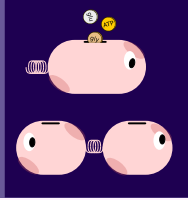
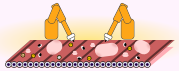


Cost of metabolic pathways

Elad Noor & Wolfram Liebermeister



LEARNING PLANET INSTITUTE

Outline

Costs and Benefits

Resource allocation: ribosomal and metabolic fractions

Toy examples for flux optimization

Factorized rate law

Enzyme Cost Minimization (for a single pathway)

Appendix 1: deriving the Haldane rate law

An economist's perspective

- What are the **costs** associated with keeping an enzyme at a certain level E?

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Cells adapt their macro-composition based on the growth conditions

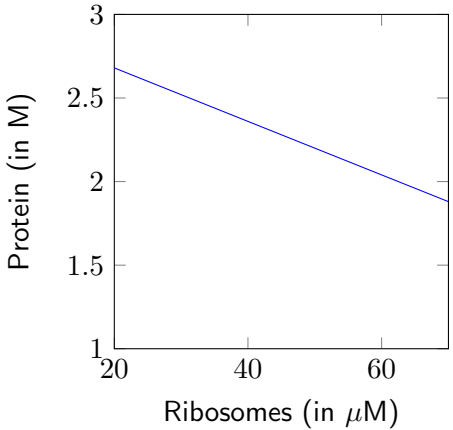

Figure: The concentration of protein (P) as a function of the concentration of ribosomes (R) in *E. coli*, which follows the line $P = 3.0 \cdot 10^6 - 1.6 \cdot 10^4 R$ [1].

The R and C sections of the proteome

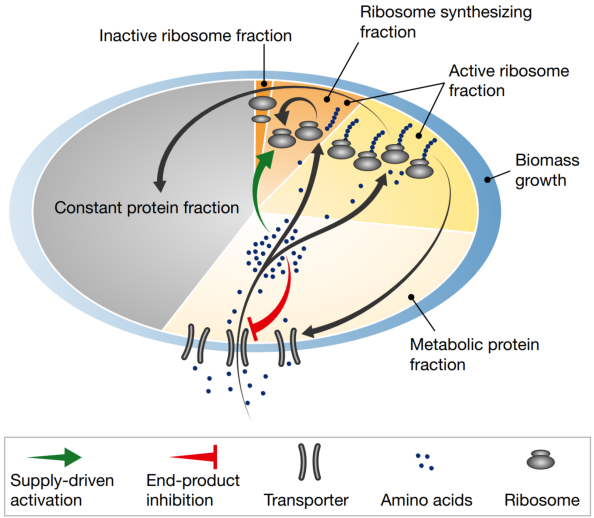

Figure: Schematic illustration of the growth model. Figure from [2].

Resource balance analysis*

- Start with a genome-scale stoichiometric model of a metabolic network
- Add reactions that describe tRNA, transcription, translation
- Obtain apparent kinetic constants for all reactions (ratio between flux and enzyme cost)
- Maximize growth rate (by non-linear optimization)

* More on this tomorrow at Anne Goelzer's talk on "Resource allocation models".

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Different approach

- Quantify only the metabolic protein fraction cost
- Focus on a single pathway or flux mode (not the entire network)
- Try to use all available data about every enzyme
- Ask general questions about optimality

Maximizing flux through an unbranched pathway

Consider the following linear pathway [3]:

$$S_0 \overset{\mathrm{v1}}{\rightleftharpoons} S_1 \overset{\mathrm{v2}}{\rightleftharpoons} \dots \overset{\mathrm{v}n}{\rightleftharpoons} S_n$$

where S_0 and S_n are the (pre-defined) concentrations of the first substrate and last product, S_i is the (variable) concentrations of intermediate i, and E_i is the (variable) concentration of the enzyme catalyzing reaction i.

