The Thermodynamics of Covalent Modification Cycles as Biological Switches

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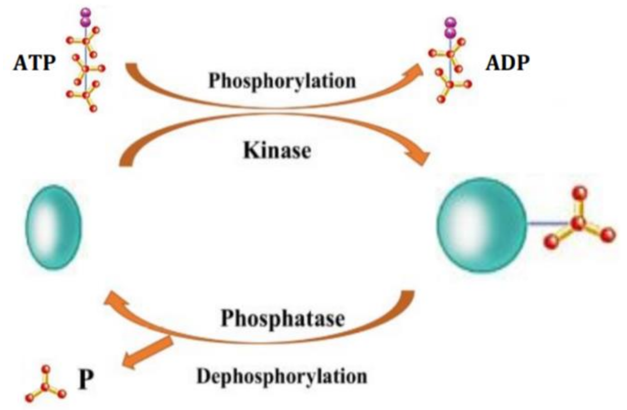
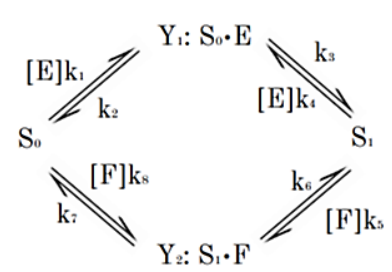
Intro to Covalent Modification — Background

Covalent modification is the addition or elimination of chemical groups to proteins through enzyme-catalyzed reactions, as illustrated below (left).





The Golbeter-Koshland Loop

We are specifically interested in reversible *modification/demodification cycles*, where one enzyme exists primarily to catalyze a reaction that modifies the substrate, and another exists primarily to catalyze a (distinct) reaction that demodifies it. Some important biological examples:

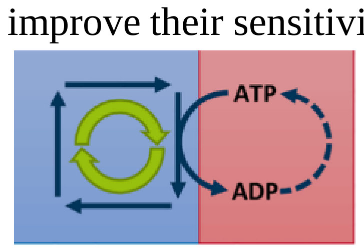
Example	Histone acetylation (gene regulation)	Pyruvate dehydrogenase (glycolysis - TCA)	G-protein coupled receptor (signal transduction)
Enzymes	Acetyltransferase, Deacetylase	PDH Kinase, PDH Phosphatase	Guanine Exchange Factor, GTPase-Activating Protein
Donor	Acetyl CoA	ATP	GTP
Substrate forms	Acetylated, Deacetylated	Phosphorylated, Dephosphorylated	With GTP, with GDP

The prototype example of a modification/demodification cycle is the *Goldbeter-Koshland Loop*, shown above (right) **[1]**.

Physics of Modification Cycles — Background

In systems with cycles, a steady state can be reached either with no net movement of matter between any two given states (equilibrium), or with the movement of matter in a cycle that keeps relative concentrations constant (NESS).

Cycles like the GK-Loop were historically referred to as "futile cycles" because they spend energy to maintain opposing reactions, making their utility unclear. However, it is now established that the energy expenditure of these cycles serves to improve their sensitivity and dynamic range as biological switches.



References and Acknowledgements

[1] Goldbeter, A., & Koshland, D. E. (1981, November). An amplified sensitivity arising from covalent modification in biological systems. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC349147/

