Cellulosome Structure Determination Employing Atomistic Simulations Combined to Experimental Assays

Report to:

Blue Waters allocations for the UIUC

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Executive Summary:

Cellulosomes are multi-enzyme complexes that target deconstruction of cellulose and hemicellulose in anaerobic cellulosome-containing bacteria. Briefly, in cellulosome assembly, a large non-catalytic polypeptide called the scaffoldin, embedded with various cohesins, anchors dockerin-containing enzymes through cohesin–dockerin interactions. Specificity of the cohesin–dockerin interaction allows incorporation of different catalytic cellulases and hemicellulases onto the scaffoldin that may or may not be bound to another domain tethered to the cell wall. For their very efficient mechanism of degrading plant-cell-wall biomass, cellulosomes can be employed in the second-generation biofuel industry, which aims to use agricultural waste to produce ethanol. Furthermore, the recent discovery of cellulosomal bacteria in the lower gut of humans is paradigm shifting as it has allowed demonstration of the capacity to degrade both hemicellulose and cellulose, at least, in the gut of some humans. Employing molecular dynamics simulations, complementing single-molecule and biochemistry experiments, we characterized cellulosome's components, showing that even a single mutation can cause a large change in the cellulosome structural stability.

Description of research activities and results:

1. Key Challenges

Here we employ single-molecule force spectroscopy with an atomic force microscope (AFM) and steered molecular dynamics (SMD) simulations to reveal force propagation pathways through mechanically stable cellulosome proteins[1]. Using a combination of network-based correlation analysis supported by AFM directional pulling experiments, we can pin down single crucial amino acids promoting force resilience. The results implicate specific force-propagation routes that are advantageous for achieving high stability under shear forces.

2. Why it matters

Bacteria play a key role in the second-generation biofuel industry since their cellulolytic enzymes, used for plant-cell-wall degradation, are employed in the production of these advanced biofuels[2]. Also, symbiont bacteria greatly influence human health and play a significant role in pathogenesis, disease predisposition, physical fitness, and dietary responsiveness[3].

Our work has been focused on investigating a key processes underlying bacterial activity, namely plant fiber metabolism. Particularly, we are investigating the structure and function of cellulosomes, the highly cooperative macromolecular complexes that are central for the metabolic process of many bacteria[4].

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3. Why Blue Waters

Investigating the structure and functional processes of large enzymatic complex machineries, such as the cellulosomes, is only possible on petascale computing resources, such as Blue Waters. Structures obtained using enhanced sampling techniques, such as GSA, are only reliable if thousands of conformations (models) are predicted. Employing GSA for the numerous linkers of the cellulosome is a well-suited task for the large-scale parallel architecture of Blue Waters.

4. Accomplishments

Combining biochemical and single molecule experiments with molecular dynamics (MD) and steered molecular dynamics (SMD) simulations, we investigated a series of cellulosomal cohesins from *Acetivibrio Cellulolyticus*. We revealed that these cellulosomal components withstand different amounts of force depending on their position in the protein network. In this study, we combined one-step in-vitro expression and specific covalent pulldown of protein constructs to assess the mechanical stability distinct highly related proteins in a parallel single molecular force spectroscopy assay. Using steered molecular dynamics simulations, the experimental results were reproduced and important amino acids were identified. In addition, the simulations performed on Blue Waters were used to suggested mutations that were experimentally performed by site-directed mutagenesis, engineering proteins in an attempt to pin down single crucial amino acids promoting force resilience.

5. Next Generation work

Our main goal is to obtain a clear picture of the cellulosome structure at work. For that, long molecular dynamics simulations of different cellulosomes, some of them with hundreds of millions of atoms, will have to be performed. Also, to investigate the enzymatic mechanism in the context of the cellulosome, hybrid QM/MM simulations will have to be performed, using multiple QM regions, requiring a massive computer power. Such complex study might only be feasible in a few years, requiring pre-exascale and exascale.

Publications:

The main publication representing this work is still in progress, especially due to new experimental validations. Three highly-related manuscripts using this or previous Blue Waters allocations were published, as listed below.

C. Schoeler, K.H. Malinowska, R.C. Bernardi, L.F. Milles, M. a Jobst, E. Durner, et al., Ultrastable cellulosome-adhesion complex tightens under load. Nat. Commun. 5 **(2014)** 5635.

C. Schoeler, R.C. Bernardi, K.H. Malinowska, E. Durner, W. Ott, E.A. Bayer, E. A., et al., Mapping mechanical force propagation through biomolecular complexes. Nano Letters, 15 **(2015)**, 7370.

R.C. Bernardi, M.C.R. Melo, K. Schulten, Enhanced Sampling Techniques in Molecular Dynamics Simulations of Biological Systems., Biochim. Biophys. Acta. 1850 **(2015)** 872.

References:

1. Schoeler, C. *et al.* Mapping Mechanical Force Propagation Through Biomolecular Complexes. *Nano Lett.* **15,** 7370–7376 (2015).
2. Dodd, D. & Cann, I. K. O. Enzymatic deconstruction of xylan for biofuel production. *Glob. Change Biol. Bioenergy* **1,** 2–17 (2009).
3. Faith, J. J. *et al.* The long-term stability of the human gut microbiota. *Science* **341,** 1237439 (2013).
4. Ben David, Y. *et al.* Ruminococcal cellulosome systems from rumen to human. *Environ. Microbiol.* (2015).
5. Smith, S. P. & Bayer, E. a. Insights into cellulosome assembly and dynamics: from dissection to reconstruction of the supramolecular enzyme complex. *Curr. Opin. Struct. Biol.* **23,** 686–94 (2013).