Fira Math

Constraints on Metabolic Network Analysis in Bacterial Physiology

Jonathan (Shao-Kai) Huang

September 8, 2025

Department of Physics, National Taiwan University /
Institute of Molecular Biology, Academia Sinica

Table of contents

Introduction

Overview

PRX Life PRX Life 3, 022001 Published 1 April 2025 [?].

[1] This is an approximation.

Overview

PRX Life PRX Life 3, 022001 Published 1 April 2025 [**?**].

Main ideas of this paper are:

- Characterize **emergent properties** of biological interactions in bacterial cells.

[1] This is an approximation.

Overview

PRX Life PRX Life 3, 022001 Published 1 April 2025 [**?**].

Main ideas of this paper are:

- Characterize **emergent properties** of biological interactions in bacterial cells.
- These constraints are equivalent to **Kirchhoff's laws** and **Ohm's law**.

[1] This is an approximation.

Overview

PRX Life PRX Life 3, 022001 Published 1 April 2025 [**?**].

Main ideas of this paper are:

- Characterize **emergent properties** of biological interactions in bacterial cells.
- These constraints are equivalent to **Kirchhoff's laws** and **Ohm's law**.
- Bacterial growth physiology can be analyzed quantitatively as **electrical circuits** $\Rightarrow$ **coarse-graining**. [1]

[1] This is an approximation.

Laws of Bacterial Physiology

Author(s):

"Life is required to make more life."

Laws of Bacterial Physiology

Author(s):

"Life is required to make more life."

Growth Laws
Many emergent behaviors can be described by simple phenomenological laws:

Laws of Bacterial Physiology

Author(s):

"Life is required to make more life."

Growth Laws

Many emergent behaviors can be described by simple phenomenological laws:

(i) Rate at which environmental materials are assimilated is balanced according to composition

Author(s):

"Life is required to make more life."

Growth Laws

Many emergent behaviors can be described by simple phenomenological laws:

(i) Rate at which environmental materials are assimilated is balanced according to composition

(ii) Rates are constrained by the autocatalytic nature of life

Exponential Growth

- When environmental nutrient is unlimited, population increases like

$$\frac{\mathrm{d}N}{\mathrm{d}t} \sim N(t) \;\Rightarrow\; N(t) = N_0 e^{\lambda t}. \tag{1}$$

Exponential Growth

- When environmental nutrient is unlimited, population increases like

$$\frac{\mathrm{d}N}{\mathrm{d}t} \sim N(t) \;\Rightarrow\; N(t) = N_0 e^{\lambda t}. \tag{1}$$

- **Balanced growth** characterizes exponential phase: In order for cells to accumulate exponentially, generating processes must happen at balanced rates.

Metabolic Networks Are Complicated

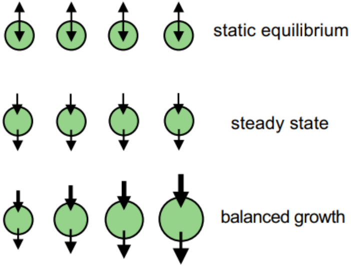

Figure 1: Comparison of equilibrium, steady state, and balanced growth [**?**].

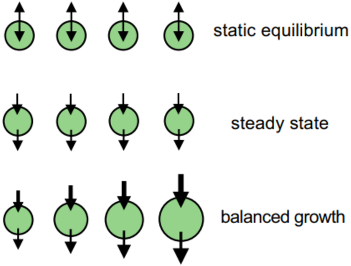

Figure 1: Comparison of equilibrium, steady state, and balanced growth [?].

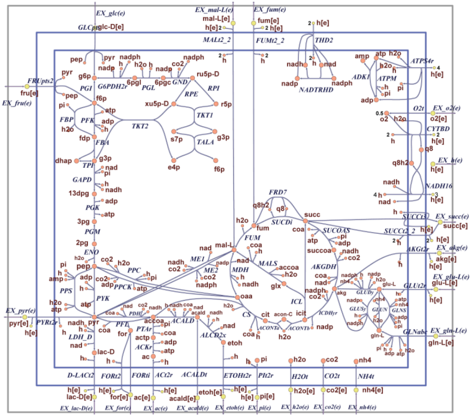

Figure 2: Core metabolic network of E. coli [?].

