34th Symposium on Biotechnology for Fuels and Chemicals
May 1, 2012

U.S. DEPARTMENT OF ENERGY

Real-time imaging of plant cell wall structure at nanometer scale, with respect to cellulase accessibility and degradation kinetics

Shi-You Ding

DOE BioEnergy Science center (BESC)

National Renewable Energy Laboratory (NREL)

NREL/PR-2700-55275

BESC BioEnergy Science Center

NREL NATIONAL RENEWABLE ENERGY LABORATORY

OAK RIDGE National Laboratory

34th Symposium on Biotechnology for Fuels and Chemicals
May 1, 2012

U.S. DEPARTMENT OF ENERGY

Cellulases Bind and Digest Cellulose from Its Planar (Hydrophobic) Faces

Shi-You Ding

DOE BioEnergy Science center (BESC)

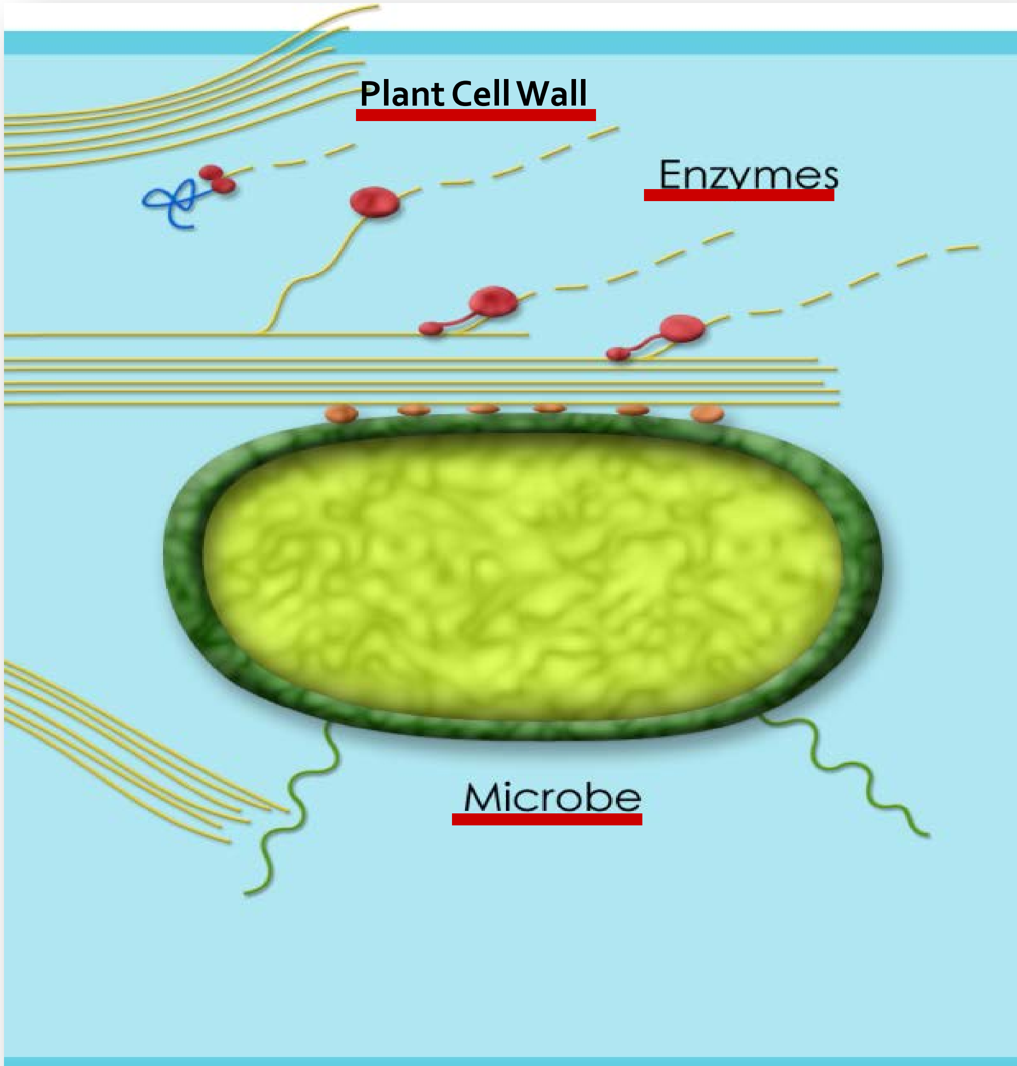
National Renewable Energy Laboratory (NREL)