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We assume that each reaction follows the rate law $v_i = E_i \cdot (k_i S_{i-1} - k_{-i} S_i)$

$$S_{i-1} \underset{\mathrm{E}_i\,\mathrm{k}_{-i}\,\mathrm{S}_i}{\overset{\mathrm{E}_i\,\mathrm{k}_i\,\mathrm{S}_{i-1}}{\rightleftharpoons}} S_i \tag{1}$$

* This rate law corresponds to unsaturated Michaelis-Menten kinetics, where $k_i = k_{\mathsf{cat}}/K_M$

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$$S_{i-1} \underset{\mathrm{E_i\ k_{-i}\ S_i}}{\overset{\mathrm{E_i\ k_i\ S_{i-1}}}{\rightleftharpoons}} S_i \tag{1}$$

Finally, assume we have a fixed amount of total enzyme concentration (E_{tot}).

$$\sum_i E_i \quad \leq \quad E_{\mathsf{tot}}$$

- Constants: S_0, S_n, k_i, k_{-i}, E_{tot}
- Variables: v_i, E_i, S_i
- Constraints: $v_i = E_i \cdot (k_i S_{i-1} - k_{-i} S_i)$ $\quad \sum_i E_i \leq E_{\text{tot}}$ $\quad v_i = J$

Blackboard

Maximizing flux through an unbranched pathway

At steady-state, all rates must be equal to the pathway flux (J):

$$
\begin{aligned}
J &= v_i = E_i \cdot (k_i S_{i-1} - k_{-i} S_i) \\
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Conjecture 1:
to achieve the highest pathway flux J, the optimal enzyme allocation [4][5] is:

Optimal allocation

$$\begin{aligned} \hat{E}_i &= E_{\text{tot}} \cdot \frac{A_i^{-1/2}}{\sum_i A_i^{-1/2}} \\ A_i &\equiv \frac{k_1}{k_{-1}} \cdot \frac{k_2}{k_{-2}} \cdots \frac{k_{(i-1)}}{k_{-(i-1)}} \cdot k_i \end{aligned}$$

Blackboard

Maximizing flux through an unbranched pathway

First, we solve a simplified version of the conjecture, where all $k_i = k_{-i} = 1$.

Conjecture 1.1: Given a pathway in steady-state with n reactions described by:

$$\begin{aligned} J &= v_i = E_i \cdot (S_{i-1} - S_i) \\ \sum_i E_i &\leq E_{\text{tot}} \end{aligned}$$

flux is maximized when the enzymes are distributed uniformly along the pathway:

$$\hat{E}_i = E_{\text{tot}}/n$$

Maximizing flux through an unbranched pathway

Proof of Conjecture 1.1:
First, we note that the constraint on E_{tot} must be realized, otherwise we can increase all enzyme amounts proportionally (until reaching the maximum) and thus increase J by the same factor.
Then, we note that there is a simple relationship between two consecutive metabolites:

$$\begin{aligned} J &= E_i \cdot (S_{i-1} - S_i) \\ S_i &= S_{i-1} - J/E_i \,. \end{aligned}$$

Starting with S_n and iteratively substituting S_i using this formula until reaching S_0:

$$\begin{aligned} S_n &= S_0 - J \sum_i E_i^{-1} \\ J &= \frac{S_0 - S_n}{\sum_i E_i^{-1}} \,. \end{aligned}$$

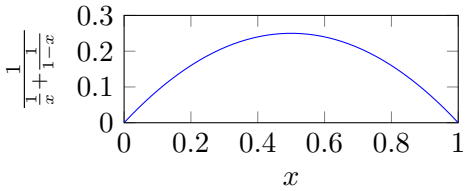
Maximizing flux through an unbranched pathway

Proof of Conjecture 1.1:

So, we find that J is proportional to the harmonic mean of E_i:

$$J = (S_0 - S_n) \cdot \left(\sum_i E_i^{-1} \right)^{-1} \tag{2}$$

and together with the fact that $\sum_i E_i = E_{\text{tot}}$, we can see[1] that the maximum is reached at $E_i = E_{\text{tot}}/n$. □



[1] Exercise: prove that the harmonic mean is maximized by a uniform distribution.

Maximizing flux through an unbranched pathway

Obviously, the assumption that $k_i = k_{-i} = 1$ is very oversimplified. Imagine what would happen if:

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- one of the enzymes was much slower (i.e. low values of k_i and k_{i-1})?

Maximizing flux through an unbranched pathway

Obviously, the assumption that $k_i = k_{-i} = 1$ is very oversimplified. Imagine what would happen if:

- one of the enzymes was much slower (i.e. low values of k_i and k_{i-1})?
- one of the reactions was thermodynamically unfavorable ($k_{-i} \gg k_i$)?

