Workshop SPT Chemical Reaction Networks

Elad Noor, Wolfram Liebermeister

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The problem: allocate enzymes to maximize a metabolic pathway flux

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Kinetic metabolic models and flux balance models

Kinetic metabolic models describe the metabolic reaction rates v_l depending on enzyme levels ε_l and metabolite concentrations c_i :

 $v_l = \varepsilon_l f_l(\mathbf{c})$

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Metabolite dynamics (for "internal" metabolites i) depends on the rates v_l and on stoichiometric coefficients n_{il} :

$$
\frac{\mathrm{d}c_i}{\mathrm{d}t} = \sum_l n_{il} v_l(\mathbf{c})
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Flux analysis models ignore dynamics and determine steady fluxes $J_l = v_l$ satisfying

$$
0 = \sum_l n_{il} J_l
$$

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What is the enzyme cost of a metabolic flux distribution?

Given a stationary flux distribution J_1 and remembering the rate laws

$$
v_l = \varepsilon_l \ f_l(\mathbf{c}),
$$

by solving for the enzyme levels ε_l we obtain the overall enzyme demand

$$
\varepsilon_{\text{tot}}(\mathbf{J}) = \sum_{l} \varepsilon_{l} = \sum_{l} \frac{J_{l}}{f_{l}(\mathbf{c})}
$$

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$$

For fixed concentrations c_i , the enzyme efficiencies $k_l^{\mathrm{app}}=f_l(\mathbf{c})$ are constant and

$$
\varepsilon_{\text{tot}}(\mathbf{J}) = \sum_{l} \frac{J_l}{k_l^{\text{app}}}
$$

The demand scales linearly with the fluxes (and hence, with the [sy](#page-5-0)s[te](#page-7-0)[m](#page-4-0)['](#page-5-0)[s](#page-6-0) ["](#page-7-0)[o](#page-0-0)[u](#page-1-0)[t](#page-10-0)[p](#page-11-0)[ut](#page-0-0) [fl](#page-10-0)[u](#page-11-0)[x"](#page-0-0)[\)](#page-43-0) 290

With "trivial" kinetics: enzyme efficiencies are inversely additive

We assume a pathway with stationary flux J and constant efficiencies, $v_l = \varepsilon_l \; k_l^{\rm app}$ $\frac{1}{l}$. Setting $v_i=J$ and solving for ε_l , we obtain the enzyme demand

$$
\varepsilon_{\text{tot}} = \sum_{l} \varepsilon_{l} = \sum_{l} \frac{1}{k_{l}^{\text{app}}} \cdot J
$$

So we can write the pathway flux as

$$
J=k_{\rm tot}^{\rm app}\,\varepsilon_{\rm tot}
$$

with the effective pathway efficiency

$$
k_{\rm tot}^{\rm app} = \frac{1}{\sum_l \frac{1}{k_l^{\rm app}}}
$$

The inver[s](#page-10-0)e k^{app} k^{app} k^{app} are tim[es](#page-7-0), and additive like resistances in a series [of](#page-8-0) [r](#page-6-0)es[is](#page-8-0)t[or](#page-1-0)s[.](#page-11-0)

[Constraint-based kinetic models](#page-8-0)

Reversible enzyme kinetics based on Haldane

For a reversible enzyme catalyzed reaction $^{1}\colon S\stackrel{\mathrm{E}}{\xrightarrow{\hspace*{0.5cm}}}P$

$$
v = \varepsilon \cdot \underbrace{\frac{k_+^{\text{cat}} s/K_{\text{S}} - k_-^{\text{cat}} p/K_{\text{P}}}{1 + s/K_{\text{S}} + p/K_{\text{P}}}}
$$

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 $^{\text{1}}s$, p , and ε are the concentrations of S , P , and E

[Constraint-based kinetic models](#page-9-0)

