1. Simple and Complex Models of Enzyme Kinetics

The flux bounds are essential constraints in flux balance analysis calculations and the convex decomposition of the stoichiometric array. Beyond their role in the flux estimation problem, the flux bounds are *integrative*, i.e., these constraints integrate many types of genetic and biochemical information into the flux estimation problem.

Flux bounds constrain the values that each reaction in a metabolic network can take. A general model for these bounds is given by:

$$-\delta_j \left[V^{\circ}_{max,j} \left(\frac{e}{e^{\circ}} \right) \theta_j(\ldots) f_j(\ldots) \right] \leq v_j \leq V^{\circ}_{max,j} \left(\frac{e}{e^{\circ}} \right) \theta_j(\ldots) f_j(\ldots)$$

where $V^{\circ}_{max,j}$ denotes the maximum reaction velocity (units: flux) computed at some characteristic enzyme abundance (units: concentration), the ratio e/e° is a correction for enzyme abundance (units: dimensioness), $\theta_j(\ldots) \in [0, 1]$ is the fraction of maximial enzyme activity (a function or measurement producing units: dimensionless), and $f_j(\ldots)$ is a function describing the substrate dependence of the reaction rate j (units: dimensionless). Both $\theta_j(\ldots)$ and $f_j(\ldots)$ could have associated parameters, e.g., saturation or binding constants, etc. Finally, the quanity $\delta_j \in \{0, 1\}$ is a *binary* variable:

- If reaction j is **reversible** $\delta_j = 1$ or,
- If reaction j is **irreversible** $\delta_j = 0$

Today, let's focus on approaches for computing the value of these bounds, i.e., the form and value of the terms in the brackets. In this lecture, we will:

1. Develop simple models of enzyme kinetics using the Michaelis–Menten approach
2. Introduce the fundamental concepts underlying more complex models of allosteric regulation (the fast control

mechanisms that we mentioned previously)
3. Introduce effective discrete regulation models to capture allosteric regulation

2. What is allosteric regulation?

Allosteric regulation modulates enzyme activity by binding effector molecules to sites other than the enzyme's active site. These events can activate (enhances rate) and inhibit (decreases rate). Allosteric regulation operates on a fast time scale compared to gene expression (synthesis of the enzyme).

Allosteric mechanisms in Central Carbon Metabolism:

- Reznik E, Christodoulou D, Goldford JE, Briars E, Sauer U, Segrè D, Noor E. Genome-Scale Architecture of Small Molecule Regulatory Networks and the Fundamental Trade-Off between Regulation and Enzymatic Activity. Cell Rep. 2017 Sep 12;20(11):2666-2677. doi: 10.1016/j.celrep.2017.08.066. PMID: 28903046; PMCID: PMC5600504.

3. Simple: Michaelis–Menten kinetics

Let's assume we have a well-mixed test tube containing an enzyme E (a protein that catalyzes chemical reactions), which converts substrate S (the starting compound) into product P according to three elementary reactions:

$$\begin{aligned} E + S &\rightleftharpoons E:S \\ E:S &\longrightarrow E + P \end{aligned}$$

The kinetics of each elementary step can be written using mass-action kinetics, i.e.,

$$\begin{aligned} r_1 &= k_1[E][S] \\ r_2 &= k_2[E:S] \\ r_3 &= k_3[E:S] \end{aligned}$$

where $[\cdot]$ denotes a species concentration, and k_j denotes the rate constant governing the jth elementary reaction:

- The rate r_1 describes the *association* rate between the enzyme and substrate,
- The rate r_2 represents the rate of *dissociation* of the enzyme-substrate complex, and
- The r_3 denotes the rate of *chemical conversion* of the bound substrate into the product (we assume the dissociation of the product from the enzyme is fast).

The enzyme must obey the relationship:

$$[E_T] = [E] + [E:S]$$

where $[E_T]$ denotes the total enzyme concentration in the tube, $[E]$ denotes the free enzyme concentration (not bound to substrate) while $[E:S]$ denotes the enzyme-substrate complex.

