A simple thermodynamic relation, very useful for metabolic modelling

or: how I learned to stop worrying and love equilibrium constants

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Overview: a useful thermodynamic relation

One-way reaction rates and thermodynamic force

$$\frac{v_+}{v_-} = \mathrm{e}^{\Theta}$$

$$\Theta = -\Delta_\mathrm{r} G'/RT$$

1. **Relationships between kinetic constants**
   Equilibrium constant and Haldane relationship

2. **Relationship between concentrations and flux directions**
   Thermodynamic force, flux directions, and metabolite levels

3. **Bounds on catalytic efficiency and enzyme demand**
   Forces $\rightarrow$ Flux ratios $\rightarrow$ Enzyme efficiency $\rightarrow$ Enzyme demand in metabolism

4. **Impact on control and generation and propagation of noise**
   Forces $\rightarrow$ Flux ratios $\rightarrow$ Elasticities $\rightarrow$ Control and fluctuations

One-way reaction rates and thermodynamic force

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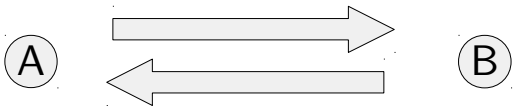
Thermodynamic equilibrium between microscopic states: detailed balance!



$$\underbrace{v_+}_{k_+\, p_A} = \underbrace{v_1}_{k_-\, p_B}$$

$$\frac{k_+}{k_-} = \frac{p_B}{p_A} = \frac{e^{-F_B/RT}}{e^{-F_A/RT}} = e^{-\Delta F/RT}$$

One-way rates and thermodynamic force

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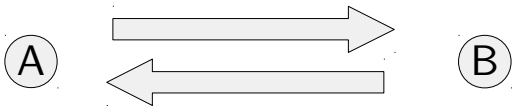

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$$\frac{k_+}{k_-} = \frac{p_B}{p_A} = \frac{e^{-F_B/RT}}{e^{-F_A/RT}} = e^{-\Delta F/RT}$$

Non-equilibrium metabolic reaction

The net reaction rate results from one-way rates $v = v_+ - v_-$

Forward-driven reaction

Small reverse rate: net rate given by forward rate!

Near-equilibrium reaction

Forward and reverse rates much larger than net rate!

$$\frac{v_+}{v_-} = \mathrm{e}^{\Theta} \qquad \Theta = \ln K_{eq} - \ln \frac{p}{s} \qquad \Theta = -\Delta_{\mathrm{r}} G'/RT$$

How are one-way rates related to reactant concentrations?

Reversible Michaelis-Menten law

$$v(s,p,e) = e\,\frac{k_+\, s/K_s - k_-\, p/K_p}{1 + s/K_s + p/K_p}$$

Rate ratio

$$\frac{v_+}{v_-} = \frac{k_+}{k_-}\frac{K_p}{K_S}\frac{s}{p}$$

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In **one** equilibrium state (v = 0), we obtain

$$\frac{v_+}{v_-} = 1$$

Equilibrium reaction

Rate ratio

$$1 = \frac{k_+}{k_-}\frac{K_p}{K_S}\underbrace{\frac{s^{\mathrm{eq}}}{p^{\mathrm{eq}}}}_{1/K_{\mathrm{eq}}}$$

Haldane relationship

$$K_{eq} = \frac{k_+}{k_-}\frac{K_p}{K_s}$$

$\rightarrow$ same mass-action ratio in **all** equilibrium states, given by equilibrium constant $K_{eq} = \dfrac{p^{\mathrm{eq}}}{s^{\mathrm{eq}}}$

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$\rightarrow$ same mass-action ratio in **all** equilibrium states, given by equilibrium constant $K_{eq} = \frac{p^{\mathrm{eq}}}{s^{\mathrm{eq}}}$

One-way rate ratio $$\frac{v_+}{v_-} = K_{eq}\,\frac{s}{p}$$

Rewrite as $\frac{v_+}{v_-} = \mathrm{e}^{\Theta}$ with thermodynamic driving force $\Theta = \ln K_{eq} - \ln\frac{p}{s}$

.. which is nothing but $\Theta = -\Delta_{\mathrm{r}}G'/RT$

1. Relationships between kinetic constants

Thermodynamic constraints for kinetic constants

Haldane relationships (between equilibrium constants and kinetic constants)

$$K_{eq} = \frac{k_+}{k_-}\,\frac{K_p}{K_s} \qquad \text{(for Michaelis-Menten rate law)}$$

Wegscheider conditions (between equilibrium constants)

$$\ln K_{\mathrm{eq}} = N^{\mathrm{T}}\,\ln c^{\mathrm{eq}} = N^{\mathrm{T}}\,x \qquad \rightarrow \text{ in the span of } \; N^{\mathrm{T}}$$

This imposes, e.g., that product of equilibrium constants around a cycle = 1!

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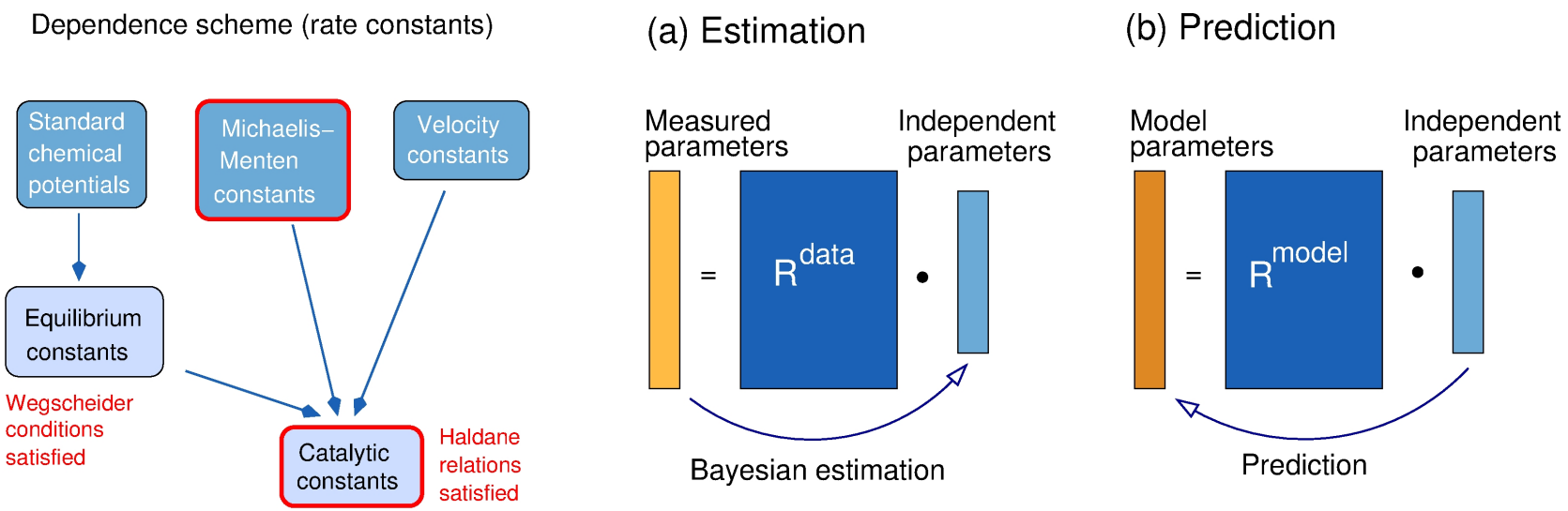
Application: Estimation of balanced kinetic model parameters



Lubitz et al. (2010) J. Phys. Chem. B 114(49):16298-16303.