BESC BioEnergy Science Center

NREL NATIONAL RENEWABLE ENERGY LABORATORY

OAK RIDGE National Laboratory

Biomass Degradation System



- ✓ Biomass: the plant cell walls
- • Cellulolytic microbes
- ✓ Enzymes

Objectives

- Develop tools to measure biomass at the nanometer scale
- Elucidate the molecular bases of biomass deconstruction
  - Native plant cell wall at the nanometer scale
  - Cell wall structure changes after delignification
  - Cellulase accessibility
  - Cellulase digestibility
- Identify factors that affect the conversion efficiency of biomass-to-biofuels

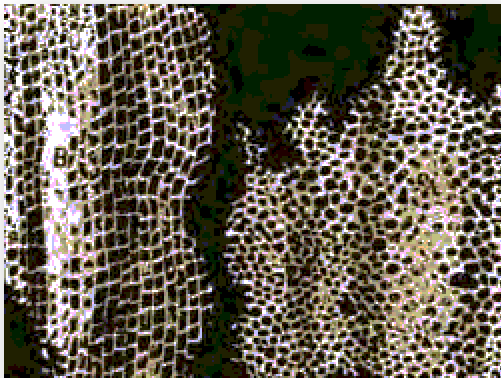
Tool Development


25 years ago…

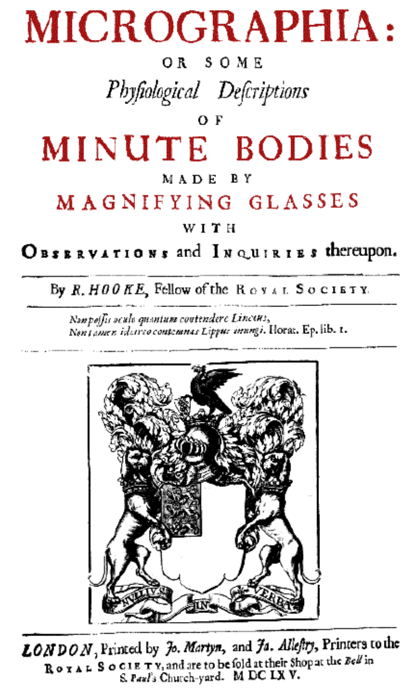
15 years ago…

Today…

A multi-Length Scale Problem
- Requires Correlative Imaging Approaches



The "cell" was firstly described based on microscopic observation of cork cell walls (Robert Hooke, 1665, *Micrographia*)

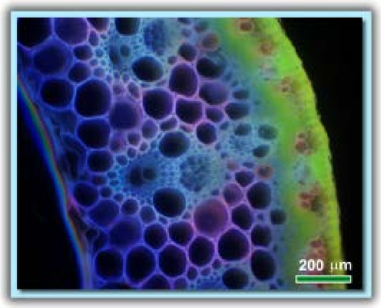

MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES
WITH
OBSERVATIONS and INQUIRIES thereupon.

By R. HOOKE, Fellow of the ROYAL SOCIETY.

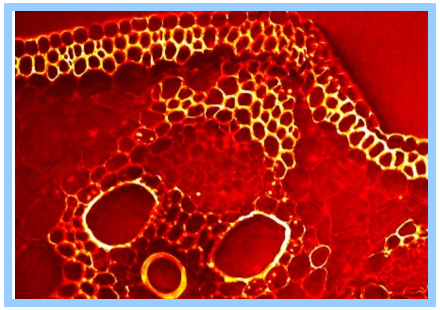
Non possis oculo quantum contendere Linceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

LONDON, Printed by Jo. Martyn, and Ja. Allestry, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the Bell in S. Paul's Church-yard. M DC LX V.

Plant

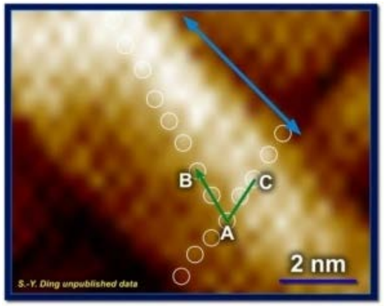


Tissues



Cells

Microfibrils



Polymers

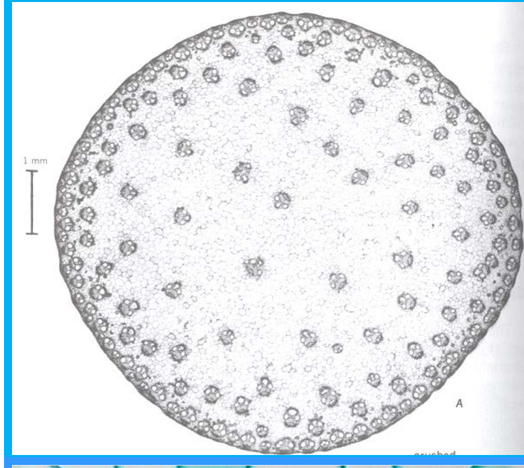
Meters: 10^{0} | Centimeters: 10^{-2} | Micrometers: 10^{-6} | Nanometers: 10^{-9} | Ångströms: 10^{-10}

