Time-resolved, super-resolution optical imaging of Cel7A cellulases

Jaemyeong Jung[1], Tiziano Gaiotto[2], Jason J. Han[1], Devin W. Close[2], Andrew R.M. Bradbury[2], Tina Jeoh[3], Gnana S. Gnanakaran[4], Peter M. Goodwin[1]

***Short Abstract* — The efficient conversion of abundant lignocellulosic biomass to biofuels such as ethanol would reduce the nation's dependency on fossil fuels. A crucial step in this process is the enzyme-catalyzed hydrolysis of cellulose to glucose. Here we use single-molecule imaging to directly monitor the activity of individual fluorescently-labeled cellulases interacting with insoluble cellulose substrates in order to elucidate molecular-level details of cellulase activity. The motion of multiple, individual cellulases was simultaneously recorded with ~10 nanometer spatial resolution. Time-resolved localization microscopy provides insights on binding and diffusion of cellulases to active sites on cellulose and cellulase processivity.**

***Keywords* — Single-molecule imaging, Cellulase**

I. Introduction

Lignocellulosic biomass is the most abundant biological material on earth. It has been projected that the available land resources of the United States are sufficient for producing the biomass needed for cellulosic biofuels to meet 30% of the nation's transportation fuel requirements by the middle of this century. [1] Processes for conversion of lignocellulosic biomass to biofuels and other useful hydrocarbon products are typically comprised of three steps [2]; (i) Pretreatment: Cellulose is rendered into a form more susceptible to enzyme degradation. (ii) Enzyme degradation: a mixture of cellulases catalyzes the hydrolysis of cellulose to glucose, a simple sugar. [3] (iii) Fermentation: microbial conversion of glucose to biofuels such as ethanol. At present, the high cost of the second step (ii) is a roadblock to the economical, large-scale conversion of biomass to fuel and its widespread use. The enzyme-catalyzed hydrolysis of cellulose is complex – it entails the cooperative (synergistic) action of three enzymes known collectively as cellulases: endoglucanase and two exoglucanases on the insoluble substrates. In contrast to homogeneous solution-phase catalysis, it is well known that the overall efficiency of this heterogeneous catalysis process depends on factors in addition to the catalytic rates of the cellulases, including: cellulase absorption, desorption and diffusion rates on the cellulose substrate, and the processivity of exoglucanase-catalyzed hydrolysis of individual cellulose molecules. To date, due in large part to limitations of the bulk analysis methods used for its study, this heterogeneous reaction remains poorly understood. Here, we used single-molecule fluorescence imaging to elucidate molecular-level details of exoglucanase (Cel7A) interactions during cellulose degradation.

[1]Materials Physics and Applications, [2]Bioscience, and [4]Theoretical Divisions Los Alamos National Laboratory
[5]Department of Biological and Agricultural Engineering, University of California at Davis
Correspondence to: Peter Goodwin pmg@lanl.gov, Jaemyeong Jung jjung@lanl.gov

II. Results

The binding and movement of single Cel7A enzyme molecules on insoluble cellulose substrates were monitored using total-internal reflection fluorescence microscopy.

A. Specific binding of cellulase on the substrate

From the co-localization of the Cy5-labeled Cel7A together with fluorescein-labeled cellulose, the specific binding of enzyme to the cellulose substrate was confirmed.

B. Single-molecule tracking

Time resolved, super-resolution imaging was applied to directly characterize the binding and movement of individual enzymes. Analysis of the single-molecule trajectories over time reveals the behavior of enzymes on the cellulose microfibrils, under various conditions.

C. Binding time analysis

Structurally distinct cellulases (Cel7A exoglucanases from *Trichoderma reesei* and *Talaromyces emersonii*) show different behavior on cellulose substrates.

III. Conclusion

The present study directly visualizes the complex nature of cellulase behavior on the cellulose surface, and provides knowledge of interactions at the molecular level that cannot be readily inferred from ensemble averages reported by conventional, bulk analyses.

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