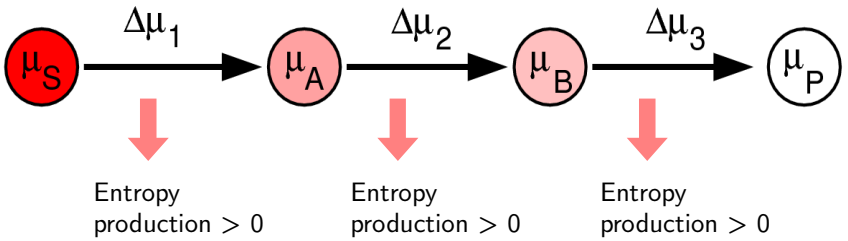
2. Metabolite concentrations, forces, and flux directions

Thermodynamic forces and flux directions

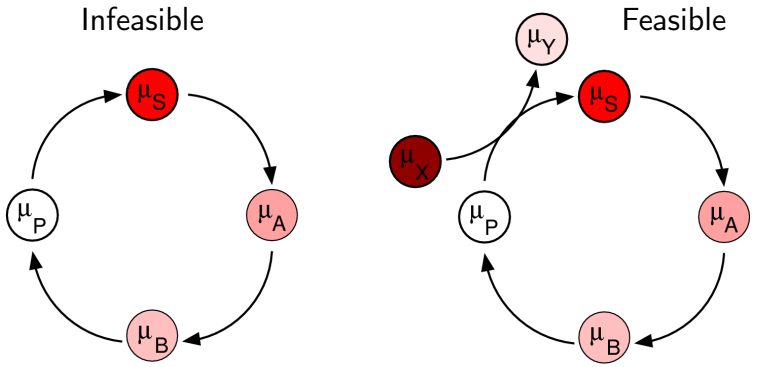
Our formula $\frac{v_+}{v_-} = \mathrm{e}^{-\Delta_\mathrm{r}\Theta}$ implies:

Sign constraint: flux and force have the same sign!

$$\mathrm{sign}(v) = \mathrm{sign}(\Theta) = -\mathrm{sign}(\Delta_\mathrm{r}G) = -\mathrm{sign}(\Delta_\mathrm{r}\mu)$$



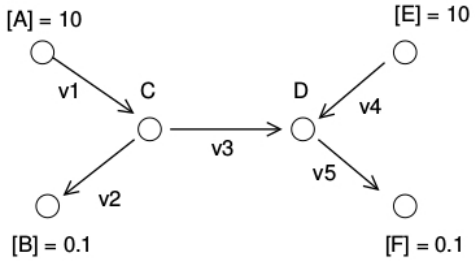
Entropy production / (time * volume) $\sigma = v\,\Theta$



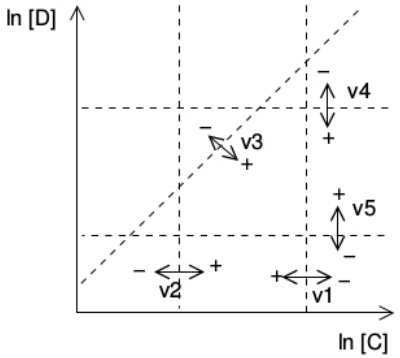
Thermodynamic constraints on fluxes and metabolite levels

The thermodynamic force is a linear function in log-metabolite space!

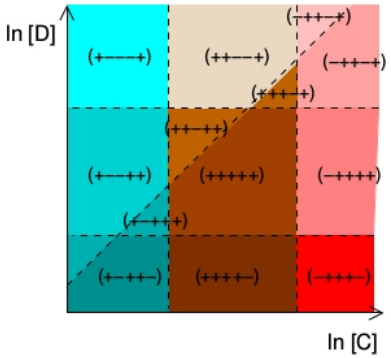
(a) Example model with nominal reaction directions



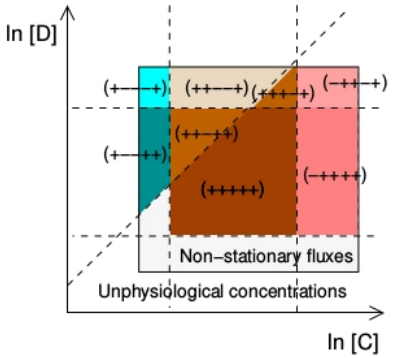
(b) Flux sign constraints in metabolite space



(c) Tesselation of metabolite space



(d) Feasible M–polytopes



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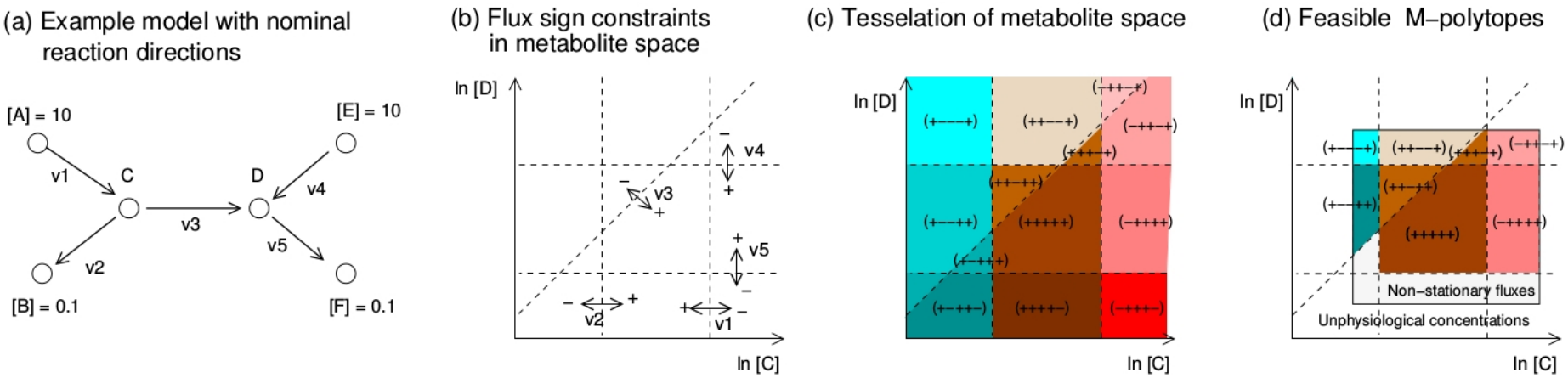


Usage in flux modelling

1. Assume metabolite levels c $\rightarrow$ compute ΔG $\rightarrow$ obtain directions of all fluxes v!
2. Assume fluxes v $\rightarrow$ obtain set of feasible metabolite profiles c

"Reversible reactions"

Every reaction is reversible in theory, but some aren't if physiological metabolite bounds are imposed (eg. 1 nm $<$ c $<$ 100 mM)

1. "Thermodynamically" feasible flux distribution

- Feasible for **some** metabolite profile (no further restrictions)
- Loopless condition: no thermodynamic flux cycles

2. "Thermo-physiologically" feasible flux distribution

- Feasible for a **plausible** metabolite profile (within physiological bounds)
- Excludes not only loops, but also pathways in "wrong direction"

Max-Min driving force criterion, avoiding distributed bottlenecks

Thermodynamic sign constraint:

Along a pathway (= following flux directions), all forces must be positive

$\rightarrow$ Feasibility criterion for fluxes: "does the metabolite polytope have a finite volume?"