To estimate the *overall* rate v, we stipulate a single rate-limiting step out of the set of elementary reactions. Let's assume that the rate of chemical conversion (r_3) is the slowest step, i.e., the substrate bounces on/off the enzyme quickly with only a tiny fraction of these binding events resulting in a successful chemical transformation. Thus, the overall rate is then given by:

$$v = k_3[E:S]$$

Let's also assume that we already know (or can estimate) the rate constants k_1, k_2 and k_3. When this is true, the only unknown is $[E:S]$. However, we can relate $[E:S]$ to variables we know (E_T and at least initially S) through the enzyme balance, and the *pseudo-steady-state assumption* for the reaction intermediate $[E:S]$:

$$\frac{d[E:S]}{dt} = k_1[E][S] - k_2[E:S] - k_3[E:S] \simeq 0$$

Rearranging and solving for $[E:S]$ gives the relationship:

$$[E:S] \simeq \frac{k_1}{k_2 + k_3}[E][S]$$

where the ratio of constants is defined as the Michaelis-Menten saturation coefficient or K_M:

$$\frac{1}{K_M} \equiv \frac{k_1}{k_2 + k_3}$$

Substituting the definition of K_M into the overall rate yields:

$$v = k_3 \frac{[E][S]}{K_M}$$

However, we do not know the free enzyme concentration of $[E]$; to find $[E]$ we subsitute $[E:S]$ into the enzyme balance and solving for $[E]$:

$$[E] = \frac{[E_T]K_M}{K_M + [S]}$$

Lastly, we substitute $[E]$ into the overall rate to arrive at the final expression for v:

$$v = V_{max} \frac{[S]}{K_M + [S]}$$

where $V_{max} \equiv k_3[E_T]$.

Limiting cases:

- when $S \gg K_M$, the rate becomes close to V_{max}.
- when $S \ll K_M$ the rate appears to be linear with respect to substrate concentration.
- when $K_M \simeq S$ the reaction rate equals $v \simeq 1/2V_{max}$.

4. Complex: MWC and Sequential kinetic models

The Monod-Wyman-Changeux model (MWC model, also known as the symmetry model) describes allosteric transitions of proteins made up of identical subunits. Effector binding modulates the state of the entire protein.

- MONOD J, WYMAN J, CHANGEUX JP. ON THE NATURE OF ALLOSTERIC TRANSITIONS: A PLAUSIBLE MODEL. J Mol Biol. 1965 May;12:88-118. doi: 10.1016/s0022-2836(65)80285-6. PMID: 14343300.

The sequential model (KNF model) of allosteric regulation posits that enzyme subunits are independent. Thus, binding substrate (or effector) to a subunit results in only slight conformational changes to adjacent subunits. KNF model can do describe both positive and negative cooperativity:

- Koshland D Jr, Némethy G, Filmer D. Comparison of experimental binding data and theoretical models in proteins containing subunits. Biochemistry. 1966 Jan;5(1):365-85. doi: 10.1021/bi00865a047. PMID: 5938952.

5. Effective discrete state kinetic models

Michaelis–Menten kinetics are easy to understand, but they neglect many factors, e.g., the influence of allosteric regulators. On the other hand, MWC/sequential models are detailed but case-specific (and too complex). It would be great if we could correct Michaelis–Menten kinetics to capture the influence of allosteric factors.

Suppose we model the rate v_j as the product of a kinetic limit (a simple model of the rate) and a correction term that accounts for the missing regulation:

$$v_j = r_j \theta(\dots)_j$$

where v_j denotes the overall rate (units: μM/time), r_j denotes the kinetic limit i.e., the maximum rate of conversion (units: μM/time) and $0 \leq \theta(\dots)_j \leq 1$ (units: dimensionless) is a control function that describes the influence of effector molecules.

5.1 Discrete state control function model

There is a wide variety of different possible ways we can build the $\theta(\dots)_j$ control functions. Ultimately, it doesn't matter what we choose, as long as it gives a good performance. However, let's introduce an idea that we will revisit later, namely a discrete probabilistic approach.

5.1.1 Theory

Suppose an enzyme E can exits in one of $s = 1, 2 \dots, \mathcal{S}$ possible microstates, where each microstate s has some pseudo energy ϵ_s. Some microstates will lead to activity (the ability to carry out the chemical reactions, while others will not). For each microstate s, let's assign a pseudo energy ϵ_s, where by definition $\epsilon_1 = 0$; we assume the base state has the lowest energy. Next, suppose the probability that enzyme E is in microstate s follows a Boltzmann distribution which says:

$$p_i = \frac{1}{Z} \times f_i \exp\left(-\beta \epsilon_i\right) \qquad i = 1, 2, \dots, \mathcal{S}$$

where p_i denotes the probability that enzyme E is in microstate $i = 1, 2, \ldots, \mathcal{S}$, f_i denotes a state-specific factor $f_i \in [0, 1]$, β denotes the thermodynamic beta and Z denotes a normalization factor (called the Partiton function in the statistical physics community). We can find Z using the summation law of discrete probolity e.g., $\sum_s p_s = 1$ which gives:

$$Z = \sum_{s=1}^{\mathcal{S}} f_i \exp\left(-\beta \epsilon_i\right)$$

which gives:

$$p_i = \frac{f_i \exp\left(-\beta \epsilon_i\right)}{\sum_{s=1}^{\mathcal{S}} f_i \exp\left(-\beta \epsilon_i\right)} \qquad i = 1, 2, \ldots, \mathcal{S}$$