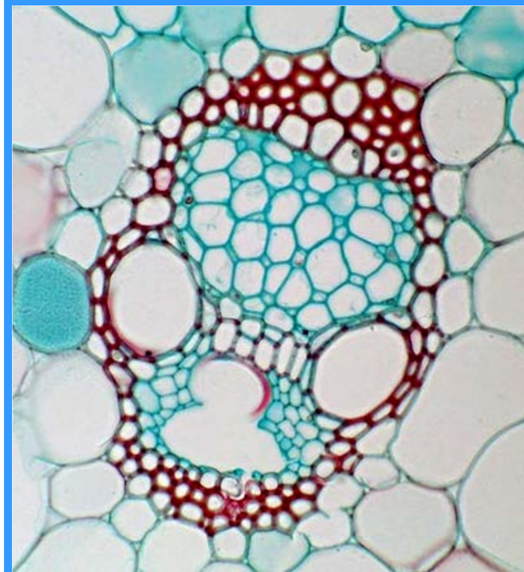
Biomass – The Plant Cell Walls

The plant cell walls are composed of cellulose, hemicelluloses, pectins, lignins, glycosylated proteins, and other minor components

…

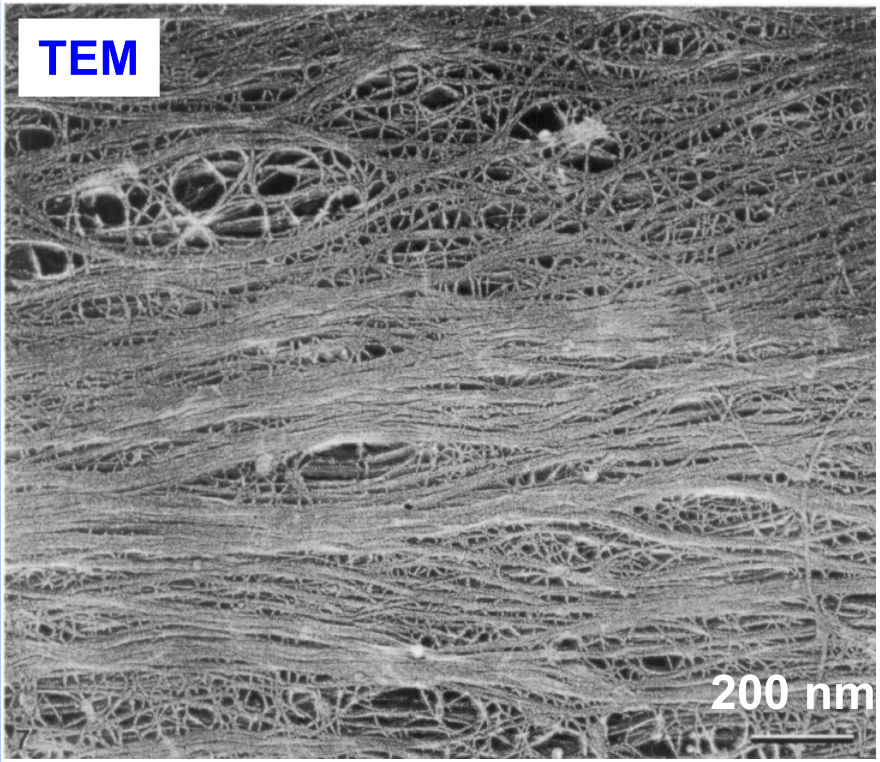
- **Where** are these polymers?
- **What** is the chemistry?
- **How** are they cross-linked, assembled during biosynthesis, and changed during biodegradation?
- **Why** are they recalcitrant to bioconversion?