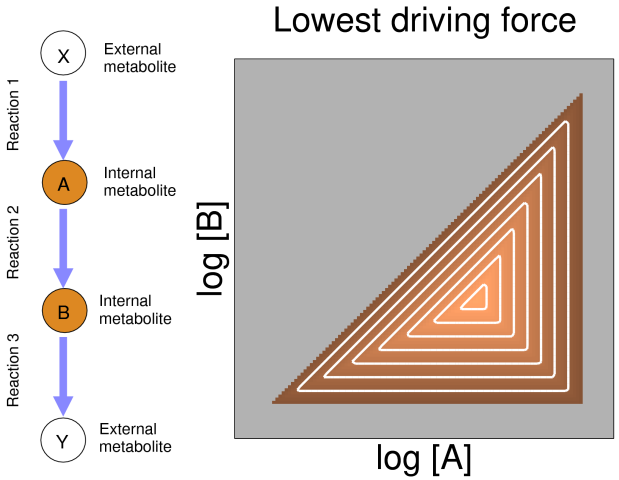
Physiological metabolite constraints can be imposed ("Thermo-physiologically feasible")

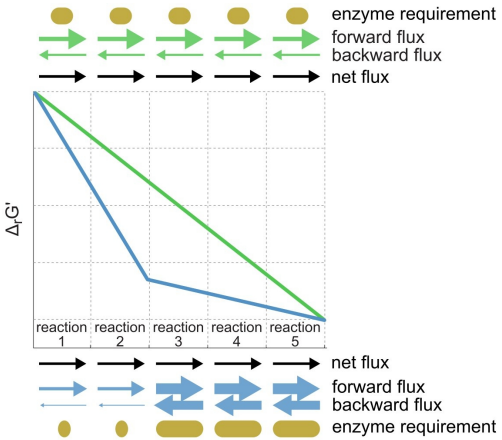
MDF criterion (assuming feasible fluxes):

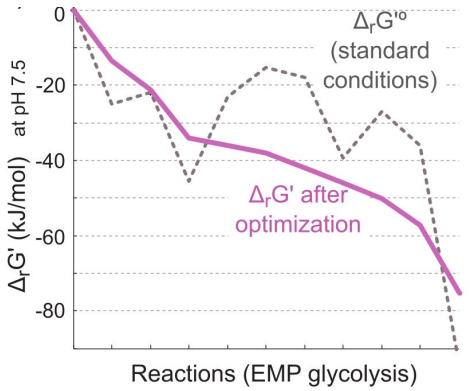
Along a pathway (=following flux directions), the smallest force must still be as large as possible!

$\Rightarrow$ Optimality criterion for metabolite levels and thermodynamic forces

Motivation: avoid near-equilibrium reactions (because of their low catalytic efficacy)!







Noor et al. (2014), PLoS Computational Biology 10 (2): e1003483.

3. Thermodynamic bounds on catalytic efficiency and enzyme demand

A small force increases microscopic rates and enzyme demand

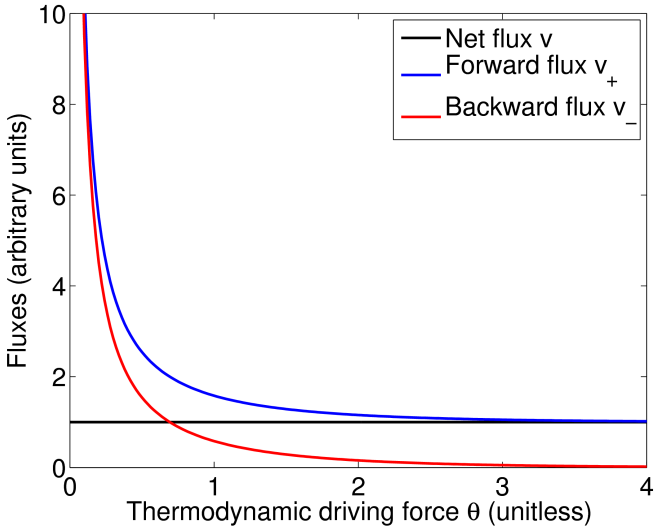
Forward-driven reaction (large force)

One-way flux resembles net flux

Near-equilibrium reaction (small force)

Large one-way fluxes / net flux!

$$v = v_+ - v_- \qquad \frac{v_+}{v_-} = \mathrm{e}^{\Theta}$$

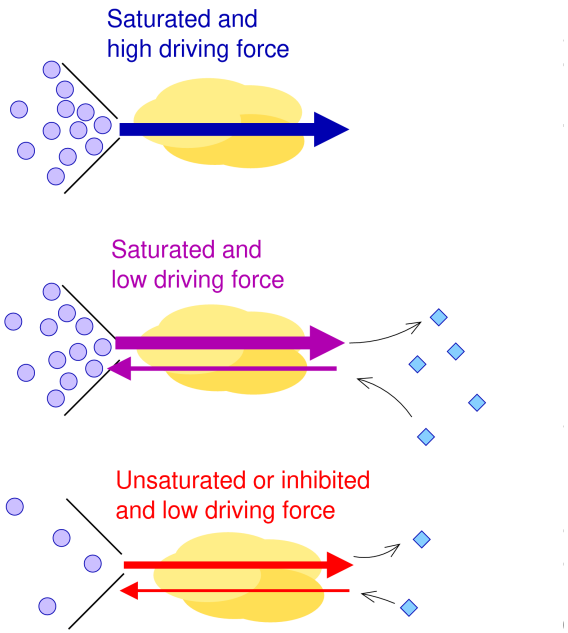


$$v_+ = \frac{e^{\Theta}}{e^{\Theta} - 1} v \qquad v_- = \frac{1}{e^{\Theta} - 1} v$$

Since microscopic fluxes are enzyme-driven, **small forces imply high enzyme demands per catalysed (net) flux!**