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$$

Haldane further showed that the equilibrium constant satisfies the following relationship:

$$
K^{\text{eq}} = \frac{k_{+}^{\text{cat}}}{k_{-}^{\text{cat}}} \frac{K_{\text{P}}}{K_{\text{S}}}
$$

 $^{\text{1}}s$, p , and ε are the concentrations of S , P , and E

[Constraint-based kinetic models](#page-10-0)

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$$

The Haldane rate law can be rewritten (Noor et al. [2013]) as²:

 $^{\text{1}}s$, p , and ε are the concentrations of S , P , and E 2 where $\Delta_r G'\equiv \Delta_r G'^\circ+R\; T\ln (p/s)$ and $\Delta_r G'^\circ=-R\; T\ln (K^{\rm eq})$

Enzyme Cost Minimization: minimize the enzyme demand of a given flux!

Given a pathway with fixed flux and with enzymatic reactions with Haldane rate laws, look for metabolite and enzyme levels that minimize the sum of all enzyme demands.

Enzyme Cost Minimization: minimize the enzyme demand of a given flux!

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Enzyme demand in one reaction

$$
\varepsilon = J \cdot \frac{1}{k_+^{\rm cat}} \; \cdot \underbrace{\left(1-e^{\frac{\Delta_r G'}{RT}}\right)^{-1}}_{1/\eta^{\rm for}} \; \cdot \; \underbrace{\frac{1+\frac{p}{K_{\rm P}}+\frac{s}{K_{\rm S}}}{\frac{s}{K_{\rm S}}}}_{1/\eta^{\rm kin}}
$$

The pathway cost per flux ($\varepsilon_{\text{tot}}/J$) is the sum of demands for all reactions: a convex function of metabolite log-concentrations (Liebermeister and Noor [2015]).

Example: the enzyme demand in a 3-step pathway

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Can we find analytical solutions to ECM?

Analytical solutions 1 are useful because they:

- **1** Ensure solution optimality
- 2 Help find hidden relationships
- 3 Inspire design principles
- 4 Suggest mechanisms

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¹Noor and Liebermeister [2024]

 L [Enzyme cost minimization \(ECM\)](#page-15-0)

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Problem

Kinetic models, where reactions have multi-substrate or Haldane rate laws, do not have known analytical solutions.

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Solution: focus only on unbranched pathways with simplified kinetics.

¹Noor and Liebermeister [2024]

 L [Enzyme cost minimization \(ECM\)](#page-17-0)

We come back to our full optimality problem

Instead of Enzyme Cost Minization (minimizing enzyme at given fluxes): Maximize the pathway flux with a bound on enzyme (and possible on metabolite) levels!

The factorized Haldane rate law and some simplifications

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The "mass-action" approximation

Haldane:

$$
v = \varepsilon \cdot \frac{k_+^{\text{cat}} \ s/K_{\text{S}} - k_-^{\text{cat}} \ p/K_{\text{P}}}{1 + s/K_{\text{S}} + p/K_{\text{P}}}
$$

If we assume:

 $s \ll K_{\rm S}, p \ll K_{\rm P}$

Therefore:

$$
v = \varepsilon \cdot \left(\underbrace{k_+^{\text{cat}}/K_{\text{S}}}_{k_+} \cdot s - \underbrace{k_-^{\text{cat}}/K_{\text{P}}}_{k_-} \cdot p \right)
$$

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Unbranched pathway with "mass-action" kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
\forall i \quad J = \varepsilon_i \cdot (k_i \ s_{i-1} - k_{-i} \ s_i)
$$

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Unbranched pathway with "mass-action" kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
\forall i \quad J = \varepsilon_i \cdot (k_i \ s_{i-1} - k_{-i} \ s_i)
$$

Optimized flux solution²

$$
J^* = \varepsilon_{\text{tot}} \cdot \frac{s_0 - s_n/K^{\text{eq}}}{\left(\sum_i \sqrt{\gamma_i}\right)^2}
$$