Flux Balance Analysis

- Stoichiometric matrix $S \in M_{m \times n}(R)$, biomass vector $X \in \mathbb{R}^n$:

$$\frac{\mathrm{d}X}{\mathrm{d}t} \equiv J = SX \tag{2}$$

  S is *underspecified* (metabolism is an open system) and *sparse*.

[2] suboptimal growth environment is fine

Flux Balance Analysis

- Stoichiometric matrix $S \in M_{m\times n}(R)$, biomass vector $X \in \mathbb{R}^n$:

$$\frac{\mathrm{d}X}{\mathrm{d}t} \equiv J = SX \tag{2}$$

  S is *underspecified* (metabolism is an open system) and *sparse*.
- Evolution selects cells that grow fast: $Z \propto \lambda$

[2] suboptimal growth environment is fine

Flux Balance Analysis

- Stoichiometric matrix $S \in M_{m \times n}(R)$, biomass vector $X \in \mathbb{R}^n$:

$$\frac{\mathrm{d}X}{\mathrm{d}t} \equiv J = SX \tag{2}$$

  S is *underspecified* (metabolism is an open system) and *sparse*.
- Evolution selects cells that grow fast: $Z \propto \lambda$
- Metabolic reaction rates must be **balanced** during steady-state [2] growth: $J = 0$.

[2] suboptimal growth environment is fine

Flux Balance is a Linear Programming Problem

Constrained Optimization

Maximize the objective function $Z = c \cdot x$
subject to

$$J = Sx = 0 \quad \text{(balanced growth)}, \tag{3}$$

and

$$\mathrm{lb}_i \leq x_i \leq \mathrm{ub}_i \quad \text{(bounded rates)}. \tag{4}$$

Flux Balance is a Linear Programming Problem

Constrained Optimization

Maximize the objective function $Z = c \cdot x$ subject to

$$J = Sx = 0 \quad \text{(balanced growth)}, \tag{3}$$

and

$$\mathrm{lb}_i \leq x_i \leq \mathrm{ub}_i \quad \text{(bounded rates)}. \tag{4}$$

Proteome Partition of E. coli

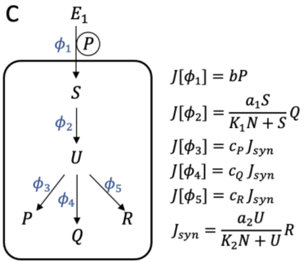

Figure 3: Three-sector proteome partition model (Lin, Wei-Hsiang, 2025) [?, ?]

Ribosomes Catalyze Protein Synthesis

- Ribosomes are optimized for **autocatalytic production**

Ribosomes Catalyze Protein Synthesis

- Ribosomes are optimized for **autocatalytic production**
- Michaelis-Menten kinetics

Ribosomes Catalyze Protein Synthesis

- Ribosomes are optimized for **autocatalytic production**
- Michaelis-Menten kinetics

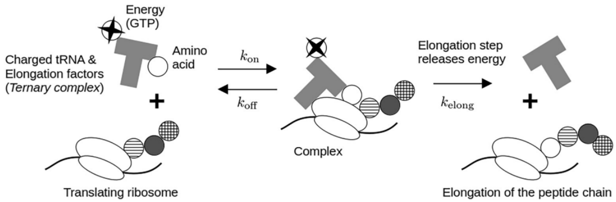

Figure 4: Ribosomes follow similar kinetics to those of enzymes: they turn charged tRNA into uncharged tRNA.

Ribosomes Catalyze Protein Synthesis

- Ribosomes are optimized for **autocatalytic production**
- Michaelis-Menten kinetics

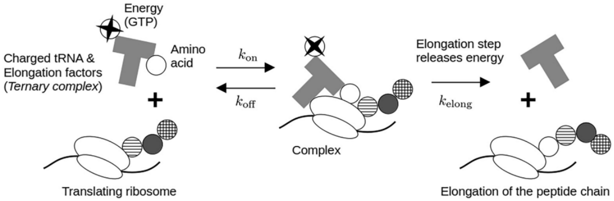

Figure 4: Ribosomes follow similar kinetics to those of enzymes: they turn charged tRNA into uncharged tRNA.