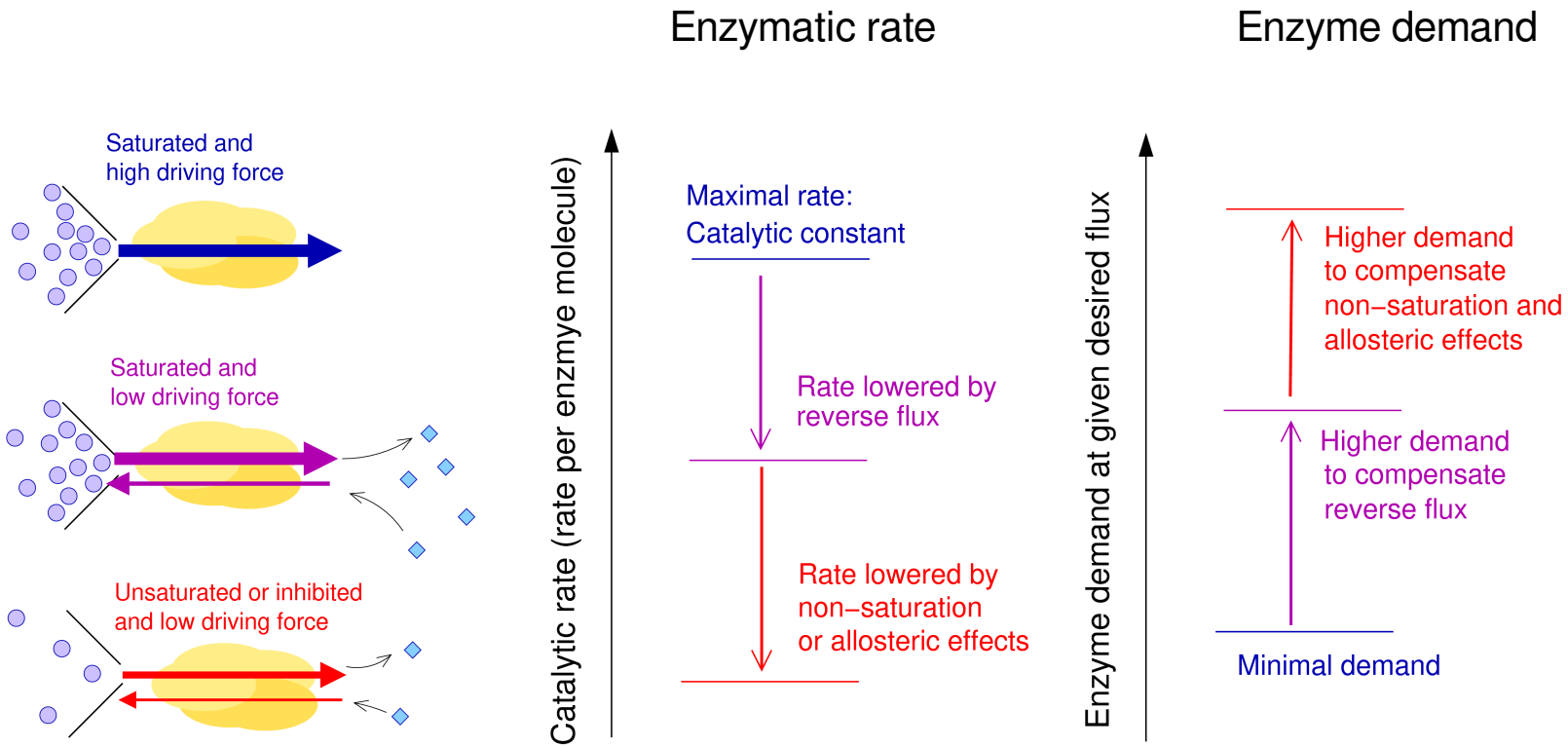
Liebermeister et al. (2010), Bioinformatics 26(12):1528-1534

Which factors determine enzymatic rates?



Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

Low enzyme efficiency implies a high enzyme demand



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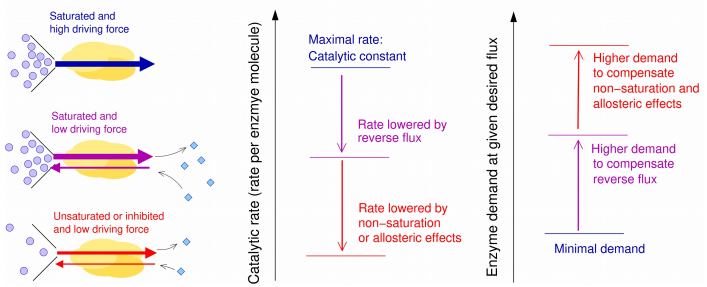
Separable rate laws: splitting rate laws into factors



Reaction rate

rate: enzyme level · forward catalytic constant · reversibility factor · saturation factor · regulation factor

$$v = E \cdot k_{\mathrm{cat}}^{+} \cdot \underbrace{[1 - \mathrm{e}^{-\theta}]}_{\eta^{\mathrm{rev}}} \cdot \underbrace{\frac{s/K_{\mathrm{S}}}{1 + s/K_{\mathrm{S}} + p/K_{\mathrm{P}}} \cdot \frac{1}{1 + x/K_{\mathrm{I}}}}_{\eta^{\mathrm{kin}}}$$

Noor et al. (2013), FEBS Letters 587(17):2772–2777

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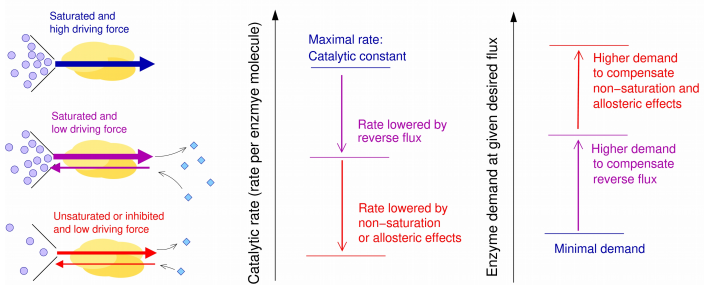
Separable rate laws: splitting enzyme cost into factors



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Enzyme demand

enzyme cost = minimum enzyme cost · $1/\eta^{\mathrm{rev}}$ · $1/\eta^{\mathrm{kin}}$

$$\underbrace{q = h_{\mathrm{E}} \cdot E}_{\text{enzyme cost}} = \overbrace{h_{\mathrm{E}} \cdot v \cdot \frac{1}{k_{\mathrm{cat}}^{+}}}^{\text{minimum enzyme cost}} \cdot \underbrace{\frac{1}{[1 - \mathrm{e}^{-\theta}]}}_{1/\eta^{\mathrm{rev}}} \cdot \underbrace{\frac{1 + s/K_{\mathrm{S}} + p/K_{\mathrm{P}}}{s/K_{\mathrm{S}}} \cdot [1 + x/K_{\mathrm{I}}]}_{1/\eta^{\mathrm{kin}}}$$