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where
$$
\gamma_i \equiv \frac{\prod_{j=i+1}^n k_j}{\prod_{j=i}^n k_{-j}}
$$
 and $K^{\text{eq}} \equiv \frac{\prod_{j=1}^n k_j}{\prod_{j=1}^n k_{-j}}$

²Waley [1964], Burns [1971]

Unbranched pathway with "thermodynamic" kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
J = \varepsilon_i k_i^{\text{cat}} \left(1 - e^{\Delta_r G_i'/RT} \right)
$$

Unbranched pathway with "thermodynamic" kinetics

$$
S_0 \xrightarrow{\text{v}_1} S_1 \xrightarrow{\text{v}_2} \dots \xrightarrow{\text{v}_n} S_n
$$

$$
J = \varepsilon_i k_i^{\text{cat}} \left(1 - e^{\Delta_r G_i'/RT} \right)
$$

Optimized flux (approximated) solution³

$$
J^* \approx \varepsilon_{\rm tot} \cdot \frac{1}{a} \left(1 - \exp \left(\frac{a}{b} \, \frac{\Delta_r G^{\prime}_{\rm tot}}{R \, T} \right) \right)
$$

where
$$
a \equiv \sum_j \frac{1}{k_j^{\text{cat}}}
$$
, $b \equiv \sum_j \frac{1}{\sqrt{k_j^{\text{cat}}}}$, and $\Delta_r G'_{\text{tot}} \equiv \sum_j \Delta_r G'_j$

³Noor and Liebermeister [2024]

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Unbranched pathway with Michaelis-Menten kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
\forall i \quad J = \varepsilon_i \cdot k_i^{\text{cat}} \frac{s_{i-1}}{s_{i-1} + K_i}
$$

K **D → K** *d* **→ K 로 → K 로 → C 로** → O Q Q +

Unbranched pathway with Michaelis-Menten kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
\forall i \quad J = \varepsilon_i \cdot k_i^{\text{cat}} \frac{s_{i-1}}{s_{i-1} + K_i}
$$

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Optimizing J would require infinite metabolite concentrations! Therefore we add a total metabolite constraint: $\sum_i s_i \leq s_{\text{tot}}$

Unbranched pathway with Michaelis-Menten kinetics

$$
S_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} \dots \xrightarrow{v_n} S_n
$$

$$
\forall i \quad J = \varepsilon_i \cdot k_i^{\text{cat}} \frac{s_{i-1}}{s_{i-1} + K_i}
$$

Optimized flux solution⁴

$$
J^* = \varepsilon_{\rm tot}\cdot \frac{1}{\sum_j 1/k^{\rm cat}_j + \left(\sum_j \sqrt{K_\mathrm{j}/k^{\rm cat}_j}\right)^2\Big/s_{\rm tot}}
$$

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⁴Noor and Liebermeister [2024]

An application of simple pathway models: a model of a growing cell

An application of simple pathway models: a model of a growing cell

Assume:

- Coarse-graining: 3 enzymatic processes $S_{sugar} \xleftarrow{\varepsilon_t} S_1 \xleftarrow{\varepsilon_m} S_2 \xleftarrow{\varepsilon_r} S_3 \xrightarrow{\mu}$
- Gontrolled parameter: s_{sugar}
- **Constraint:** $\varepsilon_t + \varepsilon_m + \varepsilon_r = \varepsilon_{\text{tot}}$
- **Optimization:** maximize growth rate (μ)
- Rate laws: mass-action, thermodynamic, or Michaelis-Menten^a
- Not included: metabolite dilution by growth

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^aExtra constraint: $s_{\text{tot}} \geq s_1 + s_2 + s_3$

