The Enzyme Cost of Metabolic Pathway Fluxes

Wolfram Liebermeister[1], Elad Noor[2], Arren Bar-Even[2], Avi Flamholz[3], Ron Milo[2]

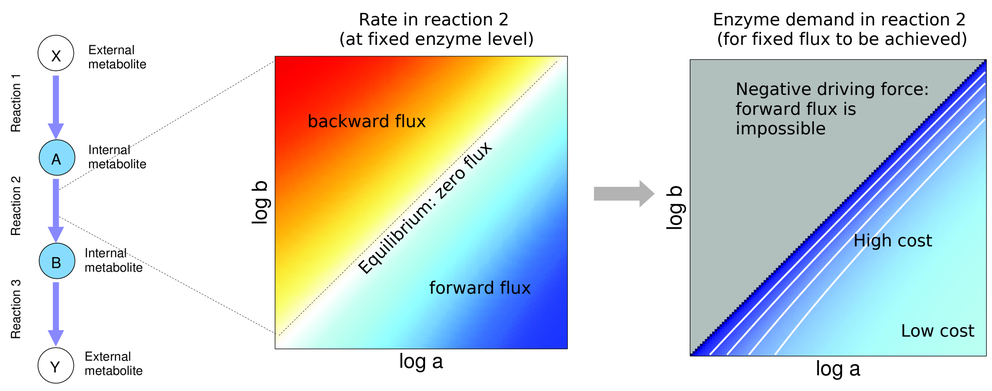
[1] Institut für Biochemie, Charité – Universitätsmedizin Berlin
[2] Department of Plant Sciences, Weizmann Institute of Science, Rehovot
[3] Department of Molecular and Cellular Biology, University of California, Berkeley

Enzyme investment plays a major role in choices between alternative metabolic routes in evolution and bioengineering. To study metabolism from an economic perspective, we propose a modelling approach in which metabolic fluxes are predefined and enzyme levels sustaining these fluxes are computed by cost minimization.

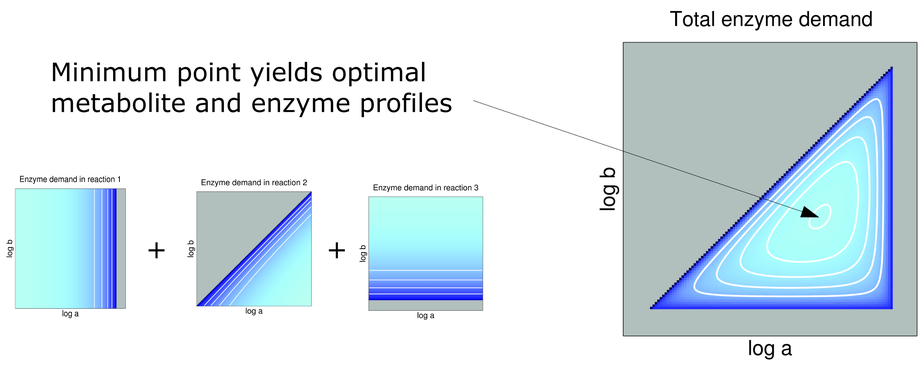
Following previous work [1,2], we define general enzyme cost functions based on known or simplified rate laws. With logarithmic metabolite levels treated as free variables, enzyme optimization becomes a convex problem. Enzyme cost minimization can help construct kinetic models with plausible metabolic and thermodynamic states.

1. Enzyme cost on the metabolite polytope

Enzyme cost in a pathway with reversible Michaeils-Menten rate laws:



Thermodynamically feasible metabolite profiles form a polytope in the space of logarithmic metabolite levels. The total enzyme cost is a convex function on this polytope. Enzyme cost function and resulting optimal metabolite and enzyme levels depend on the choice of fluxes, enzyme cost weights, and rate constants.