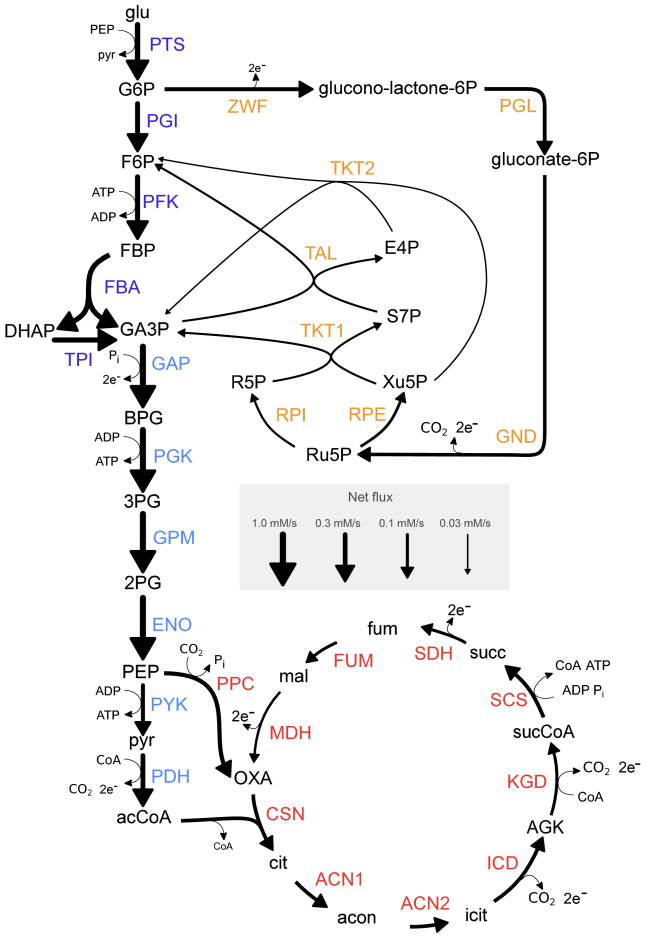
$h_{\mathrm{E}} \cdot E$: enzyme burden

Noor et al. (2013), FEBS Letters 587(17):2772–2777

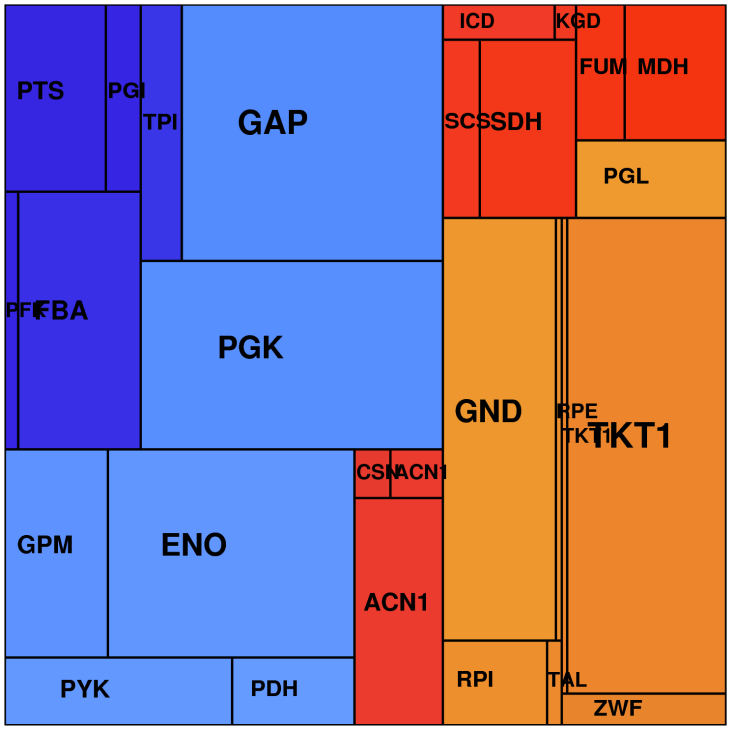
Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

How to predict the enzyme demand of given fluxes?

Measured fluxes in *E. coli* central metabolism

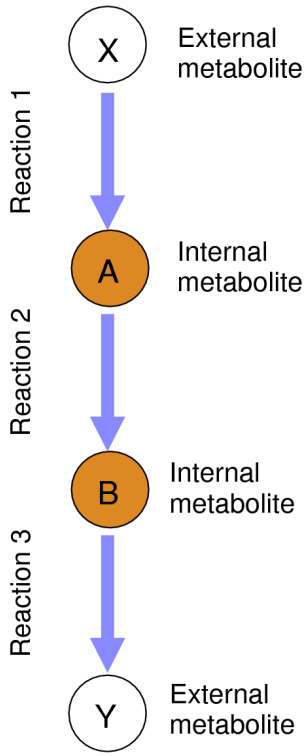


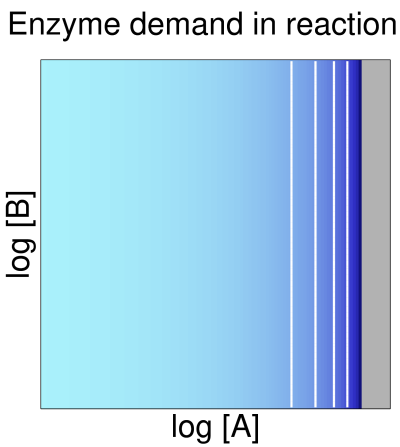
Measured enzyme levels

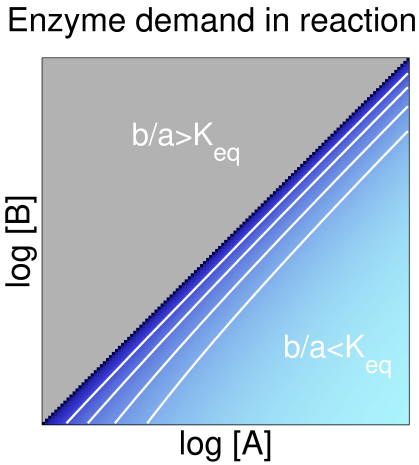


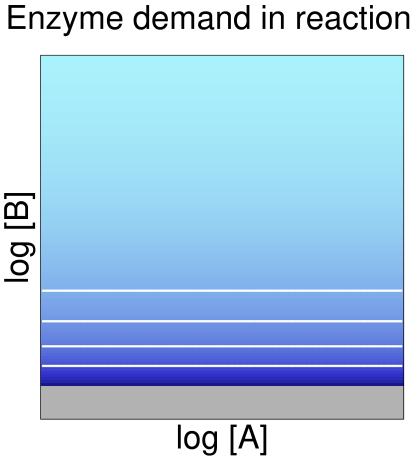
Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

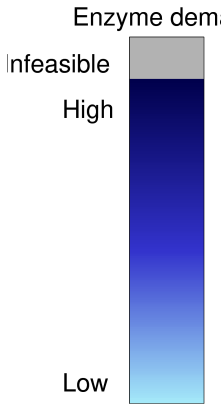
Enzyme cost minimisation: computing optimal metabolite and enzyme levels