(Haldane) Abundance of the substrate far exceeds the abundance of the enzyme.

$$\text{rate} \propto [\text{Rb}] \times \frac{[\text{tRNA}]}{K_{\text{M}} + [\text{tRNA}]}. \quad (5)$$

Global Constraints

Global Constraints

- Maaloe et al. [**?**]: Per-cell quantity of RNA, DNA, and protein increase exponentially with λ
  $\implies$ Protein concentration is **nearly constant**.

Global Constraints

- Maaloe et al. [**?**]: Per-cell quantity of RNA, DNA, and protein increase exponentially with λ
  $\implies$ Protein concentration is **nearly constant**.
- Total protein constraint: $\sum_i \phi_i = 1$.

Global Constraints

- Maaloe et al. [**?**]: Per-cell quantity of RNA, DNA, and protein increase exponentially with λ
  $\implies$ Protein concentration is **nearly constant**.
- Total protein constraint: $\sum_i \phi_i = 1$.

Protein mass fraction is a **linear** function of growth rate:

$$\phi_i = \phi_i^0 + \frac{\lambda}{\kappa_i}. \tag{6}$$

Global Constraints

- Maaloe et al. [?]: Per-cell quantity of RNA, DNA, and protein increase exponentially with λ
  $\implies$ Protein concentration is **nearly constant**.
- Total protein constraint: $\sum_i \phi_i = 1$.

Protein mass fraction is a **linear** function of growth rate:

$$\phi_i = \phi_i^0 + \frac{\lambda}{\kappa_i}. \tag{6}$$

Ohm's law: $\Delta V = I/G$.

Global Constraints

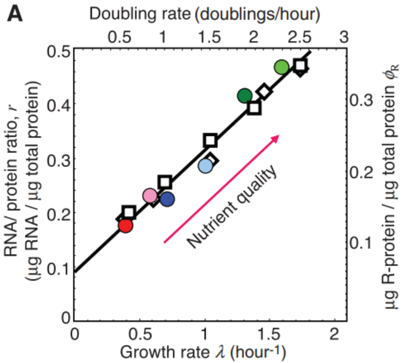

Figure 5: Growth rate is modulated by quality of nutrient [?].

Global Constraints

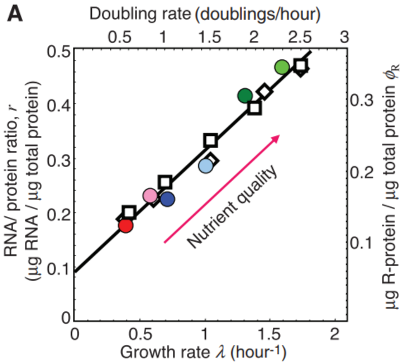

Figure 5: Growth rate is modulated by quality of nutrient [?].

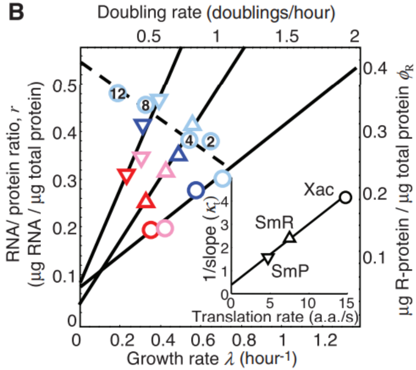

Figure 6: Growth rate is modulated by translational inhibition [?].

Proteomic Coarse-Graining and Electric Circuit

Bow-Tie Topology

Bow-Tie Topology

Conserved large-scale topological features among micro-organisms:

Conserved large-scale topological features among micro-organisms:

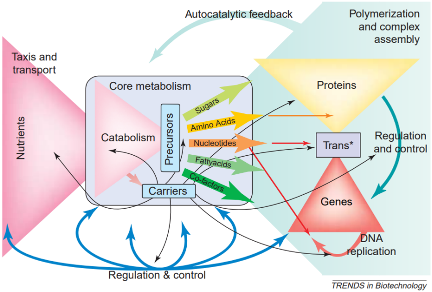

Figure 7: Common module for bacterial metabolism: diversity of inputs and outputs, processed with few intermediate common currencies [**?**].

