES]
\]

Using this conservation law the four differential equations \([I.2]\) reduce to three coupled ordinary differential equations:
\[
\frac{d[S]}{dt} = -k_1E_0[S] + (k_1[S] + k_{-1})[ES]
\]
\[
\frac{d[ES]}{dt} = k_1E_0[S] - (k_1[S] + k_{-1} + k_2)[ES]
\]
\[
\frac{d[P]}{dt} = k_2[ES] \equiv v
\]

with the initial conditions \([S]_{t=0} = S_0, [ES]_{t=0} = 0, \) and \([P]_{t=0} = 0\). Matlab code 1 solves these equations and calculates the time dependence of the concentrations \([S], [ES]\) and \([P]\) as a function of the initial concentrations \([S_0]\) and \([E_0]\) and the rate constants \(k_1, k_{-1}, \) and \(k_2\). In this case the systems can also be solved analytically. Figure 1 shows an example of the time dependence of the chemical components for \(k_1[S_0] \approx k_{-1} >> k_2\). This is often the regime of biological relevance since the substrate-enzyme binding occurs at much faster time scales than the turnover into product. The thermodynamic equilibrium or steady state \((t \to \infty)\) of this system would be \([S] = [ES] = 0; [E] = [E_0]; [P] = [S_0]\). However the relevant time-scale to consider is the time range in which \([ES]\) and \([E]\) are
relatively constant. This state is often called the quasi-equilibrium or pseudo-steady state. Under these circumstances one expects that after an initial short transient period there will be a balance between the formation of the enzyme-substrate complex and the breaking apart of complex (either to enzyme and substrate, or to enzyme and product). In the pseudo-steady state \( \frac{d[ES]}{dt} = \frac{d[E]}{dt} = 0 \) (I.4) reduces to:

\[
[ES] = \frac{k_t[S]E_0}{k_t[S] + k_{-1} + k_2}
\]

\[
v = \frac{dP}{dt} = \frac{k_2[S]E_0}{k_{-1} + k_2 + [S]}
\]  

[I.5]

In the case of many more substrate than enzyme molecules \( S_o >> E_o \), this pseudo-steady state will be achieved before there is perceptible transformation of substrate into product. In this case the equation [I.5] leads to the traditional Michaelis-Menten equation, which predicts the initial turnover rate of the enzymatic reaction \( v_o \) as a function of initial substrate concentration \( S_o \):

\[
v_o = \frac{v_{max} S_o}{K_m + S_o}
\]  

[I.6]

where the constant \( K_m = (k_{-1}+k_2)/k_t \) is called the Michaelis constant and \( v_{max} = k_2E_o \) is the maximum turn-over rate. The Michaelis constant has units of concentration and reflects the affinity of the reaction. Strong affinity means small \( K_m \). At a concentration \( K_m \) the turn-over rate is 0.5\( v_{max} \) (Fig. 2).
Figure 1. The time dependence of the substrate, enzyme, enzyme-substrate complex, and product concentration. This graph was generated by using Matlab code 1. The upper panel uses a logarithmic x-axis whereas the lower panel uses a linear scale.
Figure 2. The initial turnover rate as given by the Michaelis-Menten formula [1.6].
Matlab code 1: Michaelis-Menten kinetics

% filename: mm.m

close all;
clear;

k1=1e3;       % units 1/(Ms)
k_1=0.1;      % units 1/s
k2=0.05;      % units 1/s
E0=0.5e-3;    % units M
S0=0.001;     % units M
options=[];

[t y]=ode23('mmfunc',[0 100],[S0 0 0],options,k1,k_1,k2,E0);

S=y(:,1);
ES=y(:,2);
E=E0-ES;
plot(t,S,'.-r',t,E,'.-b',t,ES,'.-g',t,P,'.-c');
legend('S','E','ES','P',0);
xlabel('Time (s)');
ylabel('Concentration (M)');

figure
vmax=k2*E0;
Km=(k_1+k2)/k1;
v_real=k2*ES;
v_predicted=(k2*E0*S)./(Km+S);
plot(t,v_real,'.-r',t,v_predicted,'.-b');
legend('Calculatd turnover rate v','Predicted turnover rate v0',0);
xlabel('Time (s)');
ylabel('Turn-over rate (M/s)');

% filename: mmfunc.m

function dydt = f(t,y,flag,k1,k_1,k2,E0)
% [S] = y(1), [ES] = y(2), [P] = y(3)

dydt = [-k1*E0*y(1)+(k1*y(1)+k_1)*y(2);
         k1*E0*y(1)-(k1*y(1)+k_1+k2)*y(2);
         k2*y(2)];
II Equilibrium binding and cooperativity