Rate laws $$v_l = e_l \, f_l(c)$$

Enzyme cost $$q = \sum_l h_l \, e_l = \sum_l h_l \, \frac{v_l}{f_l(c)}$$

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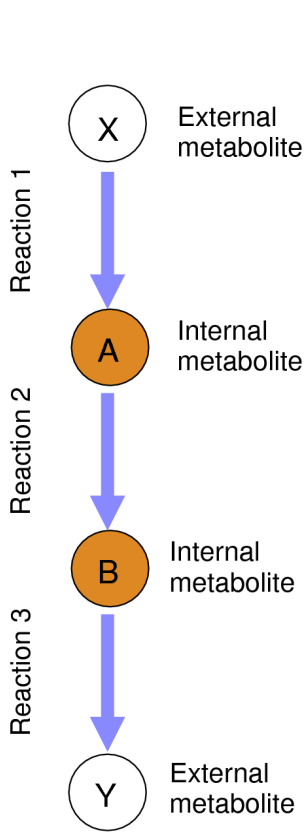
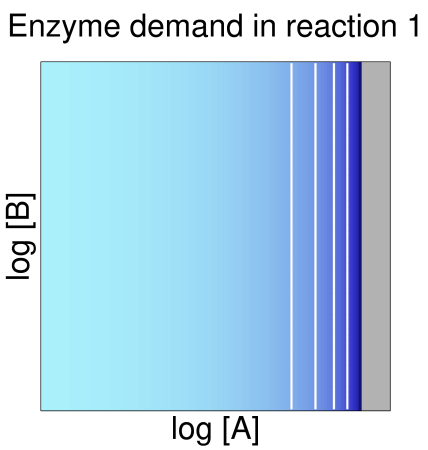
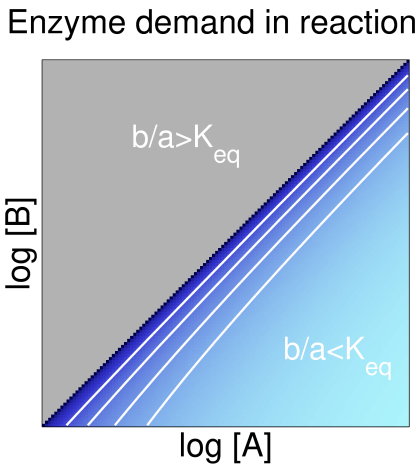
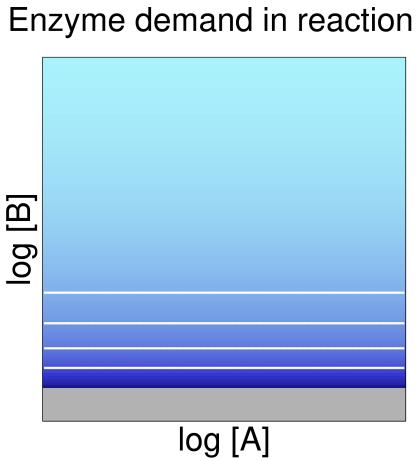
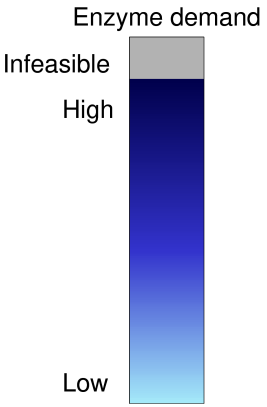
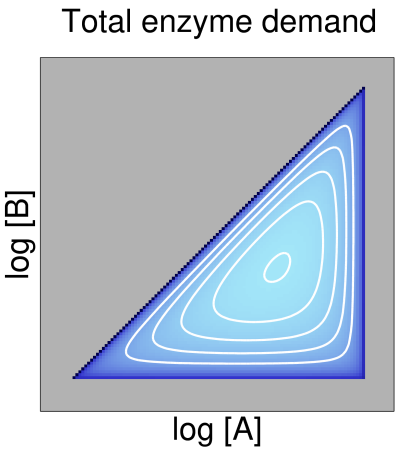


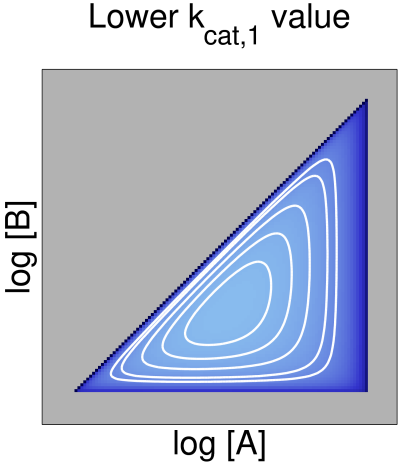


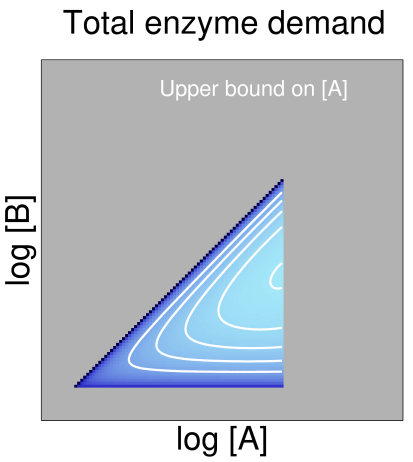








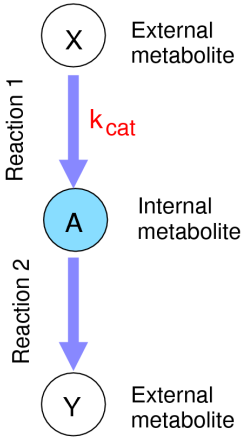




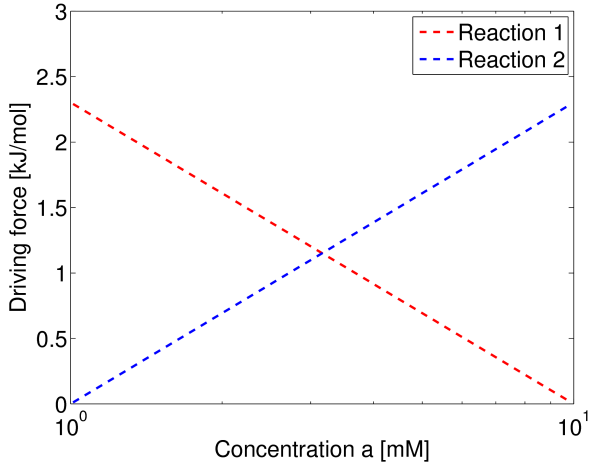
Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

Thermodynamics puts a lower bound on enzyme demand and explains steep "walls"

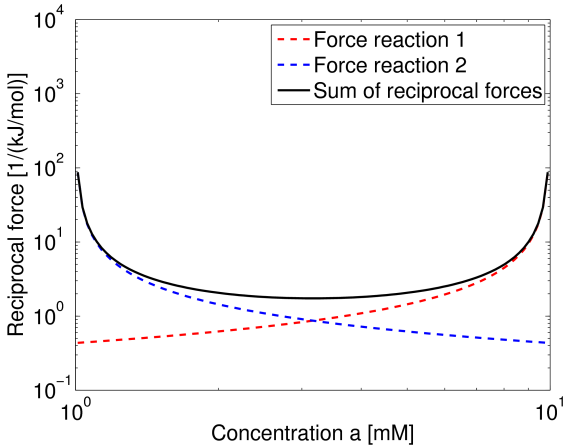
Example model



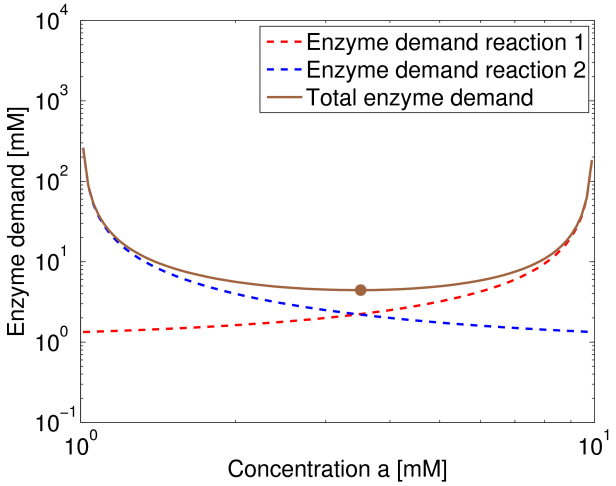
Thermodynamic forces (in metabolite space)



