Characterizing bacterial lysis by phage lytic enzymes using non-parametric data collapse

Gabriel J. Mitchell[1], Daniel C. Nelson[2], and Joshua S. Weitz[3]

***Short Abstract* — PlyC is a phage lytic enzyme currently being considered as a platform for developing phage-based alternatives to standard antibiotics. The sequence and structure of PlyC are known, but there is currently no method for characterizing the reaction mechanisms and kinetic rates of the enzyme. We present a method for analyzing the kinetics of this enzyme reaction via an indirect assay. The method is based on the principle of non-parametric data collapse and can be used to analyze biochemical reactions when direct measurements of the species are not possible.**

***Keywords* — phage lytic enzymes, data analysis, data collapse, antibiotics, enzyme kinetics, cell structure**

I. Motivation

PHAGE lytic enzymes (lysins) are produced by phages late in the infection period to burst the cell wall and release viral particles [1]. The enzymes bind to sites on bacterial cell walls specific to their native host and subsequently hydrolyze bonds crucial to cell wall integrity, which eventually results in osmotic lysis. Lysins are extremely efficient at accelerating bacterial lysis; a small quantity added exogenously will rapidly lyse many cells. For these reasons they are considered prime candidates for developing phage-based antimicrobial treatments. PlyC is a multimeric lysin from Streptococcal bacteriophage C_1. It is formed from two separate gene products that code for binding and catalytic domains [1]. A qualitative analysis of the effectiveness of the enzyme also indicates that it is the most active of all lysins studied to date. A quantitative analysis of the kinetics of PlyC and its cell wall targets can provide functional information that would allow directed engineering of enzyme properties.

II. Optical Density Assay and Data Collapse

The effectiveness of a lysin against a bacterial strain is currently assayed by recording the optical density time series of solutions after adding known titers of enzyme and bacteria. As cells rupture the optical density decreases. The exact shape of the time series is a result of the combined effects of bond hydrolysis via lysins and the cellular susceptibility to rupture as a function of the number of bonds cleaved. We present a method that can simultaneously estimate reaction parameters associated with PlyC and the population level distribution of the critical number of bonds that must be hydrolyzed before osmotic lysis ensues.

Our method is based on the principle of non-parametric data collapse. If we assume an enzyme reaction model, we can compute a reaction time series and transform the optical density as a function of time into an optical density as a function of the number of cleaved bonds. The function describing the susceptibility to lysis is invariant with respect to changes in the initial concentration of enzymes or bacteria. Hence, a series of transformed optical density curves should collapse only when we have chosen the correct reaction model and kinetic parameters. We employ standard methods for nonlinear optimization to vary the parameters of the reaction model until optimal data collapse is obtained over a range of initial enzyme and cell concentrations. In addition to estimating kinetic parameters, the method estimates the distribution of susceptibility to lysis within the population. We apply this method to study the lysis of Streptococcus strains by PlyC and obtain estimates of the binding, unbinding and forward reaction rates for the enzyme.

III. Conclusion

The data collapse method we developed can be used to analyze the kinetics of any lytic enzyme and represents a novel tool in protein engineering. The principle of data collapse has previously been applied to study biochemical reactions [2]. A method for automated data collapse has also been developed [3]. The unique feature of our method is that it does not require an explicit scaling relation, which makes it generally applicable for probing the dynamics of biological systems though indirect measurements.

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[1]School of Biology, Georgia Institute of Technology, Atlanta, GA 30332.
E-mail: gabriel.mitchell@gatech.edu
[2]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland 20850.
E-mail: nelsond@umbi.umd.edu
[3]School of Biology and Physics, Georgia Institute of Technology, Atlanta, GA 30332.
E-mail: jsweitz@gatech.edu

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