Kinetic Cooperativity in Monomeric Enzymes

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INTRODUCTION

Cooperativity is a phenomenon in which multiple and spatially distinct binding sites communicate. Typically, cooperative binding has been found in enzymes with multiple ligand-binding sites. Experimentally, for an enzyme with no cooperativity, the rate of product formation as a function of substrate concentration can be described by the Michaelis-Menten equation, $V = \frac{V_{max}\cdot[s]}{K_m + [s]}$ which has a hyperbolic form. In contrast, an enzyme with cooperative binding gives a **sigmoidal curve**.

Often, the sigmoidal curve will be fitted with a Hill function, $H(x) = \frac{[s]^n}{1+[s]^n}$ and a Hill coefficient will be assigned. The Hill coefficient n, which is often interpreted as the minimal number of interacting binding sites, shows the sharpness of the function in response to changing of substrate concentration. However, in previous work in our lab, **position-steepness** of the first derivative of the product formation rate is used for quantitative description of a sigmoidal graph.

Human glucokinase (GCK), acts as the primary glucose sensor in the body, phosphorylating glucose in the presence of ATP. It regulates glucose concentration through a sigmoidal response. Only one glucose-binding site is observed in the crystal structure of GKC. Kinetic cooperativity describes such a **monomeric enzyme** with a sigmoidal response to substrate concentration.

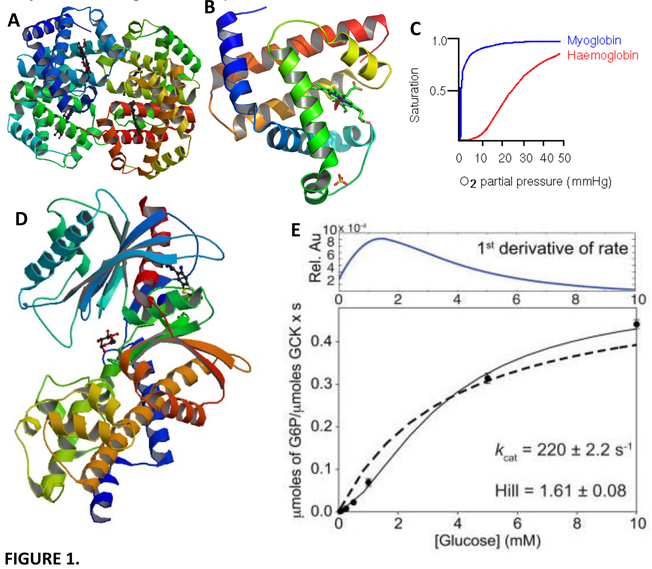

FIGURE 1.

A) Crystal structure of hemoglobin. B) Crystal structure of myoglobin. C) Hyperbolic and sigmoidal shape of function of saturation over oxygen partial pressure with myoglobin and hemoglobin respectively. D) Crystal structure of glucokinase. E) Bottom: Sigmoidal shape and fitted Hill function with Hill coefficient 1.61 of product formation over glucose concentration. Top: first derivative of product formation.

RESULTS

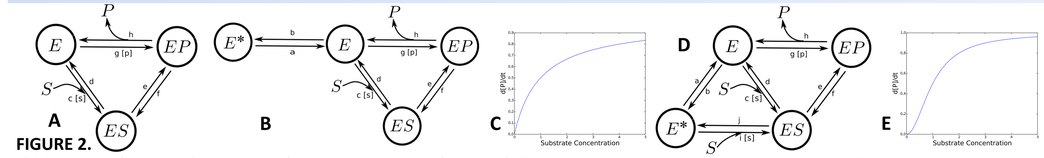

FIGURE 2.