Sum of inverse forces



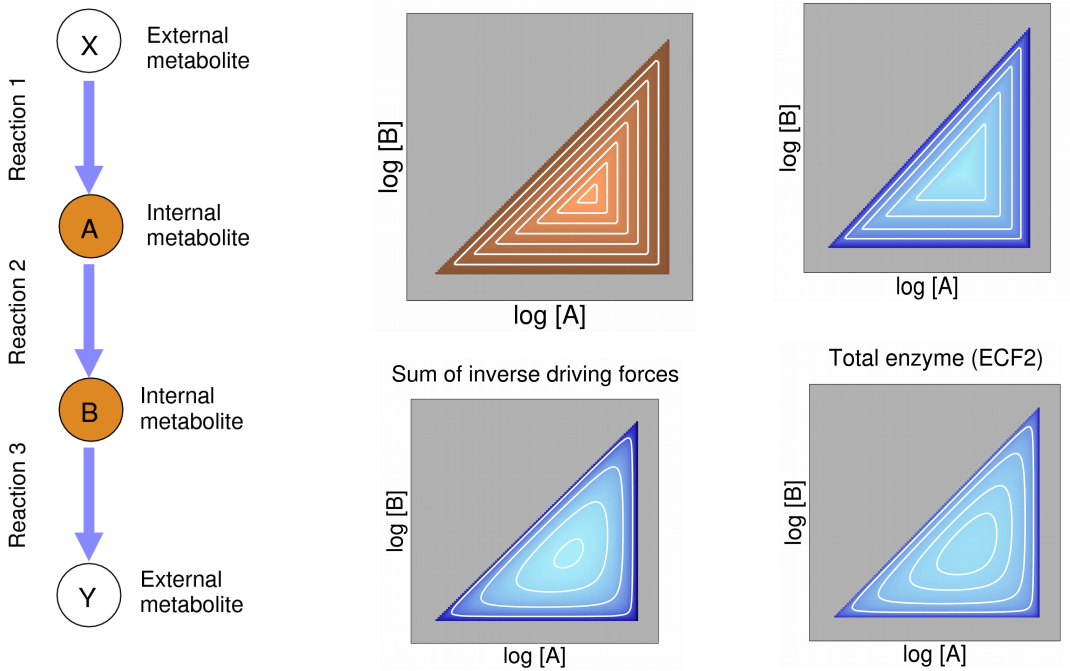
Enzyme cost in kinetic model



Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

Enzyme cost can be approximated by thermodynamic forces

1. Approximating enzyme cost functions on the metabolite polytope



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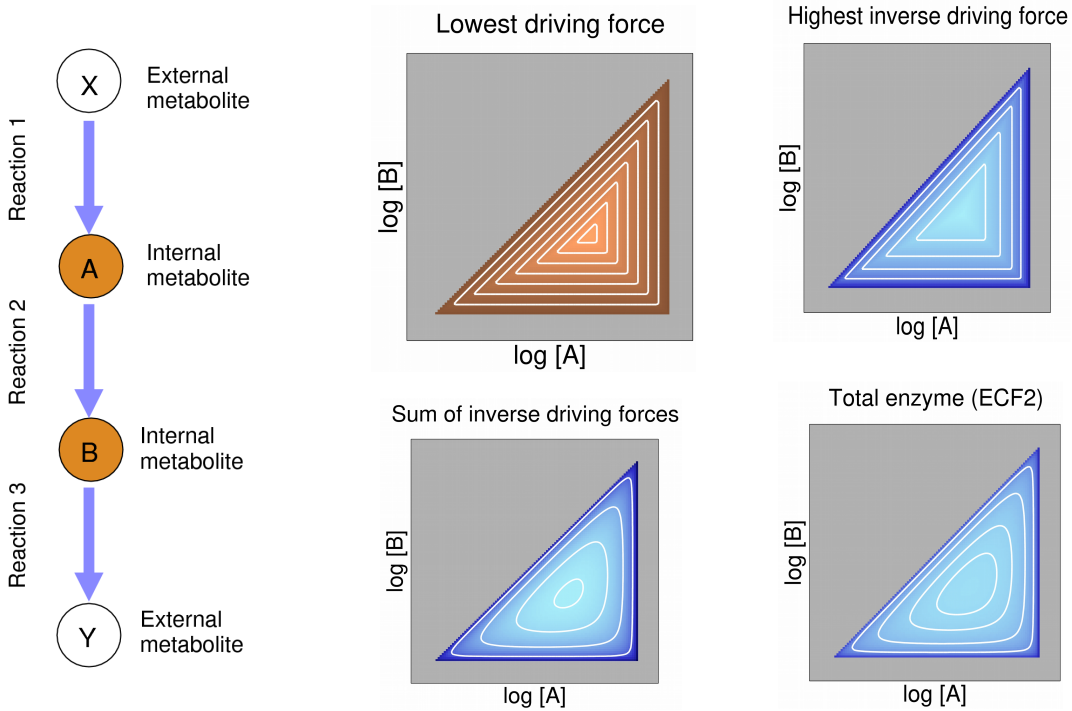


2. Minimal "affordable" driving force (tighter bound than just positivity)

Requirement: $e \leq e_{\max}$

Resulting bound on the force: $\Theta > \dfrac{v}{k_+ \, e_{\max}}$

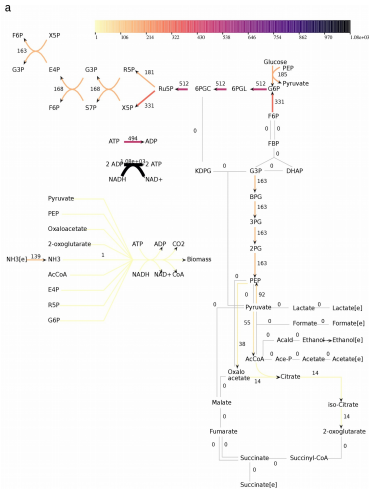
Derivation:

$$\frac{v}{k_+ \, e_{\max}} \leq \frac{v}{k_+ \, e} = \eta^{\mathrm{rev}} \, \eta^{\mathrm{sat}} < \eta^{\mathrm{rev}} = 1 - e^{-\Theta} < \Theta$$

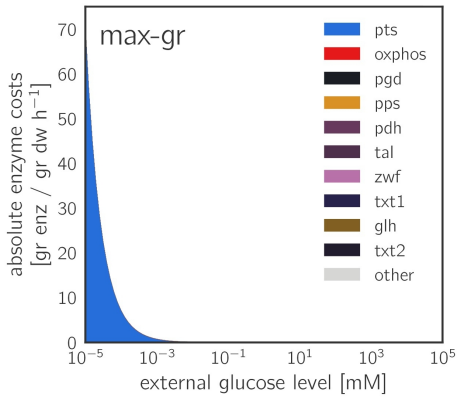
Noor et al. (2016), PLoS Computational Biology 12 (10): e1005167.