2. A hierarchy of enzyme cost functions

Since parameter values are often missing, we construct simple formulae for enzyme costs with different demands for parameters to be known. All enzyme cost scores are derived from separable rate laws [3]:

Example: Reaction A ↔ B with Michaelis-Menten law, non-competitive inhibition, and thermodynamic driving force $\theta = -\Delta G/RT$

$$v = E \cdot k_{\mathrm{cat}} \cdot \left[1 - \mathrm{e}^{-\theta}\right] \cdot \frac{c_{\mathrm{A}}/K_{\mathrm{A}}}{1 + c_{\mathrm{A}}/K_{\mathrm{A}} + c_{\mathrm{B}}/K_{\mathrm{B}}} \cdot \frac{1}{1 + c_{\mathrm{I}}/K_{\mathrm{I}}}$$

Rate = Enzyme level • Forward catalytic constant • Thermo-dynamic efficiency • Kinetic efficiency • Non-competitive allosteric regulation

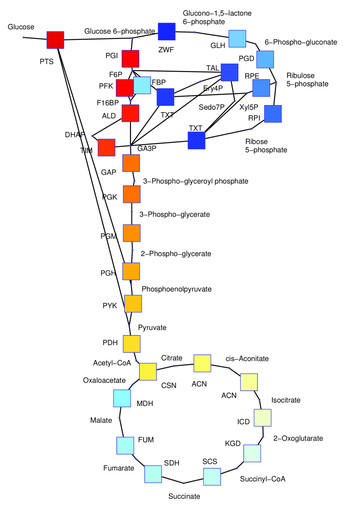
The pathway enzyme cost is a sum $Q = \sum_l q_l = \sum_l h_l \cdot E_l$ of reaction enzyme costs

$$q = \underbrace{h \cdot v \cdot \frac{1}{k_{\mathrm{cat}}}}_{\text{ECF1}} \cdot \underbrace{\frac{1}{\left[1 - \mathrm{e}^{-\theta}\right]}}_{\text{ECF2}} \cdot \underbrace{\frac{1 + c_{\mathrm{A}}/K_{\mathrm{A}} + c_{\mathrm{B}}/K_{\mathrm{B}}}{c_{\mathrm{A}}/K_{\mathrm{A}}} \cdot \left[1 + c_{\mathrm{I}}/K_{\mathrm{I}}\right]}_{\text{ECF3}}$$

By omitting some terms, simplified enzyme cost functions (ECF) with fewer parameters are obtained. The cost scores hold for any reaction stoichiometries.

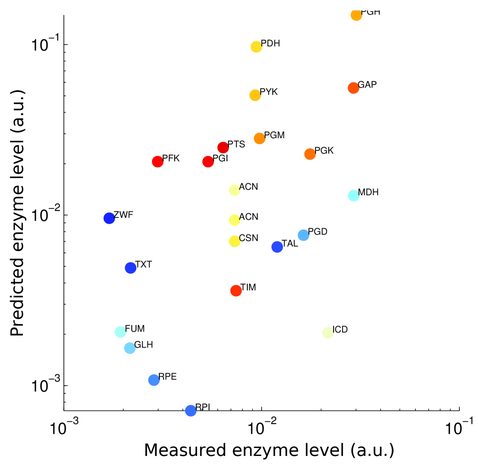
Short name	Enzyme cost	Catalytic constant	Thermodyn.	Enzyme saturation	Depends on / can predict
	h	k_{cat}^{+}	$\eta^{\mathrm{th}}(\Theta)$	$\eta^{\mathrm{kin}}(\mathbf{c}, K_{\mathrm{M}})$	
ECF0	-/√	-	-	-	Enzyme
ECF1	-/√	√	-	-	Enzyme
ECF2	-/√	√	√	-	Enzyme + Thermodynamic force
ECF3	-/√	√	√	√	Enzyme + Metabolite levels
ECF4	-/√	√	√	√	Enzyme + Metabolite levels

3. Enzyme levels predicted for *E. coli* central metabolism



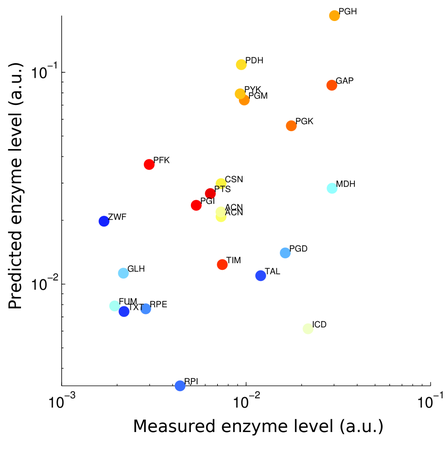
Enzyme cost score ECF1

Spearman rank correlation r = 0.48 [p-value 0.015]