Bow-Tie Topology

Conserved large-scale topological features among micro-organisms:

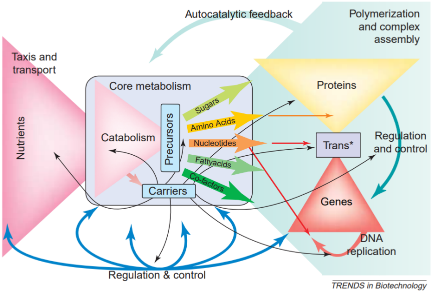

Figure 7: Common module for bacterial metabolism: diversity of inputs and outputs, processed with few intermediate common currencies [**?**].

- Bacterial metabolism and transcriptional machinery exhibits **bow tie architecture**

Bow-Tie Topology

Conserved large-scale topological features among micro-organisms:

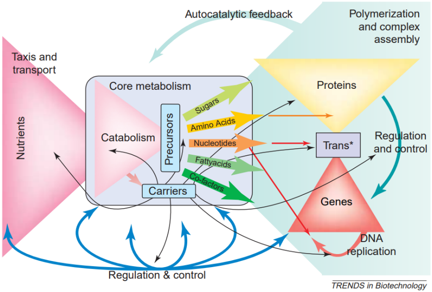

Figure 7: Common module for bacterial metabolism: diversity of inputs and outputs, processed with few intermediate common currencies [?].

- Bacterial metabolism and transcriptional machinery exhibits **bow tie architecture**
- Proteins can be partitioned into only few classes

Equivalent Circuits and Kirchhoff's Laws

Kirchhoff's Laws

Governing laws for DC circuits

$$\sum_{\text{node } m} j_n = 0 \text{ (current law)}, \tag{7}$$

$$\sum_i \phi_i = 0 \text{ (voltage law)}. \tag{8}$$

j_n is proportional to λ.

Equivalent Circuits and Kirchhoff's Laws

Kirchhoff's Laws

Governing laws for DC circuits

$$\sum_{\text{node } m} j_n = 0 \text{ (current law)}, \tag{7}$$

$$\sum_{i} \phi_i = 0 \text{ (voltage law)}. \tag{8}$$

j_n is proportional to λ.

Thevenin's Law

A network of voltage sources and resistors can be replaced by an equivalent circuit with one voltage source and one resistor.

Anti-Correlation Among Proteome Sectors

- **Proteome partition**: coarse-graining proteins into sectors that behave similarly under specific probes, e.g. functionality

Anti-Correlation Among Proteome Sectors

- **Proteome partition**: coarse-graining proteins into sectors that behave similarly under specific probes, e.g. functionality
- Two sectors: $\phi_{\mathrm{M}}^{0} + \phi_{\mathrm{R}}^{0} = 1$

Anti-Correlation Among Proteome Sectors

- **Proteome partition**: coarse-graining proteins into sectors that behave similarly under specific probes, e.g. functionality
- Two sectors: $\phi_{\mathrm{M}}^{0} + \phi_{\mathrm{R}}^{0} = 1$
- Antibiotic decreases λ without affecting ϕ_{M}^{0}: modulates κ_{R} alone.
- Nutrient quality modulates κ_{M}.

Ohmics

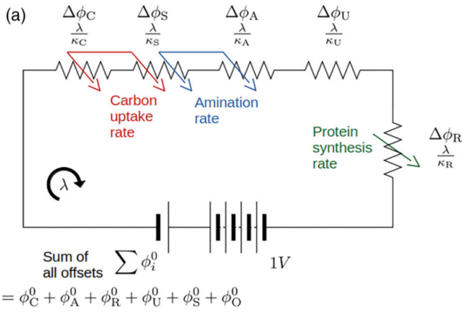

Figure 8: Six-sectors: ribosomes (R), carbon uptake (C), a.a. biosynthesis (A), carbon uptake + a.a. biosynthesis (S), λ-dependent but not inhibited (U), not λ-dependent [**?**].