Assessing the enzyme demand of elementary flux modes and predicting condition-dependent growth rates

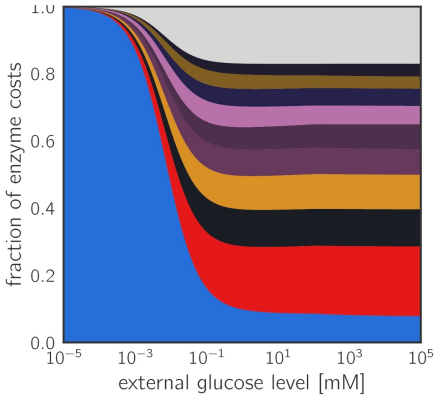
Network model (E. coli)



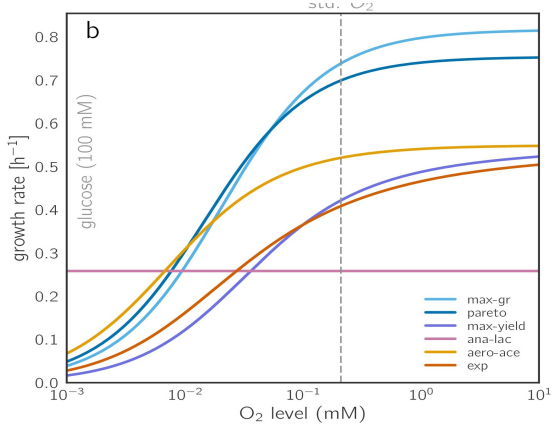
Predicted protein demand



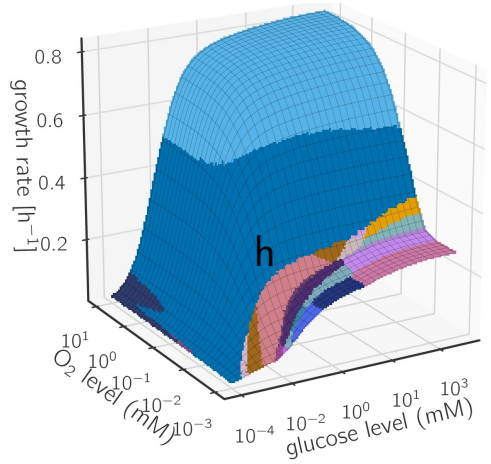
Predicted protein demand (relative)



Predicted Monod curves (oxygen, for EFMs)



Predicted Monod surface (glucose and oxygen)



Wortel et al. (2018), PLoS Computational Biology .

4. Forces, control, and metabolic noise

How do changing reactant levels change the reaction rate?

Forward-driven reaction

Small reverse flux
→ little effect of changing product levels

Near-equilibrium reaction

Similar forward and reverse fluxes
→ similar effects of substrate and product, huge relative changes in net flux

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Reaction elasticities

Forward-driven reaction tend to have (relatively) low product elasticities.

$$E^v_{c_i} = E^{\mathrm{rev}}_i + E^{\mathrm{kin}}_i$$

$$E^{\mathrm{rev}}_{li} = \frac{v^+ m^{\mathrm{S}}_i - v^- m^{\mathrm{P}}_i}{v} = \frac{e^{\Theta} m^{\mathrm{S}}_i - m^{\mathrm{P}}_i}{e^{\Theta} - 1}$$

with molecularities m

Liebermeister et al. (2010), Bioinformatics 26(12):1528-1534
Liebermeister (2013), arXiv:1309.0267

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with molecularities m

Flux control coefficients

In linear reactions chains, forward-driven reactions have (relatively) high flux control.

Example: linear chain with identical kinetics:,

Fully saturated enzymes: $C^J_{v_l} \propto \mathrm{e}^{\Theta} - 1$

Enzymes in linear range: $C^J_{v_l} \propto \frac{\mathrm{e}^{\Theta} - 1}{\prod^i_{m=1} \mathrm{e}^{\Theta_m}}$

Liebermeister et al. (2010), Bioinformatics 26(12):1528-1534
Liebermeister (2013), arXiv:1309.0267
Noor et al. (2014), PLoS Computational Biology 10 (2): e1003483.

Near-equilibrium reactions generate more noise; forward-driven reactions act as rectifiers

1. Chemical noise, generated in individual reactions

Assumption: number of microscopic events is Poisson-distributed

$\rightarrow$ Approximately white noise in net reaction rate, proportional to sqrt(microscopic rates)!

Forward-driven reaction

- Noise production in forward flux
- Noise as expected from net rate

Near-equilibrium reaction

- Noise production in forward and reverse flux
- Much bigger than expected from net rate!

Gillespie (2000) J. Chem. Phys., 113(1):297–306

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2. Noise propagation

- Resembles propagation of dynamical perturbations
- Forward-driven reactions act as rectifiers
- Study by computing spectral densities (derived from spectral response coefficients)

Gillespie (2000) J. Chem. Phys., 113(1):297–306

Liebermeister (2013), arXiv:1309.0267

Conclusions

Thermodynamics, kinetics, protein demand,
and choice of pathway fluxes are tightly entangled!

Which metabolic modelling methods use the methods described?

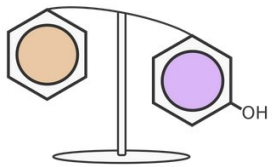
- Kinetic models with reversible rate laws (and Parameter balancing)
- Variants of flux balance analysis (EBA, TMFA, Loopless FBA, ...)
- Enzyme cost prediction (ECM, EFCM)
- Elasticity sampling (for models with reversible rate laws)

Advantage of thermodynamics over full kinetic description:

Thermodynamics yields **general** constraints – not enzyme-specific!

Thermodynamic data

Equilibrium constants / Gibbs free energies



eQuilibrator

Type a name of a compound, reaction or enzyme Search

equilibrator.weizmann.ac.il

Thanks to my collaborators ..

Ron Milo
Weizmann Institute of Science

Elad Noor
ETH Zürich

Arren Bar-Even
MPI for Molecular Plant Physiology

Avi Flamholz
UC Berkely

Dan Davidi
Weizmann Institute of Science

Meike Wortel
CEES, Oslo

Michael Ferris
U. Wisconsin, Madison

Frank Bruggeman
Vrije Universiteit, Amsterdam

Thank you!

Who has used thermodynamics in metabolic modelling, and how?

Which thermodynamic relationships did you use?

What results / constraints did you obtain by using these constraints?

How did this capture properties of a more complicated (e.g. kinetic) description?