Blackboard

Maximizing flux through an unbranched pathway

Proof of Conjecture 1: We can define the following aggregate kinetic parameters:

$$A_i \equiv \frac{k_1}{k_{-1}} \cdot \frac{k_2}{k_{-2}} \cdots \frac{k_{(i-1)}}{k_{-(i-1)}} \cdot k_i$$

Then, solving for the flux J given a set of enzyme concentrations E_i [6]:

$$J = \left(S_0 - S_n \prod_{i=1}^{n} (k_{-i}/k_i) \right) \left(\sum_i (A_i E_i)^{-1} \right)^{-1}$$

In other words, J is proportional to a *weighted* harmonic mean of the E_is. This function is maximized (for a given total $\sum_i E_i = \text{const}$) when:

$$E_i \propto A_i^{-1/2}$$

□

A historical overview of kinetic rate laws

Consider a reaction $S \rightleftharpoons P$ catalyzed by an enzyme E.

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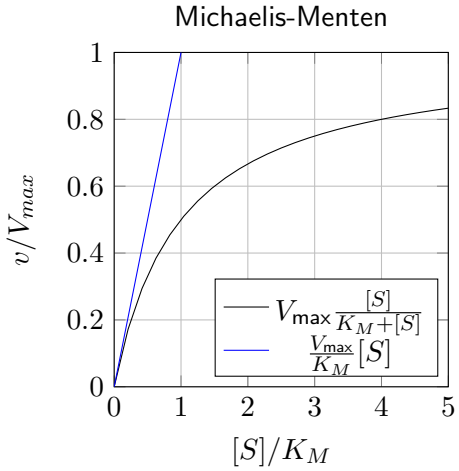

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$$v = [E_0] \frac{k^+_{\mathsf{cat}}[S]/K_S - k^-_{\mathsf{cat}}[P]/K_P}{1 + [S]/K_S + [P]/K_P} \qquad (3)$$

(* for the unimolecular case)

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- Based on this derivation, Haldane also found a relationship that always holds between the kinetic parameters of the enzyme and the equilibrium constant of the reaction (K_{eq}).

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$$\frac{k^+_{\mathsf{cat}}}{k^-_{\mathsf{cat}}} \cdot \frac{K_P}{K_S} = K_{\mathsf{eq}} \qquad (4)$$

The Factorized Rate Law

Using the Haldane relationship, the Haldane rate law can be rewritten[2] in the following form [11]:

$$v = [E_0] \underbrace{k_{\text{cat}}^{+} \cdot \left(1 - e^{\Delta_r G'/RT}\right) \cdot \frac{[S]/K_S}{1 + [S]/K_S + [P]/K_P}}_{k_{\text{app}}} \tag{5}$$

where

$$\begin{aligned} \Delta G'_r &= \Delta G'^{\circ}_r + R \cdot T \cdot \ln\left([P]/[S]\right) \\ \Delta G'^{\circ}_r &= -R \cdot T \cdot \ln K_{\text{eq}} \end{aligned}$$

[2] Exercise: show that equation 5 is equivalent to standard Haldane rate law formulation.

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Note that $v = [E_0] k_{\text{app}}$ is the rate law used in Resource Balance Analysis, where k_{app} is a constant estimated from empirical data.

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The Factorized Rate Law

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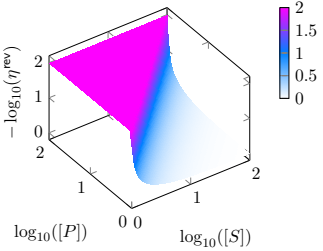
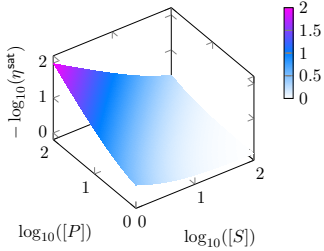
- $[E_0]k^+_{\text{cat}} = 1$ mM/s
- $\Delta_r G'^{\circ} = 0$
- $K_S = 1$ mM
- $K_P = 1$ mM





Enzyme Cost Minimization

Let's go back to our linear pathway:

$$S_0 \overset{\mathrm{v}_1}{\rightleftharpoons} S_1 \overset{\mathrm{v}_2}{\rightleftharpoons} \dots \overset{\mathrm{v}_n}{\rightleftharpoons} S_n$$

But now, all enzymes have general kinetics based on the factorized rate law:

$$\begin{aligned} v_i &= E_i \cdot k^{+}_{\text{cat, i}} \cdot \eta_i^{\text{rev}} \cdot \eta_i^{\text{sat}} \\ \sum_i E_i &\leq E_{\text{tot}} \end{aligned}$$

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How to solve

In this case, it is not possible to express J as a function of E_i. But, we can use the following trick: minimizing $\sum_i E_i$ for a given J is equivalent to maximizing J for a given $\sum_i E_i = E_{\text{tot}}$. The only free variables will be the metabolite concentrations.

