Cracking the Code of Metabolic Regulation: Optimal Control and Reinforcement Learning of Regulation and Enzyme Activities

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Overview

Experimental measurement or computational inference/prediction of the enzyme regulation needed in a metabolic pathway is hard problem. Consequently, regulation is known only for well-studied reactions of central metabolism in various model organisms. In this study, we use statistical thermodynamics and metabolic control theory as a theoretical framework to calculate enzyme regulation policies for controlling metabolite concentrations to be consistent with experimental values. A reinforcement learning approach is utilized to learn optimal regulation policies that match physiological levels of metabolites while maximizing the entropy production rate and minimizing the heat loss. The learning takes a minimal amount of time, and efficient regulation schemes were learned that either agree with theoretical calculations or result in a higher cell fitness using heat loss as a metric. We demonstrate the process on four pathways in the central metabolism of *Neurospora crassa* (gluconeogenesis, glycolysis-TCA, Pentose Phosphate-TCA, and cell wall synthesis) that each require different regulation schemes.

Prediction of Dynamics & Regulation

A new approach to the law of mass action does not require rate parameters but instead uses chemical potentials (1). Due to the statistical formulation of the theory, the approach can directly integrate metabolomics and proteomics data.

Governing Equations

Chemical Reaction: $\nu_A A \underset{-1}{\overset{1}{\rightleftarrows}} \nu_B B \underset{-2}{\overset{2}{\rightleftarrows}} \nu_C C$

Mass Action Reaction Flux: $J_{net,1} = k_1 n_A^{\nu_A} - k_{-1} n_B^{\nu_B}$

Marcelin-De Donder Equation:
$$J_{net,1} = k_{-1} n_B^{\nu_B}\left(\frac{k_1 n_A^{\nu_A}}{k_{-1} n_B^{\nu_B}}\right) - k_1 n_A^{\nu_A}\left(\frac{k_{-1} n_B^{\nu_B}}{k_1 n_A^{\nu_A}}\right)$$
$$= k_{-1} n_B^{\nu_B}\left(K_1 Q_1^{-1}\right) - k_1 n_A^{\nu_A}\left(K_{-1} Q_{-1}^{-1}\right)$$

1910 Marcelin Equation: $\Rightarrow c_1\left(K_1 Q_1^{-1}\right) - c_1\left(K_{-1} Q_{-1}^{-1}\right)$

$n_A, n_B, n_C,$	Counts of Molecular Species
ν_A, ν_B, ν_C	Unsigned Stoichiometric Coefficients
$k_{\pm i}$	Forward and Reverse Rate Constants
K_I	Equilibrium Constants
$J_{net,i}$	Reaction Flux

The Marcelin Equation sets each of the decay rates of the forward and reverse forces to the same rate. Assuming the same decay rate for *all* reactions removes any kinetic bottlenecks in phase space of the system such that the dynamics are governed only by the thermodynamics and the energy surface is convex.

Equivalent Optimization

Reconstruction of the flux allows an optimization routine to a steady state as well as the usual ODE solver approach. Since the flux can be rewritten using a maximum entropy assumption as the Marcelin Equation, a steady state is achieved by solving the coupled system

$$\frac{dn}{dt} = S^T J$$
$$= S^T\left(KQ^- - K^- Q\right),$$

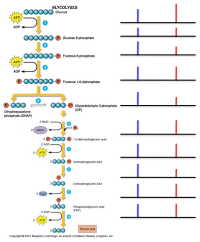
Given appropriate fixed boundary values for metabolites, the system has a unique solution.

Steady state concentrations and fluxes can be gleaned by solving a convex optimization problem:

$$\min \left\| S_V{}^T\left(KQ^- - K^- Q\right)\right\|_2^2$$

The predicted concentrations are a maximum entropy prediction.

Agreement with Experiment



$$\Delta S_{reg} = \sum_j^k \log\left(\frac{\theta_j}{\rho_j}\right)$$

A maximum entropy approach will predict some concentrations well above physiological levels. Enzyme activities need to be adjusted until the predicted and experimental measurements agree.