Ohmics

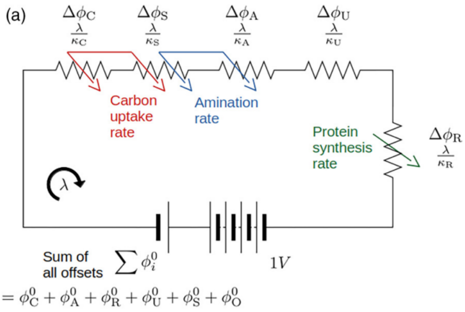

Figure 8: Six-sectors: ribosomes (R), carbon uptake (C), a.a. biosynthesis (A), carbon uptake + a.a. biosynthesis (S), λ-dependent but not inhibited (U), not λ-dependent [?].

Coarse-grain according to proteins' response to probes.

$$\lambda = \frac{1 - \phi_C^0 - \phi_A^0 - \phi_R^0 - \phi_U^0 - \phi_S^0 - \phi_O^0}{1/\kappa_C + 1/\kappa_A + 1/\kappa_R + 1/\kappa_U + 1/\kappa_S} \tag{9}$$

Ohmics

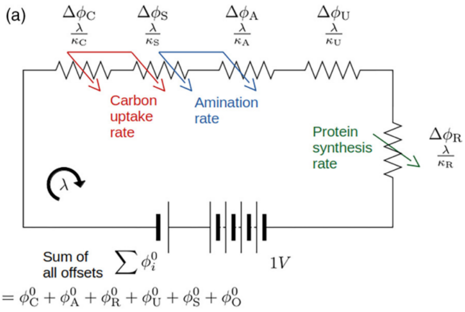

Figure 8: Six-sectors: ribosomes (R), carbon uptake (C), a.a. biosynthesis (A), carbon uptake + a.a. biosynthesis (S), λ-dependent but not inhibited (U), not λ-dependent [?].

Coarse-grain according to proteins' response to probes.

$$\lambda = \frac{1 - \phi_C^0 - \phi_A^0 - \phi_R^0 - \phi_U^0 - \phi_S^0 - \phi_O^0}{1/\kappa_C + 1/\kappa_A + 1/\kappa_R + 1/\kappa_U + 1/\kappa_S} \tag{9}$$

Growth on N carbon sources:

$$\frac{1}{\kappa_C} \longrightarrow \frac{1}{\kappa_{C_1} + \kappa_{C_2} + \cdots + \kappa_{C_N}} \tag{10}$$

Applications

Antibiotic Transport and Binding

Antibiotic Transport and Binding

- Ribosome-targeting antibiotics can modulate conductance κ_{R}.

Antibiotic Transport and Binding

- Ribosome-targeting antibiotics can modulate conductance κ_{R}.
- "Ohmics" assumption for antibiotics-growth rate relationship:

$$\begin{cases} \frac{\mathrm{d}a}{\mathrm{d}t} &= -\lambda a - k_{\mathrm{on}} a r_{\mathrm{u}} + k_{\mathrm{off}} r_{\mathrm{b}} + P_{\mathrm{in}} a_{\mathrm{ex}} - P_{\mathrm{out}} a, \\ \frac{\mathrm{d}r_u}{\mathrm{d}t} &= -\lambda r_{\mathrm{u}} - k_{\mathrm{on}} a r_{\mathrm{u}} + k_{\mathrm{off}} r_{\mathrm{b}} + s(\lambda), \\ \frac{\mathrm{d}r_b}{\mathrm{d}t} &= -\lambda r_{\mathrm{b}} + k_{\mathrm{on}} a r_{\mathrm{u}} - k_{\mathrm{off}} r_{\mathrm{b}}. \end{cases} \tag{11}$$

$a_{\mathrm{ex}}, k_{\mathrm{on}}, k_{\mathrm{off}}, P_{\mathrm{in}}, P_{\mathrm{out}} \in \mathbb{R}_{\geq 0}$. $s(\lambda)$ is undetermined!