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Given a flux J find the set of metabolite concentrations **S** that minimizes:

$$\sum_i E_i \;=\; \sum_i \frac{J}{k^{+}_{\text{cat, i}} \cdot \eta_i^{\text{rev}} \cdot \eta_i^{\text{sat}}}$$

where η_i^{rev} and η_i^{sat} are both functions of **S**.

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How to solve

Solving this problem analytically is not possible in general, but it can be done numerically using convex optimization [12].

ECM example for a toy model

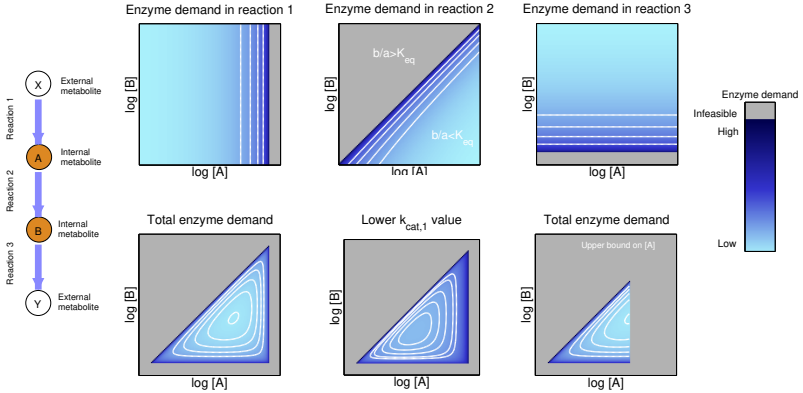

Figure: A 3-step toy model showing the enzyme cost as a function of metabolite concentrations.

Examples using Jupyter Notebook

click here

Example of two glycolyses

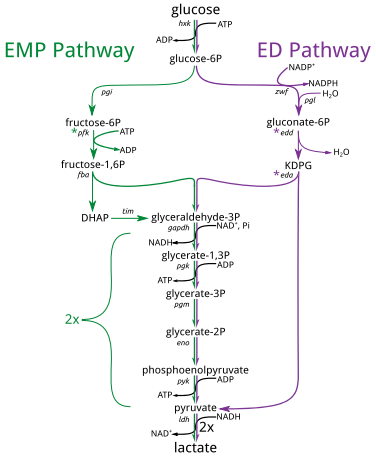

Figure: Metabolic network showing both types of glycolysis.

A tale of two glycolyses

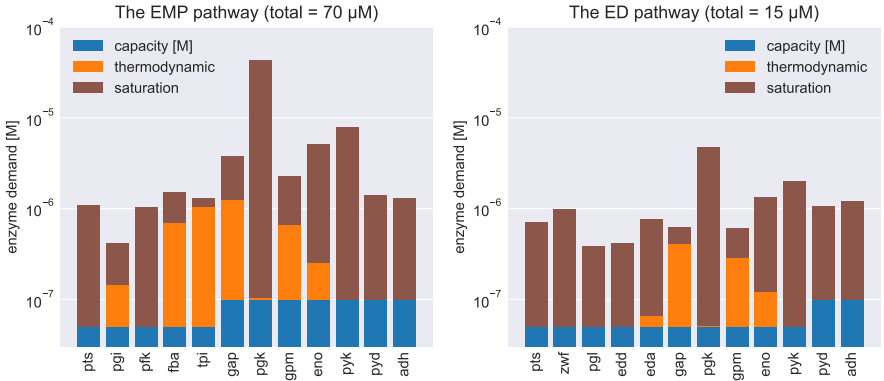

Figure: Optimized enzyme concentrations based on ECM results. Pathway flux $= 0.01mM/s$, $k_{\text{cat}} = 200s^{-1}$, $K_M = 200\mu M$, enzyme MW $= 40kDa$

Running ECM on a model of *E. coli* central metabolism

Reaction	flux	k_{cat}^{+}	K_S	$\Delta G'^{\circ}$
PGI	0.39 mM/s	17.8 1/s	0.15 mM	2.5 kJ/mol
PFK	0.44 mM/s	12.5 1/s	0.07 mM	-16.1 kJ/mol
FBA	0.44 mM/s	19.0 1/s	0.22 mM	21.4 kJ/mol
TPI	0.44 mM/s	967.7 1/s	8.43 mM	5.49 kJ/mol
GAP	0.92 mM/s	170.2 1/s	0.61 mM	5.23 kJ/mol
		⋮		

The data was collected from online databases such as BRENDA and eQuilibrator.