In the previous Section we considered Michaelis-Menten kinetics. We found that the traditional form of the Michaelis-Menten equation [I.6] is derived by assuming a quasi-steady state in which the concentration of enzyme-substrate complex is fairly constant over time. Additionally we had to assume that initially the substrate is in excess. In this Section, we first will take a step back and focus on the steady state behavior of reversible reactions and introduce the concept of multiple binding sites. Initially we will consider multiple binding sites that are independently binding substrates. However for most protein complexes the binding of substrates is not independent. For example, after binding the first substrate molecule the binding probability of the second substrate is affected. This phenomenon is called cooperativity.

In the previous section it was assumed that one substrate molecule binds to one enzyme molecule. In biological reactions however proteins often bind multiple substrates. Assume a protein has n binding sites for a substrate. $P_j$ denotes the protein bound to j substrate molecules S. The reactions describing this process are:

$$S + P_{j-1} \leftrightarrow P_j$$  \[II.1\]

where $j = 1, 2, \ldots, n$.

The time-evolution of the concentration of unbound protein $P_o$ is (j=1):

$$\frac{d[P_o]}{dt} = -k_{+1}[P_o][S] + k_{-1}[P_1]$$ \[II.2\]

where $k_{+1}$ and $k_{-1}$ are the forward and backward rate constants of [II.1] for j=1. The association (1/dissociation constants) is defined as:

$$K_a = \frac{k_{+1}}{k_{-1}}$$ \[II.3\]

In steady state, $d[P_o]/dt = 0$:

$$K_a = \frac{[P_1]}{[P_o][S]}$$ \[II.4\]
To characterize all n reactions, we introduce the n association constants $K_j$, $j = 1, 2, \ldots, n$.

$$K_j = \frac{[P_j]}{[P_{j-1}][S]} \quad \text{[II.5]}$$

It is experimentally difficult to measure $[P_j]$, a more convenient quantity is the average number $r$ ($0 < r < n$) of substrates bound to the protein. Because there are $j$ substrates bound to $P_j$, $r$ is given by:

$$r = \frac{[P_1] + 2[P_2] + 3[P_3] + \ldots + n[P_n]}{[P_0] + [P_1] + [P_2] + \ldots + [P_n]} \quad \text{[II.6]}$$

Combining [II.5] and [II.6] gives Adair’s equation:

$$r = \frac{K_1[S] + 2K_2[S]^2 + 3K_3[S]^3 + \ldots + nK_n[S]^n}{1 + K_1[S] + K_2[S]^2 + \ldots + K_n[S]^n} \quad \text{[II.7]}$$

Note that $0 < r < n$, one often uses the normalized form, called the saturation function $Y = r/n$ ($0 < Y < 1$).

**Identical and independent binding sites**

For now let’s assume we have n identical binding sites and that binding at a given site is independent of the state of binding of all other sites. The rate constants $k_+$ and $k_-$ characterize the binding and unbinding rates respectively. In steady state, [II.2] can now be written as:

$$0 = -nk_+ [P_0][S] + k_- [P_1] \quad \text{[II.8]}$$

The factor n takes into account that there are n possible binding sites available for binding the first substrate. On the other hand there is only one possibility to loose a substrate going from state $P_1$ to $P_0$. Similarly for $j=2$ we can deduce:

$$0 = -(n-1)k_+ [P_1][S] + 2k_- [P_2] \quad \text{[II.9]}$$

because there are $(n-1)$ possibilities to add a substrate and only 2 possibilities to remove a substrate. If the intrinsic association constant $K$ is defined as:

$$K \equiv \frac{k_+}{k_-} \quad \text{[II.10]}$$

we find that $K_1 = nK$ and $K_2 = (n-1)K/2$. In general, one can write:

$$K_j = \frac{(n-j+1)K}{j} \quad \text{[II.11]}$$
for \( j = 1, 2, \ldots, n \). By substituting [II.11] in [II.7] an explicit equation for \( r \) as a function of \( K, n, \) and \([S]\) is found. We will not go through the details of the derivation. If you are interested, see for example Bisswanger (2002, p. 11-16). The final result is elegantly simple:

\[
\frac{nK[S]}{1+K[S]} \quad [\text{II.12}]
\]