Adjust Enzyme Activities using Metabolic Control Analysis

Deterministic Regulation. Postulating a linear response, the choice of which reaction is to be regulated at a given steady state can be solved as an optimal control problem using Metabolic Control Analysis. Concentration control coefficients, $C_i^j = \frac{d\log(n_i)}{d\log(\alpha_j)}$, represent the correlated sensitivity of the i-th metabolite and the j-th activity and can predict beneficial regulation. Initially, activities aj for each reaction j is set to 1.0. Activities are then selected for adjustment to achieve the goal $\Delta S_{reg} \leq 0$. Reactions are scored for selection by normalizing the potential change in metabolites by the current value

$$V_j = \frac{\sum_{i=1}^M \left[n_i \cdot \max\left(C_i^j, 0\right)\right]}{\sum_{i=1}^M n_i}$$

Reactions chosen to be regulated maximize V_j. The activity α_i for reaction j is then adjusted and a new steady state solution is found,

$$min \|S^T \alpha (KQ^- - K^- Q)\|_2^2$$

Enzyme activities continue to be adjusted until $\Delta S_{reg} \leq 0$.

Reinforcement Learning Based Regulation

Calculation of Regulation

MCA presumes that the concentrations will change linearly with enzyme activity, but this is not necessarily true. Reinforcement Learning (RL) can be used to learn optimal regulation instead.

Regulation is applied to reactions by changing the scalar valued activity of the j-th enzyme, $\alpha_j \in [0,1]$, where activity values of 0 and 1 represent complete reaction regulation and no enzyme regulation respectively. The activity for each reaction j is applied as a multiplier to the net reaction flux.

- Net reaction flux: $J_j = \alpha_j\left(K_j Q_j^- - K_j^- Q_j\right)$.
- Regulation step size: $\Delta\alpha_j = \alpha_j \frac{dn_i}{n_i^*}\left(-\sum_{k=1}^N B_{ik} S_{kj} J_j\right)$
- Linear stability matrix: $A = n_i^* \sum_{k=1}^M S_{ik} \frac{\partial J_k}{\partial n_i}|_{n=n^*}$

Learning Regulation

The agent (Figure 1) is meant to determine an optimal regulation policy that results in a maximal reward. This is achieved by utilizing an n-step SARSA algorithm and neural network function approximation for the value function.

- States are represented as a set of regulation values: $\alpha = [\alpha_1, \ldots \alpha_N]$.
- Actions are defined by advancing a single MCA calculated step.

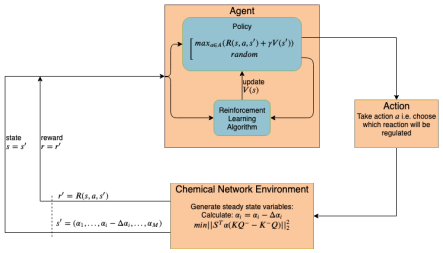


Figure 1

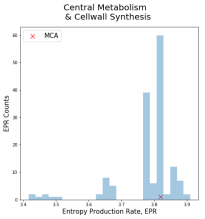


Figure 2

Regulation predictions for both metabolic control (red 'X') and RL (grey lines) based methods are shown for each reaction (Figure 3). Corresponding entropy production rates or cell fitness from the respective methods are compared (Figure 2).

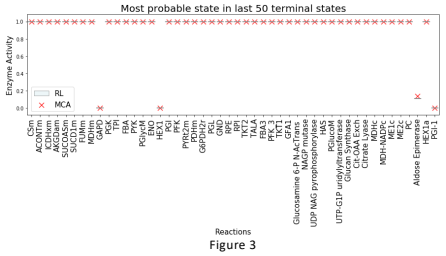


Figure 3

References

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2. Cannon, W.R., *et al.,* (2018) Prediction of metabolite concentrations, rate constants and post-translational regulation using maximum entropy-based simulations with application to central metabolism of *Neurospora crassa*. Processes. 6(6) https://doi.org/10.3390/pr6060063

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