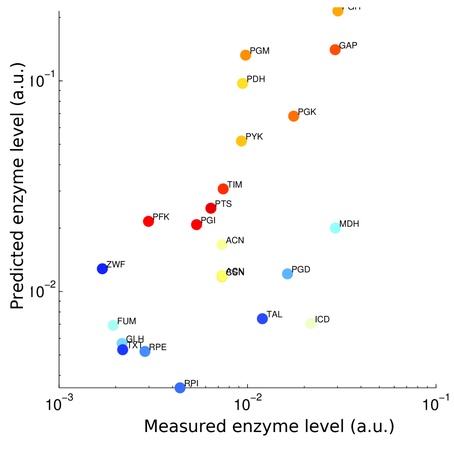
Enzyme cost score ECF2

Spearman rank correlation r = 0.49 [p-value 0.018]



Enzyme cost score ECF3

Spearman rank correlation r = 0.57 [p-value 0.004]



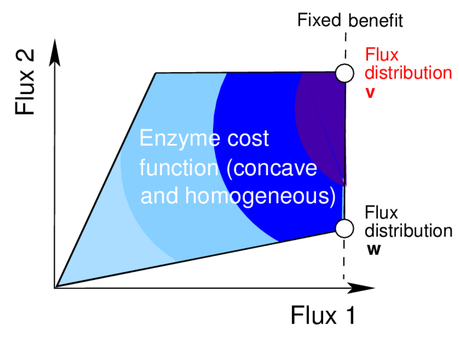
Date sources: Equilibrium constants computed using component contributions [4];
Rate constants from Brenda, processed by parameter balancing [5]; Flux data: [6]; Protein data: [7]

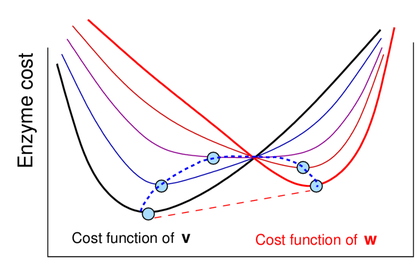
4. Mathematical results relevant to flux analysis

Enzyme cost functions as defined here show two general properties:

(i) The enzyme cost function is convex on the metabolite polytope.

(ii) The optimized enzyme cost is a concave function on the segments of the flux polytope (i.e. flux polytopes with defined flux directions)





A convex combination of two flux modes **v** and **w** has typically a higher cost than the costs of **v** and **w** together. Moreover, separating the pathway fluxes in cell compartments or distinct temporal phases can provide an advantage.

Among the stationary flux distributions with predefined benefit, the enzyme cost is minimized by an elementary mode, as previously shown in [8,9].

FBA methods based on flux minimization ignore the exact relationship between enzyme levels and stationary fluxes. Our enzyme costs could replace the linear flux costs typically used in FBA.

[1] Flamholz *et al.* (2013), PNAS 110(24), 10039–10044
[2] Noor *et al*. (2014), PLoS Comp. Biol. 10:e100348
[3] Noor *et al.* (2013), FEBS Letters 587 (17), 2772-2777
[4] Noor *et al*. (2013), PLOS Comp. Biol. 9:e1003098
[5] Lubitz *et al*. (2010) J. Phys. Chem. B 114 (49), 16298-16303
[6] Chen *et al*. (2010), Metabolic Engineering 13 (1), 38-48
[7] Valgepea *et al*. (2013), Mol. Biosyst. 9 (9), 2344-23458
[8] Wortel *et al*. (2014), FEBS Journal 281 (6), 1547-1555
[9] Müller *et al*. (2014). J. Theor. Biol. 190, 347-182

Funded by DFG Deutsche Forschungsgemeinschaft