Antibiotic Transport and Binding

- Ribosome-targeting antibiotics can modulate conductance κ_{R}.
- "Ohmics" assumption for antibiotics-growth rate relationship:

$$\begin{cases} \frac{\mathrm{d}a}{\mathrm{d}t} &= -\lambda a - k_{\mathrm{on}} a r_{\mathrm{u}} + k_{\mathrm{off}} r_{\mathrm{b}} + P_{\mathrm{in}} a_{\mathrm{ex}} - P_{\mathrm{out}} a, \\ \frac{\mathrm{d}r_u}{\mathrm{d}t} &= -\lambda r_{\mathrm{u}} - k_{\mathrm{on}} a r_{\mathrm{u}} + k_{\mathrm{off}} r_{\mathrm{b}} + s(\lambda), \\ \frac{\mathrm{d}r_b}{\mathrm{d}t} &= -\lambda r_{\mathrm{b}} + k_{\mathrm{on}} a r_{\mathrm{u}} - k_{\mathrm{off}} r_{\mathrm{b}}. \end{cases} \tag{11}$$

  $a_{\mathrm{ex}}, k_{\mathrm{on}}, k_{\mathrm{off}}, P_{\mathrm{in}}, P_{\mathrm{out}} \in \mathbb{R}_{\geq 0}$. $s(\lambda)$ is undetermined!
- Qualitatively different behavior based on binding affinity.

Antibiotic Transport and Binding

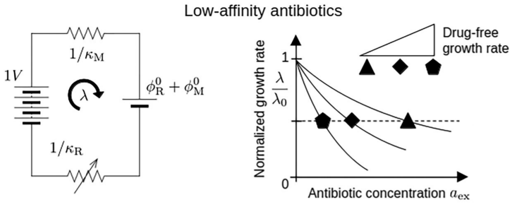

Figure 9: Low-affinity antibiotics [?].

Antibiotic Transport and Binding

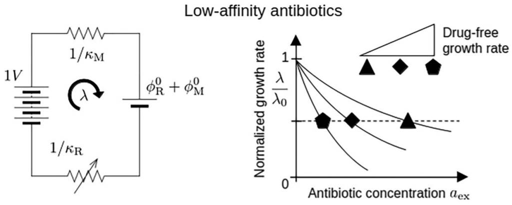

Figure 9: Low-affinity antibiotics [?].

- Langmuir-like inhibition curves.

Antibiotic Transport and Binding

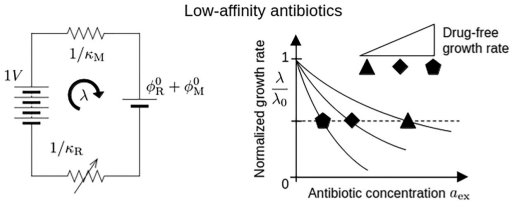

Figure 9: Low-affinity antibiotics [?].

- Langmuir-like inhibition curves.
- Half-inhibition conc. anti-correlated with growth rate:

$$\lambda_0 = \lambda \left(1 + \frac{a_{\text{ex}}}{\text{IC}_{50}}\right).$$

Antibiotic Transport and Binding

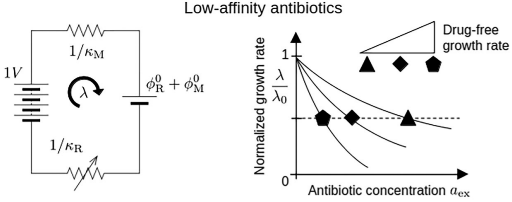

Figure 9: Low-affinity antibiotics [?].

- Langmuir-like inhibition curves.
- Half-inhibition conc. anti-correlated with growth rate:

$$\lambda_0 = \lambda \left(1 + \frac{a_{\mathrm{ex}}}{\mathrm{IC}_{50}}\right).$$

- Effective against **fast-growing** bacteria.