Note that the mathematical form of this equation is very similar to Michaelis-Menten kinetics. However this result is a steady-state (equilibrium) property while Michaelis-Menten equation is not. Equation [II.12] can also be derived in a more hand waving manner. As the \( n \) binding sites are identical and independent, it is not important to view them as clustered in one protein. If \([F]\) is the concentration of free binding site and \([B]\) the concentration of bound sites in steady state, then the association constant for this equilibrium is given by:

\[
K = \frac{[B]}{[F][S]} \quad [\text{II.13}]
\]

The total number of sites is: \( n[P] = [F] + [B] \), this combined with [II.13] gives:

\[
\frac{[B]}{[P]} = \frac{nK[S]}{1+K[S]} \quad [\text{II.14}]
\]

**Non-identical and independent binding sites**

Now consider the case in which the binding sites are non-identical. Each binding site family (with \( n_j \) binding sites) is characterized by its own association constant \( K_j \). At low concentrations first the binding sites with the high affinities will be occupied, the lower affinity binding site will only be occupied at larger \([S]\). As the binding site are independent the binding equation [II.12] holds for each binding site family and \( r \) is just the sum of the different individual processes:

\[
r = \frac{n_1K_1[S]}{1+K_1[S]} + \frac{n_2K_2[S]}{1+K_2[S]} + \ldots + \frac{n_mK_m[S]}{1+K_m[S]} \quad [\text{II.15}]
\]
Identical and interacting binding sites

In the following discussion we will confine ourselves to two binding sites (n=2). First, let us assume that both binding sites are identical. In this case we only have to consider three states for the protein-substrate complex: no substrate bound, one substrate molecule bound, and two substrate molecules bound. The rate constants \( k_+ \) and \( k_- \) characterize the transitions between the unbound and single-bound state, and \( k_+^* \) and \( k_-^* \) the transitions between single-bound and double-bound states. The intrinsic association constants are defined by: \( K = \frac{k_+}{k_-} \) and \( K^* = \frac{k_+^*}{k_-^*} \). Analogous to [II.10] and [II.11] we find:

\[
K_1 = 2K \\
K_2 = \frac{1}{2}K^* 
\]

[II.16]

By using Adair’s equation [II.7] we find:

\[
r = \frac{2K[S] + 2KK^*[S]^2}{1 + 2K[S] + KK^*[S]^2} 
\]

[II.17]

The saturation function \( Y = r/n \) is:

\[
Y = \frac{K[S] + KK^*[S]^2}{1 + 2K[S] + KK^*[S]^2} 
\]

[II.18]

For \( K = K^* \) we recover the hyperbolic (Michaelis-Menten like) equation [II.12]:

\[
\tilde{Y} = \frac{K[S]}{1 + K[S]} 
\]

[II.19]

Let’s compare the functional forms of [II.18] and [II.19] in more detail. The difference between the two functions is:

\[
Y - \tilde{Y} = \frac{(K^* - K)K[S]^2}{(1 + K[S])(1 + 2K[S] + KK^*[S]^2)} 
\]

[II.20]

Positive cooperativity is often defined as \( Y - \tilde{Y} > 0 \), and negative cooperativity as \( Y - \tilde{Y} < 0 \). In other words, positive cooperativity occurs when the affinity of binding a second ligand is larger than binding the first ligand \( (K^* > K) \). For negative cooperativity the binding affinity for the second ligand is smaller than for the first \( (K^* < K) \).
Another, often used, definition for cooperativity is sigmoidality (from ‘S shaped’). For a sigmoidal curve the second derivative should change sign. Let’s introduce the dimensionless variables $\beta = K^*/K$ and $x = K[S]$:

\[
Y = \frac{x(1+\beta x)}{1+2x+\beta x^2}
\]

\[
\frac{dY}{dx} = \frac{1+2x\beta + \beta x^2}{(1+2x+\beta x^2)^2}
\]

\[
\frac{d^2Y}{dx^2} = \frac{2\beta - 2 - \beta \left[3 + 3\beta x + \beta x^2\right]}{(1+2x+\beta x^2)^3}
\]

[II.21]

The second derivative can only change sign if $\beta > 2$. Note that this definition yields a different criterion for cooperativity. According to the first definition a reaction is cooperative for $\beta > 1$, whereas according to the second definition $\beta > 2$. During the rest of the course we will use the first definition.