A) Catalytic cycle of enzyme E. B) Catalytic cycle of enzyme E with another conformation E. C) Both models in A and B gives Michaelis-Menten equations. D) Mnemonic Model with 2 conformations of enzyme E and E*. E) Sigmoidal curve from model in D.*

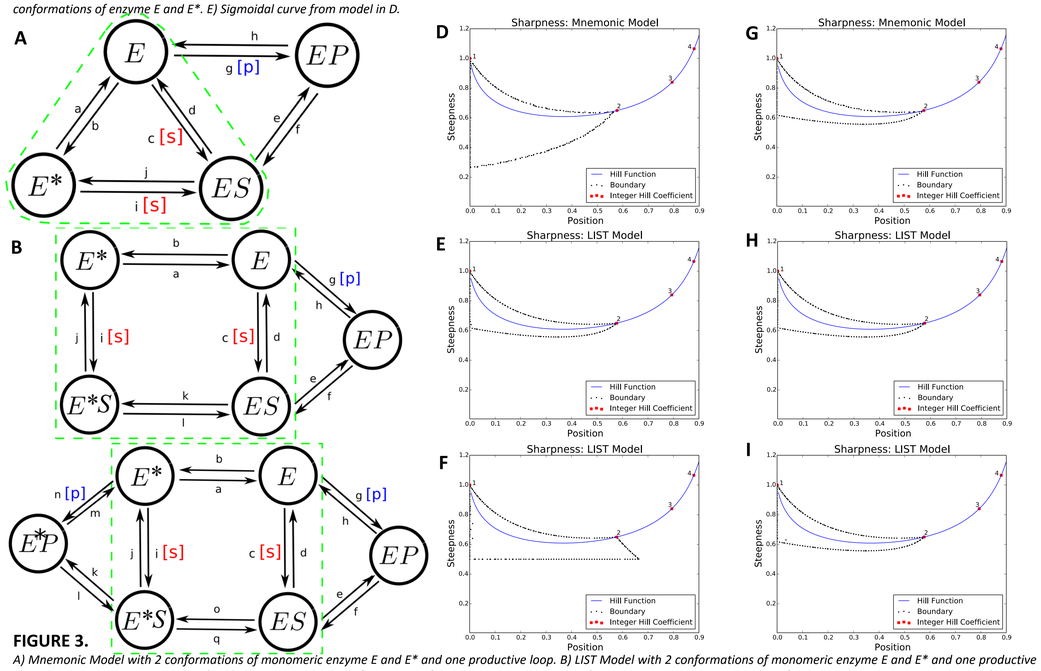

FIGURE 3.

A) Mnemonic Model with 2 conformations of monomeric enzyme E and E and one productive loop. B) LIST Model with 2 conformations of monomeric enzyme E and E* and one productive loop with one more binding state E*S. C) LIST Model with 2 conformations of monomeric enzyme E and E* and two productive loops with conformational change between E and E*, ES and E*S. D), E), F) Boundary-Finding algorithm applied to parameters in A, B, C respectively with comparison to Hill function. G), H), I) Only the imbalance of chemical potential between substrate and product is driving product formation. The vertices in green dashed line, if taken as a separate graph, would be consistent with thermodynamic equilibrium.*

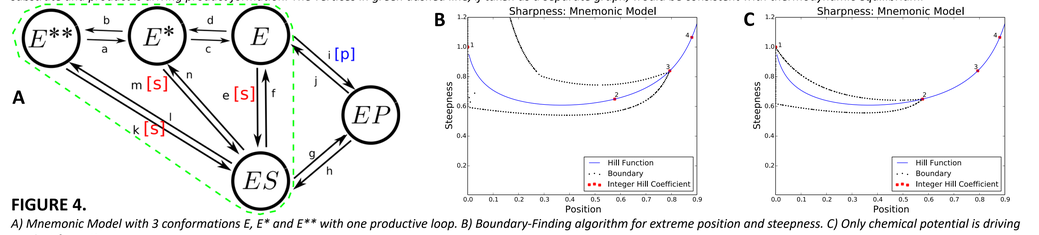

FIGURE 4.