Optimal enzyme costs can predict actual in vivo concentrations

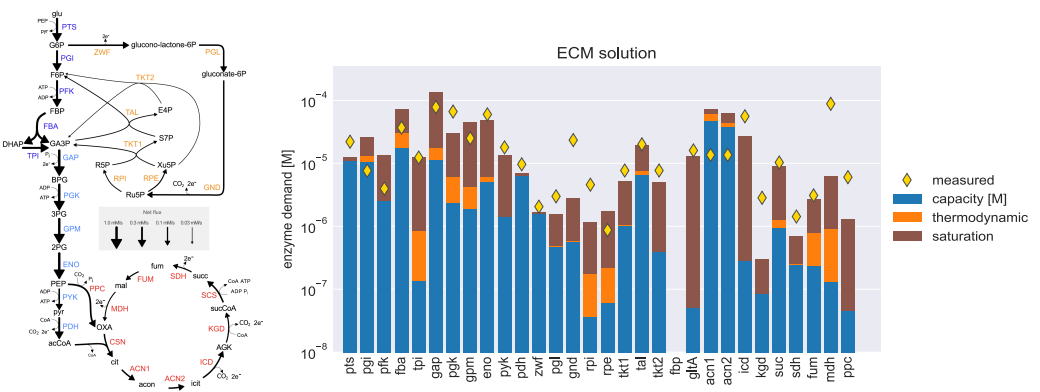

Figure: A flux map of central metabolism (left) and the optimized enzyme concentrations (right).

* note that enzymes with a small minimial cost (blue bar) tend to have higher thermodynamic (orange) and saturation (brown) costs.

Optimal enzyme costs can predict actual in vivo concentrations

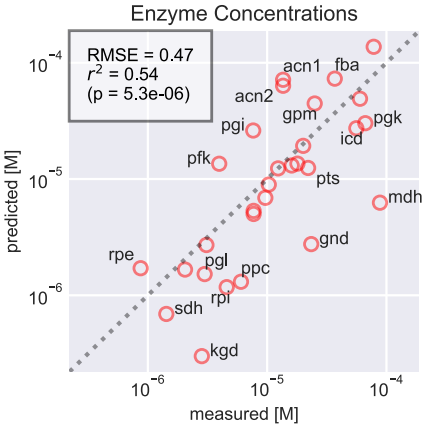

Figure: Comparing the optimized enzyme concentrations from ECM to a quantitative proteomics dataset.

Max-min Driving Force

Enzyme Cost Minimization requires full knowledge of all enzyme kinetic parameters, but often our knowledge is limited. One approximation would be to only consider thermodynamic constraints.

Max-min Driving Force is a method based on the argument avoiding close-to-equilibrium reactions reduces enzyme cost (i.e. η^{rev} should be as high as possible).

$$v = [E_0] \cdot \underbrace{k_{\text{cat}}^{+}}_{\text{assume constant}} \cdot \underbrace{\left(1 - e^{\Delta_r G'/RT}\right)}_{\eta^{rev}} \cdot \underbrace{\frac{[S]/K_S}{1 + [S]/K_S + [P]/K_P}}_{\text{assume } \eta^{\text{sat}}=1}$$

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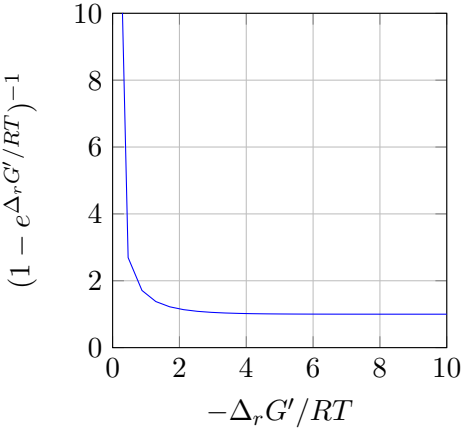
$$[E_0] \propto (1 - e^{\Delta_r G'/RT})^{-1}$$