.

Finally, we relate the probability that enzyme E is in microstate s back to the θ control function by computing the overall probability that the desired event happens, e.g., enzyme E catalyzes the reaction of interests. We know if $\Omega = \{1, 2, \ldots, \mathcal{S}\}$, then we can define the subset $\mathcal{A} \subseteq \Omega$ in which the desired event happens. Given $\mathcal{A}$, the θ function becomes:

$$\theta = \sum_{s \in \mathcal{A}} p_s$$

5.1.2 Conceptual exampleTo illustrate this idea, consider an enzyme inhibited by a downstream product (this is a common allosteric motif known as feedback inhibition). In this case, suppose enzyme E, which is inhibited by compound I, can exist in one of three possible microstates:

- **State s = 1**: No substrate S is bound, E is floating around in solution minding its own business (base state, no reaction)
- **State s = 2**: Substrate S is bound to enzyme E, but inhibitor I is not bound (reaction possible)
- **State s = 3**: Both the substrate S and inhibitor I are bound to

enzyme E (no reaction possible)

Given these microstates (and their functional assignment) we know that enzyme E can only catalyze its reaction in microstate $s = 2$, thus:

$$\theta = \frac{f_2 \exp\left(-\beta \epsilon_i\right)}{\sum_{s=1}^{3} f_s \exp\left(-\beta \epsilon_s\right)}$$

5.1.3 What are the state-specific factors?

The state-specific factors $f_i \in [0, 1]$ control the reachability of a microstate:

- if $f_\star = 0$, then microstate $\star$ can **never** be obtained
- if $f_\star = 1$, then microstate $\star$ can **always** be obtained

As a modeler, you can choose the form (or value) that $f_\star$ takes. These factors can be set to a specific value by definition (depending upon the state) or can be used to describe events associated with state i, e.g., such as binding events. For example, suppose state i involved binding an effector molecule x to the enzyme E. In this case, we could model the state-specific factor f_i as:

$$f_i = (x/K_i)^{n_i} / (1 + (x/K_i)^{n_i})$$

where $x \geq 0$ denotes effector abundance, $K_i \geq 0$ denotes a binding constant and $n_i \geq 0$ denotes a binding order parameter.

6. Summary and Conclusions

In this lecture we:

1. Developed simple models of enzyme kinetics using the Michaelis–Menten approach
2. Introduced the fundamental concepts underlying more complex models of allosteric regulation (the fast control mechanisms that we mentioned previously)
3. Introduced effective discrete regulation models to simulate allosteric regulation

7. Next Time

- What about multiple substrates?
- Does the discrete state model work?
- How do we implement these bounds models in a flux balance analysis calculation?

```
begin

    # external packages -
    using PlutoUI
    using Plots
    using PrettyTables
end
```

```
html"""
<script>
    // initialize -
    var section = 0;
    var subsection = 0;
    var subsubsection = 0;
    var headers = document.querySelectorAll('h3, h5,
    h6');

    // main loop -
    for (var i=0; i < headers.length; i++) {

        var header = headers[i];
        var text = header.innerText;
        var original = header.getAttribute("text-
    original");
        if (original === null) {

            // Save original header text
            header.setAttribute("text-original", text);
        } else {

            // Replace with original text before
           adding section number
            text = header.getAttribute("text-
    original");
        }

        var numbering = "";
        switch (header.tagName) {
            case 'H3':
                section += 1;
                numbering = section + ".";
                subsection = 0;
                break;
            case 'H5':
                subsection += 1;
                numbering = section + "." + subsection;
                break;
            case 'H6':
```

```
html"""
<style>
main {
    max-width: 860px;
    width: 70%;
    margin: auto;
    font-family: "Roboto, monospace";
}

a {
    color: blue;
    text-decoration: none;
}
</style>"""
```