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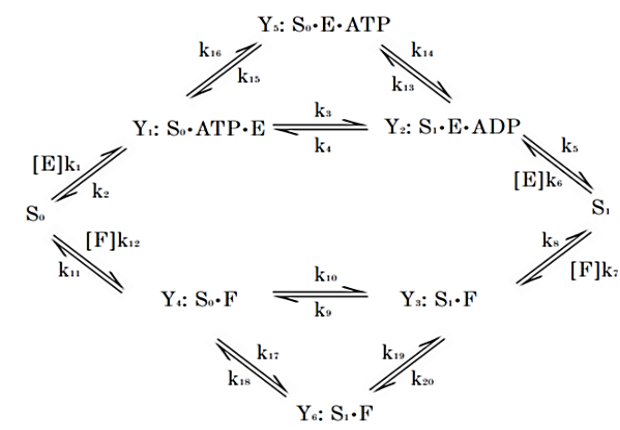
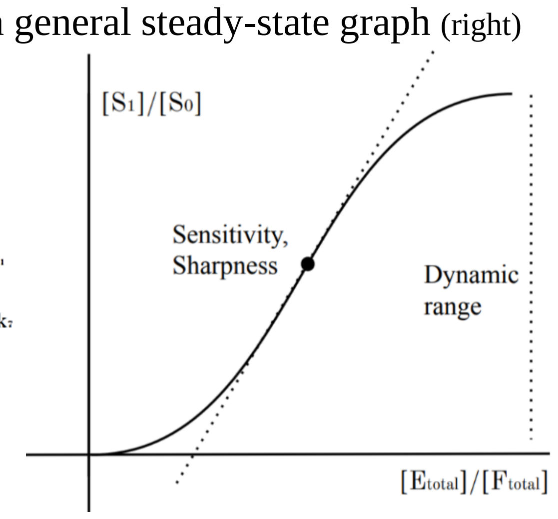
Biological Switches — Background

A prototype substrate graph (left) and a general steady-state graph (right)





Sensitivity

Definition: The maximum derivative of the substrate ratio taken with respect to the ratio of total enzyme concentrations

Significance: Sometimes systems need to be sensitive to small stimuli. For example, in insect larvae segmentation, the change in gene expression over various anatomical regions must be sharp

Dynamic Range

Definition: The substrate ratio's maximum value divided by its minimum value

Significance: The overall change in substrate concentrations must be large enough, because otherwise the switch would have very little overall effect and therefore very little utility

Thermodynamic Force (for cycles)

Definition: $\log\left(\frac{\prod_{forwards} k_i}{\prod_{backwards} k_i}\right)$ (Related to energy dissipated, entropy produced)

Significance: At equilibrium, a cycle's force is 0. Farther away from equilibrium, its magnitude increases. The connection between this quantity and the above two switch properties was the main focus of my research.

Some Previous Research Results

Some invariants and identities that are helpful starting points for observing the dynamic range/sensitivity of a system. Mostly just kinetic analysis, but some qualitative thermodynamic research as well.

$$\frac{[S_1]}{[S_0]} = \frac{c_0^E[E] + c_0^F[F]}{c_1^F[F] + c_1^E[E]} \quad \textbf{[3]}$$

$$\frac{E_{tot}}{F_{tot}}\left(\frac{1}{[S_0]} + \frac{1}{K_0^F} + \frac{1}{K_1^F}\frac{[S_1]}{[S_0]}\right)\left(c_0^E - c_1^E\frac{[S_1]}{[S_0]}\right) = \left(\frac{1}{[S_0]} + \frac{1}{K_0^E} + \frac{1}{K_1^E}\frac{[S_1]}{[S_0]}\right)\left(c_1^F\frac{[S_1]}{[S_0]} - c_0^F\right) \quad \textbf{[2]}$$

Dynamic Range Theorems — Results

For systems with only one cycle containing both substrates or with multiple such cycles with the same thermodynamic force,

$$\frac{\max_t\left(\frac{[S_1]}{[S_0]}\right)}{\min_t\left(\frac{[S_1]}{[S_0]}\right)} = \exp\left(\frac{\Delta\mu}{k_B T}\right),$$

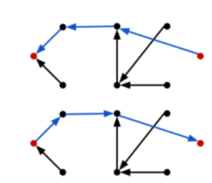
where $t := \frac{[E_{total}]}{[F_{total}]}$ and $\frac{\Delta\mu}{k_B T}$ is the thermodynamic force.

For systems with multiple internal cycles $C_1, C_2, \ldots, C_k$,

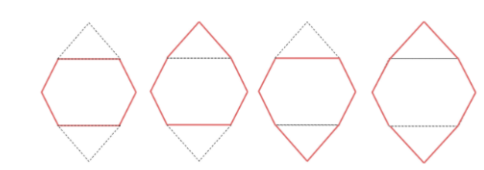
$$\min_{C_1, C_2, \cdots, C_k}\left\{\exp\left(\frac{\Delta\mu}{k_B T}\right)\right\} \le \frac{\max_t\left(\frac{[S_1]}{[S_0]}\right)}{\min_t\left(\frac{[S_1]}{[S_0]}\right)} \le \max_{C_1, C_2, \cdots, C_k}\left\{\exp\left(\frac{\Delta\mu}{k_B T}\right)\right\}.$$

Components of Proofs

- Spanning trees are used in the Matrix Tree Theorem to find relative steady-state concentrations.
- Bijection between spanning trees.