Antibiotic Transport and Binding

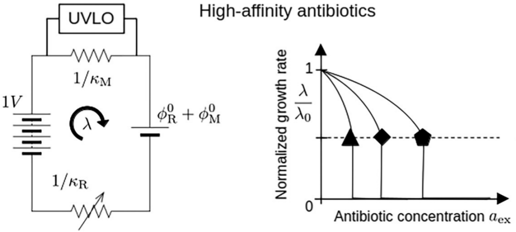

Figure 10: High-affinity antibiotics [?].

Antibiotic Transport and Binding

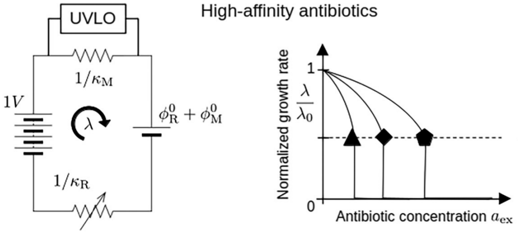

Figure 10: High-affinity antibiotics [?].

- Sigmoidal inhibition curves.

Antibiotic Transport and Binding

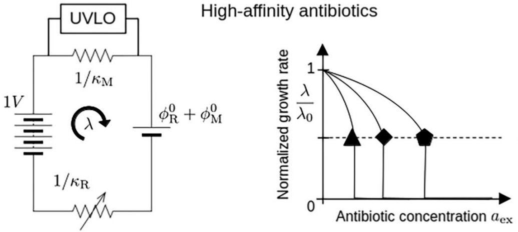

Figure 10: High-affinity antibiotics [?].

- Sigmoidal inhibition curves.
- Half-inhibition conc. correlated with growth rate:

$$\lambda_0 = \lambda \left[\frac{1}{2} \left(1 + \sqrt{1 - \frac{a_{\mathrm{ex}}}{\mathrm{IC}_{50}}} \right) \right]^{-1}.$$

- Abrupt drop of λ at IC_{50} analogous to an undervoltage lockout (UVLO)

Antibiotic Transport and Binding

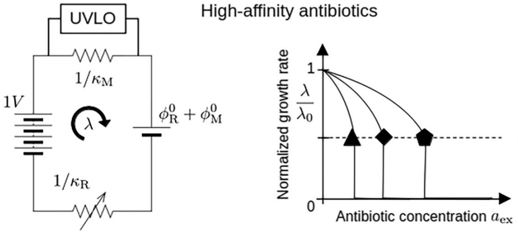

Figure 10: High-affinity antibiotics [?].

- Sigmoidal inhibition curves.
- Half-inhibition conc. correlated with growth rate:

$$\lambda_0 = \lambda \left[\frac{1}{2} \left(1 + \sqrt{1 - \frac{a_{ex}}{IC_{50}}} \right) \right]^{-1}.$$

- Abrupt drop of λ at IC_{50} analogous to an undervoltage lockout (UVLO)
- Effective against **slow-growing** bacteria.

Evolutionary Adaptation Studies

Evolutionary Adaptation Studies

Ohmic constraints help direct evolutionary adaptation trajectories by projecting genetic changes to a small set of circuit parameters, e.g. six-sector partition.

Evolutionary Adaptation Studies

Ohmic constraints help direct evolutionary adaptation trajectories by projecting genetic changes to a small set of circuit parameters, e.g. six-sector partition.

Adapting E. coli to growth in glucose and citrate [?]

- Parameters unchanged except for decrease in $\phi^0_\mathsf{O}, \phi^0_\mathsf{A}, \phi^0_\mathsf{S}$.

Evolutionary Adaptation Studies

Ohmic constraints help direct evolutionary adaptation trajectories by projecting genetic changes to a small set of circuit parameters, e.g. six-sector partition.

Adapting E. coli to growth in glucose and citrate [?]

- Parameters unchanged except for decrease in $\phi^0_{\mathrm{O}}, \phi^0_{\mathrm{A}}, \phi^0_{\mathrm{S}}$.
- Mechanistic explanation:
  - ϕ^0_{O}:decrease in porin *OmpF*
  - $\phi^0_{\mathrm{A}}, \phi^0_{\mathrm{S}}$: enzymes associates with pyruvate kinase *PykF*.

