Kinetic Cooperativity in Monomeric Enzymes

Mark Xiang1,2, John W. Biddle2, Jeremy Gunawardena2
1University of Massachusetts Amherst
2Department of Systems Biology, Harvard Medical School

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, \( V = \frac{V_{\text{max}}[S]}{K_m + [S]} \) 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, \( H(s) = \frac{s^m}{s^m + \text{Hill coefficient}^m} \) and a Hill coefficient will be assigned. The Hill coefficient, \( m \), 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 GCK. Kinetic cooperativity describes such a monomeric enzyme with a sigmoidal response to substrate concentration.

RESULTS

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 see the exact 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.

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 sigmoidity 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.

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 approximated 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.

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REFERENCES


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Contact Information: yankaixiang@umass.edu