Cell model example (1): mass-action kinetics

$$
\mu = \varepsilon_{\text{tot}} \cdot \frac{s_0 - s_n/K^{\text{eq}}}{\left(\sum_i \sqrt{\gamma_i}\right)^2}
$$

where

$$
\begin{split} \gamma_t &\equiv \frac{K_{\mathrm{M;t}}}{k_\mathrm{t}^{\mathrm{cat}}} \cdot K_r^{\mathrm{eq}} \cdot K_m^{\mathrm{eq}} \cdot K_t^{\mathrm{eq}} \\ \gamma_m &\equiv \frac{K_{\mathrm{M;m}}}{k_\mathrm{m}^{\mathrm{cat}}} \cdot K_r^{\mathrm{eq}} \cdot K_m^{\mathrm{eq}} \\ \gamma_r &\equiv \frac{K_{\mathrm{M;t}}}{k_r^{\mathrm{cat}}} \cdot K_r^{\mathrm{eq}} \end{split}
$$

Cell model example (1): mass-action kinetics

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$$

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Cell model example (2): "thermodynamic" kinetics

$$
\mu \approx \varepsilon_{\rm tot} \cdot \frac{1}{a} \left(1 - e^{-\frac{a}{b} \cdot \ln \left(s_{\rm sugar} \frac{K^{\rm eq}}{s_3} \right)} \right)
$$

where

$$
\begin{aligned} a &\equiv \frac{1}{k_\mathrm{t}^\mathrm{cat}} + \frac{1}{k_\mathrm{m}^\mathrm{cat}} + \frac{1}{k_\mathrm{r}^\mathrm{cat}}\\ b &\equiv \frac{1}{\sqrt{k_\mathrm{t}^\mathrm{cat}}} + \frac{1}{\sqrt{k_\mathrm{m}^\mathrm{cat}}} + \frac{1}{\sqrt{k_\mathrm{r}^\mathrm{cat}}}\\ K^\mathrm{eq} &\equiv K^\mathrm{eq}_r \cdot K^\mathrm{eq}_m \cdot K^\mathrm{eq}_t \end{aligned}
$$

L[Growing cell model](#page-32-0)

Cell model example (2): "thermodynamic" kinetics

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\mu \approx \varepsilon_{\text{tot}} \cdot \frac{1}{a} \left(1 - e^{-\frac{a}{b} \cdot \ln \left(s_{\text{sugar}} \frac{K^{\text{eq}}}{s_3} \right)} \right)
$$

where

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$$

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Cell model example (3): Michaelis-Menten kinetics

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Summary of three cell models

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Take-home messages

Enzyme Cost Minimization is a convex problem

Take-home messages

- **Enzyme Cost Minimization is a convex problem**
- For some special cases, we provide analytical solutions

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Take-home messages

- **Enzyme Cost Minimization is a convex problem**
- \blacksquare For some special cases, we provide analytical solutions
- We can use these results to build corse-grained cell models

Activities around Economic Principles in Cell Biology

Economic Principles in Cell Biology

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Main | Welcome | Forum | Young scholars | Book | Summer school | Teaching materials | Workshop | Blog | About Book main - Chapters - Lectures - Code - Contributors - For authors

ECONOMIC PRINCIPLES IN CELL BIOLOGY

the economic cell collective

Economic Principles in Cell Biology A free textbook

How can a cell maintain itself as a living being? Living cells, shaped by billions of years of evolution, have developed many ways to adapt to their environment, for example by regulation of gene expression, But the rules of physics and chemistry enforce certain boundaries on what cells can achieve and how they can allocate their own resources. Shaped by evolution, cells are able to "do certain things right", and our goal is to uncover some of the principles behind this.

In our free and open textbook, to which anyone can contribute, we give an overview of established approaches to cellular economics. from descriptions of simple metabolic systems to cell growth, variability, and dynamic behaviour.

For more information about our project, please see our website for authors.

To join us in writing this textbook, please get in touch!

Activities around Economic Principles in Cell Biology

■ Online seminar Economic principles in cell physiology 1st Thursday of each month

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[Optimal enzyme profiles in metabolic pathways: principles and simple solutions](#page-0-0)

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- Summer school at Learning Planet Institute Paris July 8-11, 2024 register for free online participation

For more information, see https://principlescellphysiology.org

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