Now consider the limit for which intermediate states can be neglected. In this example, that would mean that single-bound states are very unlikely. The effective reaction would be:

\[
P_o + 2S \leftrightarrow P_2
\]

[II.22]

The saturation function is now:

\[
Y = \frac{K[S]^2}{1+K[S]^2}
\]

[II.23]

where $K = P_2/([P_o][S]^2)$ is the association constant of reaction [II.22]. Note that in this case the units of $K$ are (M)$^{-2}$. This limit was first consider by Hill who proposed a graphical way to represent equations such as [II.23]. In a Hill plot one plots $\ln[Y/(1-Y)]$ versus $\ln[S]$. The slope of this graph is called the Hill number which is in this case equals 2. The Hill number is often used as an estimation of the number of binding sites of a protein. However one should be very careful as [II.23] involves a major assumption (no intermediate states). Let’s calculate the Hill number $n_H$ for the case [II.21] in which intermediate states are allowed:

\[
n_H = \frac{d}{d(ln[S])} \ln\left[\frac{Y}{1-Y}\right] = x \frac{d}{dx} \ln\left[\frac{Y}{1-Y}\right] = 1+ \frac{(\beta-1)x}{(1+x)(1+\beta x)}
\]

[II.24]
The Hill number is plotted in Fig. 3 as a function of x at different values of $\beta$. The Hill number only approaches 2 for very large $\beta$ and small x.

**Figure 3.** The Hill number as a function of the dimensionless concentration at different values of $\beta$ for a protein with two identical interacting binding sites. The mathematical form is given by equation [II.24].

![Diagram of two independent interacting binding sites](image)

**Figure 4.** Two independent interacting binding sites.
Non-identical and interacting binding sites

How would the analysis above change if the two binding sites are non-identical? The ligand binding to the two binding sites is now characterized by the rate constants $k_{\pm 1}$, $k_{\pm 2}$, $k_{\pm 3}$, and $k_{\pm 4}$ (Fig. 4) and the four intrinsic association constants $K_j = k_{+j}/k_{-j}$ ($j = 1,2,3,4$). In this case there are four states of the protein-ligand complex: nothing bound, site 1 bound, site 2 bound, and two sites bound. The principle of detailed balance (thermodynamic equilibrium) does not allow any net fluxes between states. Therefore:

$$ K_1 = \frac{[P_1]}{[P_0][S]}, K_2 = \frac{[P_1]}{[P_0][S]}, K_3 = \frac{[P_2]}{[P_1][S]}, K_4 = \frac{[P_2]}{[P_1][S]} \quad \text{[II.25]} $$

Rewriting [II.25] gives:

$$ K_1 K_3 = K_2 K_4 \quad \text{[II.26]} $$

The saturation function is given by:

$$ Y = \frac{1}{2} \frac{[P_1] + [P_2] + 2[P_2]}{[P_0][S]} = \frac{[P_0][S][K_1 + K_2] + 2[P_1][S]K_3}{[P_0][S][P_0][S][K_1 + K_2] + [P_1][S][S][P_0][S][K_3]} \Rightarrow $$

$$ Y = \frac{K_1[S] + K_2[S] + 2K_4 K_3[S]^2}{1 + K_1[S] + K_2[S] + K_1 K_3 [S]^2} \quad \text{[II.27]} $$

Note that [II.27] is independent of $K_4$ as expected because of the detailed balance equation [II.26].

If we define

$$ J = \frac{1}{2} (K_1 + K_2) $$

$$ J' = \frac{2K_4 K_3}{K_1 + K_2} \quad \text{[II.28]} $$

$$ x' = J[S] $$

$$ \beta' = \frac{J'}{J} $$

The saturation function can be written in the same form as for the identical interacting binding sites:

$$ Y = \frac{x'(1 + x'\beta')}{1 + 2x' + \beta'x'^2} \quad \text{[II.29]} $$

In the limit $K_1 = K_2$ we find $x = x'$ and $\beta = \beta'$ (identical interacting sites). In the limit $K_1 = K_3$ and $K_2 = K_4$ we recover the independent binding case:
In the case we can write $\beta'$ as:

$$\beta' = \frac{4K_1K_2}{(K_1 + K_2)^2} = \frac{4K_1K_2}{4K_1K_2 + (K_1 - K_2)^2}$$  \[\text{II.31}\]

Note that $\beta' = 1$ for identical sites and $\beta' < 1$ for non-identical sites. This implies that binding curves exhibiting negative cooperativity could arise from a protein that has independent binding sites or from a protein that has two interacting sites in which the second binding event is less likely than the first.

**Further reading on enzyme kinetics and cooperativity**

D. Fell. Understanding the control of metabolism (Portland Press, 1997)
L. A. Segal. Biological kinetics (Cambridge University Press, 1991)