Understanding Enzyme Activity Using Single Molecule Tracking

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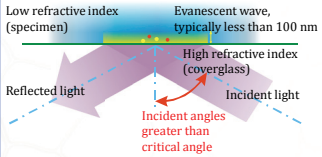
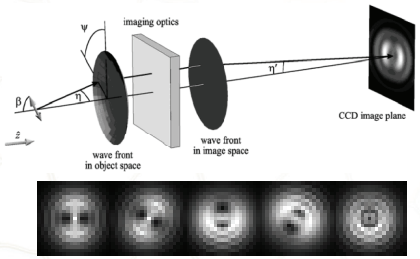
Single Molecule Tracking and TIRF

Total Internal Reflection Fluorescence Microscopy (TIRF-M) is used to measure focused and defocused fluorescence of fluorescent-tagged CBMs. The goal of this study is understanding the dynamic behavior of enzymes.



Schematic of TIRF system

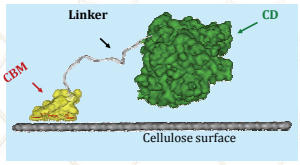
TIRF system utilizes this evanescent wave to excite single molecules in the thin section (<100 nm), thus produce fluorescence images with high signal-to-noise (S/N) ratio.



For the geometry of imaging system (top), with slight movement of the optics toward the molecule, the image can be defocused to yield characteristic interference patterns (bottom). From the pattern, we infer the molecule orientation.

Cellulase and CBM

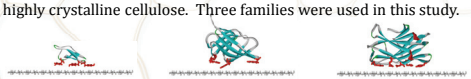
Model of cellulase depolymerizing cellulose



Carbohydrate-binding module (CBM): Bind to the substrate

Catalytic domain (CD): Hydrolyze/depolymerize carbohydrate polymers

Type A Surface binding CBMs — This class of CBM binds to insoluble, highly crystalline cellulose. Three families were used in this study.

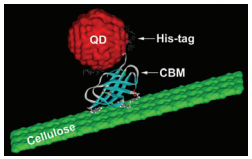


Family 1 CBM	Family 2 CBM	Family 3 CBM
(from *T. reesei* CBHI)	(from *C. fimi*)	(from *C. thermocellum*)
36 amino acids,	110 amino acids,	155 amino acids,
3 nm x 1.8 nm x 1 nm	4.5 nm x 2.5 nm x 2.5 nm	3 nm x 3 nm x 4.5 nm

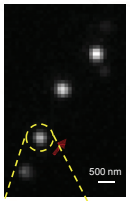
Does CBM Move on Cellulose?

Approach1:Tracking Quantum Dots (QDs)-labeled CBM

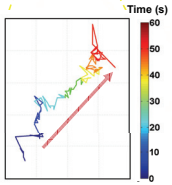
Model of cellulose/CBM/QD system



Four QDs lined up on the cellulose



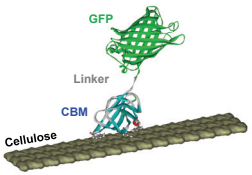
QD are anchored to the CBM by the dual histidine-tags (His-tag) and fused at *N*-and *C*-termini

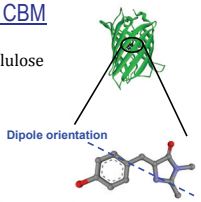


- Trajectory showed CBM moving along the direction of the cellulose
- Although QDs are bright, the emission intermittency (blinking) is difficult to distinguish one QD moving or two QDs blinking

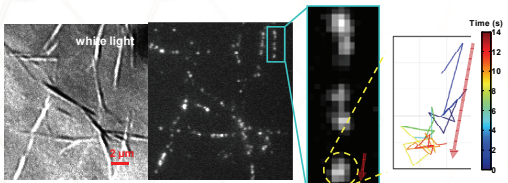
Approach2:Tracking GFP-labeled CBM

Model of GFP labeled CBM bound on cellulose





GFP has chromophore in its center with a fluorescence dipole allowing us to study the single molecule orientation



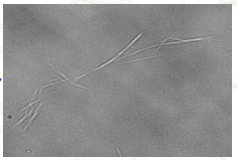
CBM binding on cellulose is specific

- CBM moving along the cellulose direction

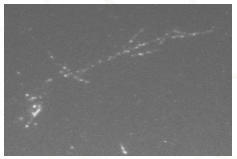
How does CBM Bind to Cellulose?

Analysis:
Image processing sequence

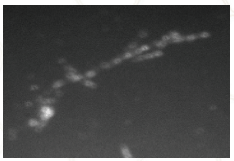
White light image of *Valonia* fibers



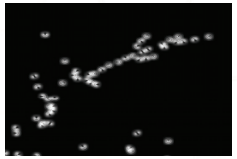
Fluorescence image of GFP-labeled CBMs bound to these *Valonia* fibers



Defocused fluorescence image



Simulated defocused orientational and positional image (DOPI)

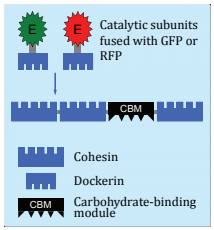


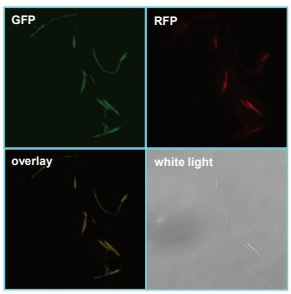
3-D Reconstruction

- Preliminary results suggest that GFP-CBM preferentially bind perpendicular to the fiber

Cellulosome Assembly

Model of cellulosome





We have developed three families of enzyme (GH5, GH12, GH74) labeled with GFP or RFP. Future work includes applying single molecule imaging technique to study cellulosome structure as well as dynamic behavior.

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