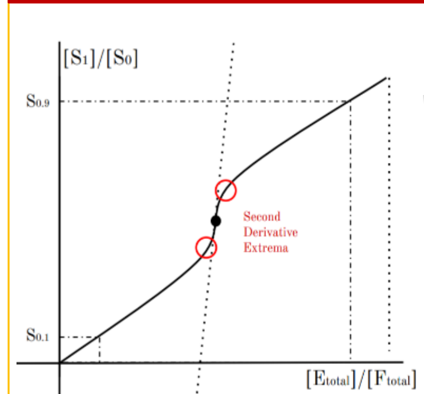
- Limits in E_{total}, F_{total}, or limits in the rate constants $k_1, k_2, k_3, \ldots \Rightarrow$ endpoints of dynamic range phase space

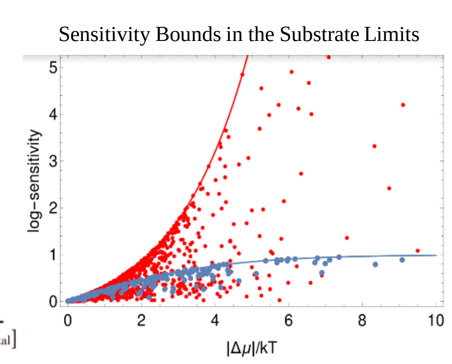


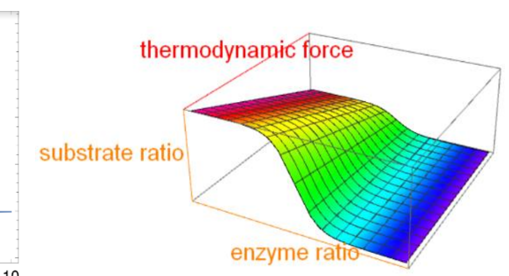
Implications

- The more energy dissipated by a mod/demod cycle, the larger the dynamic range it will have
- There is a benefit for cells incurring the cost of maintaining higher potentials and a non-equilibrium steady state
- Systems in equilibrium have dynamic range of 1
- Can get useful bounds on $[S_1]/[S_0]$ that can be applied elsewhere

Sensitivity Bounds — Results







Other related investigations

- 90-10 Rule/2nd derivative extrema improve with increased force (above, right)
- *Conjecture*: Sensitivity is monotonically increasing in S_{total} so high-substrate bound is a loose overall bound (above, middle)

Implications

- Increasing the energy dissipation increases sensitivity
- Systems in equilibrium have zero sensitivity
- New relationship between thermodynamics and switch efficacy

In the high substrate limit ($S_{total} \to \infty$), for systems with cycles $C_1, \ldots, C_k$,

$$\frac{\partial\left(\log\frac{[S_1]}{[S_0]}\right)}{\partial\left(\log\frac{[E_{total}]}{[F_{total}]}\right)} \le \frac{\sqrt{\max_{C_1, C_2, \cdots, C_k}\left\{\exp\left(\frac{\Delta\mu}{k_B T}\right)\right\}} - 1}{2}.$$

In the low substrate limit ($S_{total} \to 0$), *(shown independently by J. Owen)*

$$\frac{\partial\left(\log\frac{[S_1]}{[S_0]}\right)}{\partial\left(\log\frac{[E_{total}]}{[F_{total}]}\right)} \le \tanh\left(\max_{C_1, C_2, \cdots, C_k}\left\{\frac{\Delta\mu}{4 k_B T}\right\}\right).$$

Components of Proofs

- Inverse function theorem ($f: \frac{E_{total}}{F_{total}} \mapsto \sigma$ is bijective)
- Power Mean Inequality
- Bijection between spanning trees