Max-min Driving Force

For MDF analysis, we assume that costs are inversly proportional to the thermodynamic term in the factorized rate law.

$$[E_0] \quad \propto \quad (1 - e^{\Delta_r G'/RT})^{-1}$$

Instead of minimizing the sum of costs (like in Enzyme Cost Minimization), we try to maximize the driving force ($-\Delta_r G'/RT$) of all reactions simultaneously [11].



Elementary Flux Modes and global optimization of enzyme cost

Final thoughts

In many cases, we don't have only a pair of pathways to choose between. Rather, we have a complex metabolic map with a huge number of possible steady-state flux solutions.

We will address that scenario in the next talk, given by Meike Wortel.

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Quantifying the effects of enzyme and reactant concentration

The Haldane derivation is based on this model of catalysis:

$$\mathrm{S} + \mathrm{E} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{ES} \underset{\mathrm{k_4}}{\overset{\mathrm{k_3}}{\rightleftharpoons}} \mathrm{EP} \underset{\mathrm{k_6}}{\overset{\mathrm{k_5}}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}$$

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which translates to the following ODE system:

$$\begin{aligned}
\frac{d[ES]}{dt} &= [E] \cdot [S] \cdot k_1 + [EP] \cdot k_4 - [ES] \cdot (k_2 + k_3) \\
\frac{d[EP]}{dt} &= [E] \cdot [P] \cdot k_6 + [ES] \cdot k_3 - [EP] \cdot (k_4 + k_5) \\
\frac{d[P]}{dt} &= [EP] \cdot k_5 - [E] \cdot [P] \cdot k_6
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\frac{d[P]}{dt} &= [EP] \cdot k_5 - [E] \cdot [P] \cdot k_6
\end{aligned}$$

Haldane assumed the system quickly reaches a quasi-steady-state and therefore all time derivatives are equal to 0. In addition, the total enzyme concentration $[E_0] = [E] + [EP] + [ES]$ does not change over time.

Quantifying the effects of enzyme and reactant concentration

The easiest way to solve this system of equations is by using a matrix notation:

$$\begin{pmatrix} [S]k_1 & -(k_2+k_3) & k_4 \\ [P]k_6 & k_3 & -(k_4+k_5) \\ 1 & 1 & 1 \end{pmatrix} \begin{pmatrix} [E] \\ [ES] \\ [EP] \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ [E_0] \end{pmatrix}, \tag{6}$$

where the first two rows of the matrix correspond to $\frac{d[ES]}{dt} = 0$ and $\frac{d[EP]}{dt} = 0$, and the last row represents conservation of total enzyme concentration (note, that the equation $\frac{d[P]}{dt} = 0$ is redundant and therefore not used).

The reversible Haldane rate law

Solving[3] equation 6 yields:

$$v = [E_0] \frac{k^+_{\text{cat}}[S]/K_S - k^-_{\text{cat}}[P]/K_P}{1 + [S]/K_S + [P]/K_P} \tag{7}$$

where:

$$\begin{aligned} K_S &= \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_1(k_3 + k_4 + k_5)} \\ K_P &= \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_6(k_2 + k_3 + k_4)} \\ k^+_{\text{cat}} &= \frac{k_3k_5}{k_3 + k_4 + k_5} \\ k^-_{\text{cat}} &= \frac{k_2k_4}{k_2 + k_3 + k_4} \end{aligned}$$

[3] Exercise: solve the linear ODE system using Gaussian elimination.

The Haldane relationship

In addition, Haldane noticed that there is a dependency between the four kinetic parameters:

$$\frac{k_{\text{cat}}^{+}}{k_{\text{cat}}^{-}} \frac{K_P}{K_S} = \frac{k_1 k_3 k_5}{k_2 k_4 k_6} . \tag{8}$$

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According to mass-action kinetics, this fraction is equal to the equilibrium constant of the $S \rightleftharpoons P$ reaction.

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Today, this is commonly known at the *Haldande relationship*. Since K_{eq} is a physical constant independent of the enzyme, this means that uni-uni enzyme kinetic parameters have only three degrees of freedom (rather than four).