Concluding Remarks

Additional Thoughts

Additional Thoughts

- The Ohmics model is phenomenological.

Additional Thoughts

- The Ohmics model is phenomenological.
- Fow nutrient quality, we can recover linearity directly from the ODE

Additional Thoughts

- The Ohmics model is phenomenological.
- Fow nutrient quality, we can recover linearity directly from the ODE

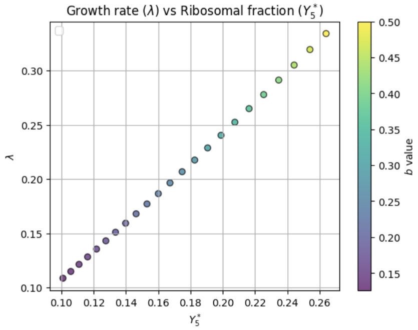

Figure 11: Three-sector partition (low nutrient).

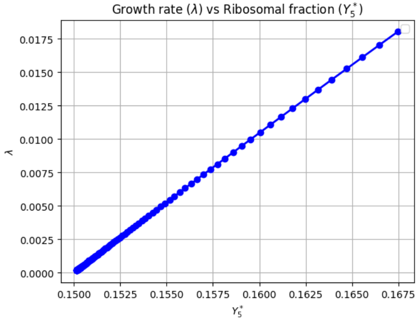

Figure 12: Six-sector partition (low nutrient).

Conclusion

- **(Near)-invariant protein concentration + enzyme kinetics** provides linkage between protein fraction and growth rate.

Conclusion

- **(Near)-invariant protein concentration + enzyme kinetics** provides linkage between protein fraction and growth rate.
- Mechanistic justification for coarse-graining complex biochemical networks with circuits.

Conclusion

- **(Near)-invariant protein concentration** $+$ **enzyme kinetics** provides linkage between protein fraction and growth rate.
- Mechanistic justification for coarse-graining complex biochemical networks with circuits.
- Wealth of large-Ohmics data $\longrightarrow$ opportunity for **synthetic biology**.

Thank You

Q & A

References

J. E. Barrick, D. S. Yu, S. H. Yoon, H. Jeong, T. K. Oh, D. Schneider, R. E. Lenski, and J. F. Kim.
Genome evolution and adaptation in a long-term experiment with escherichia coli.
Nature, 461(7268):1243–1247, Oct. 2009.

S. Cooper.
Bacterial Growth and Division: Biochemistry and Regulation of Prokaryotic and Eukaryotic Division Cycles.
Academic Press, San Diego, CA, 1st edition, 1991.
Also available as an eBook (ISBN: 978-0080917474).

M. Csete and J. Doyle.
Bow ties, metabolism and disease.
Trends in Biotechnology, 22(9):446–450, 2004.

S. Hui, J. M. Silverman, S. S. Chen, D. W. Erickson, M. Basan, J. Wang, T. Hwa, and J. R. Williamson.
Quantitative proteomic analysis reveals a simple strategy of global resource allocation in bacteria.
Molecular Systems Biology, 11(2):784, 2015.

W.-H. Lin.
Biomass transfer on autocatalytic reaction network: a delay differential equation formulation, 2025.

W.-H. Lin, E. Kussell, L.-S. Young, and C. Jacobs-Wagner.
Origin of exponential growth in nonlinear reaction networks.
Proceedings of the National Academy of Sciences, 117(45):27795–27804, 2020.

J. D. Orth, I. Thiele, and B. Palsson.
What is flux balance analysis?
Nature Biotechnology, 28(3):245–248, Mar. 2010.

M. Scott, C. W. Gunderson, E. M. Mateescu, Z. Zhang, and T. Hwa.
Interdependence of cell growth and gene expression: Origins and consequences.
Science, 330(6007):1099–1102, 2010.

M. Zim, C. Euler, and M. Scott.
Constraints on metabolic network analysis in bacterial physiology.
PRX Life, 3:022001, Apr 2025.