A) Mnemonic Model with 3 conformations E, E and E** with one productive loop. B) Boundary-Finding algorithm for extreme position and steepness. C) Only chemical potential is driving product formation.*

DISCUSSION

- A monomeric enzyme cannot exhibit cooperativity at thermodynamic equilibrium. A reaction that converts substrate to product has a preferred direction and so must be away from equilibrium. Kinetic cooperativity describes the sigmoidality of product formation rate of a monomeric enzyme in a system out of thermodynamic equilibrium.
- The presence of multiple conformations involving in substrate binding gives complexity to equation, which allows the conformational shifts to yield cooperativity.
- Matrix-tree Theorem provides the analytic expression of product formation rate for the system out of thermodynamic equilibrium.
- Using position-steepness to quantitatively measure the enzyme cooperativity preserves more information than assigning a Hill coefficient.
- If the imbalance between substrate and product is the only driving force of product formation, this places constraints on the extremes of position and steepness. If there is energy input elsewhere, these constraints can be exceeded.
- Possible future direction: apply ideas of kinetic cooperativity to enzymes with multiple conformations and multiple binding sites.

Maximum Hill Coefficient in Multiple-Conformations and Multiple-Binding Sites Enzyme

	One Conformation	Two Conformation	Three Conformation	
Thermodynamic Equilibrium	1	1	1	One Binding Site
	2	?	?	Two Binding Sites
Chemical Potential	1	2	2	One Binding Site
	2	?	?	Two Binding Sites
Non Thermodynamic Equilibrium	1	2	3	One Binding Site
	2	?	?	Two Binding Sites

TABLE 1

Maximum Hill coefficients for multiple-conformation and multiple-binding site enzymes in thermodynamic equilibrium, only driven by chemical potential or non-thermodynamic equilibrium. Question marks indicate possible future work.

REFERENCES

[1]J Estrada, F Wong, A DePace, J Gunawardena "Information integration and energy expenditure in gene regulation" Cell 166:234-44 2016.

[2]Kim P, Zhao J, Lu P, Zhao Z (2016) mutLBSgeneDB: mutated Ligand Binding Site gene DataBase. Nucleic Acids Res.2016 Oct 7. pii: gkw905.

[3]Larion M, Hansen AL, Zhang F, Bruschweiler-Li L, Tugarinov V, Miller BG, Brüschweiler R. Kinetic cooperativity in human pancreatic glucokinase originates from millisecond dynamics of the small domain. Angew Chem Int Ed. 2015; 54:8129–8132.

[4]Miele, A.E., Draghi, F., Arcovito, A., Bellelli, A., Brunori, M., Travaglini-allocatelli, C., Vallone, B. "Control of heme Reactivity by Diffusion: Structural Basis and Functional Characterization in Hemoglobin Mutants." Bio Crystal Facility. July 28, 2018.

[5]T. Ahsendorf*, F. Wong*, R Eils, J. Gunawardena. "A framework for modelling gene regulation which accommodates non-equilibrium mechanisms" BMC Biol, 12:102 2014.

[6]G. Robert Ainslie, Jr., Jonathan P. Shill, and Kenneth E. Neet. The Journal of Biological Chemistry Vol.247. No. 21, Nov 10, 1972

METHODS

Linear Framework

The linear framework uses graphs to model biochemical systems. In our case, each vertex in the graph denotes a microstate of the enzyme and the edges are the possible transitions between the microstates with edge labels representing the transition rates. The stochastic master equation from the framework is linear while the non-linear factors are accommodated by the edge labels of the graph. The linear framework can apply not only to systems in thermodynamic equilibrium, but also to non-equilibrium systems.

Matrix Tree Theorem

In a system in thermodynamic equilibrium, detailed balance is observed so that the edge labels along any single path between two vertices can be used to compute their relative probabilities. However, in a system out of thermodynamic equilibrium, detailed balance no longer holds and every path needs to be considered. Probabilities of each microstate can be calculated using the matrix-tree theorem (MTT).

Find-Boundary Algorithm

The MTT allows us to use the each edge labels to calculate the probability of each vertex, resulting in an expression for the rate of production formation in the form of rational function. By changing all the parameters of a point by random small values, the algorithm generates a scatters around the point. We then keep only the extreme points of position and steepness, and repeat the process for these points. The Find-Boundary algorithm allows us to numerically find the extreme edges in the plot of position versus steepness.

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