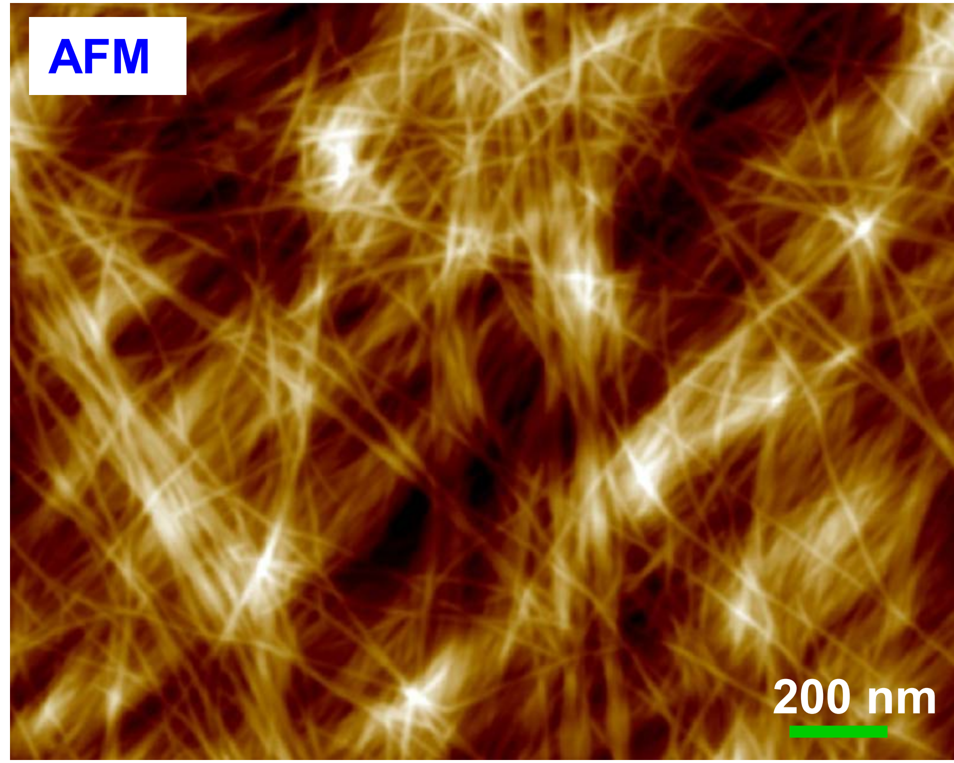




TEM and AFM

U.S. DEPARTMENT OF ENERGY





Onion cell wall material after extraction with CDTA, Na_2CO_3 and 1M KOH to remove pectins and some hemicellulosic polymers. Removal of some hemicelluloses allows lateral association of microfibrils in bundles of two to more than 20 fibres. Many cross-links are still present in the wall.

High-resolution AFM height image showing a typical maize primary cell wall surface structure. Microfibrils are parallel-arranged, and the macrofibrils scatter only on the wall surface.

McCann et al., 1990, JCS
Ding et al.,2006 *J. Agric. Food Chem. Himmel & Ding et al., 2007 Science*

BESC
BioEnergy Science Center

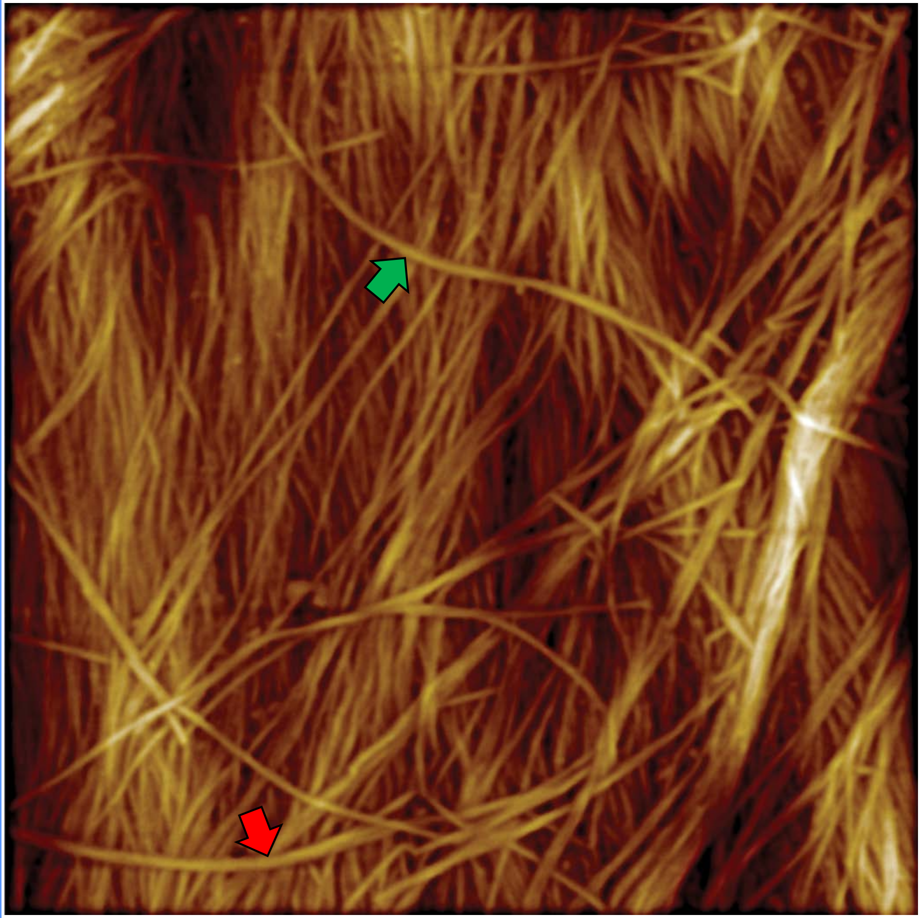
Microfibril in Dry and in Buffer

U.S. DEPARTMENT OF ENERGY

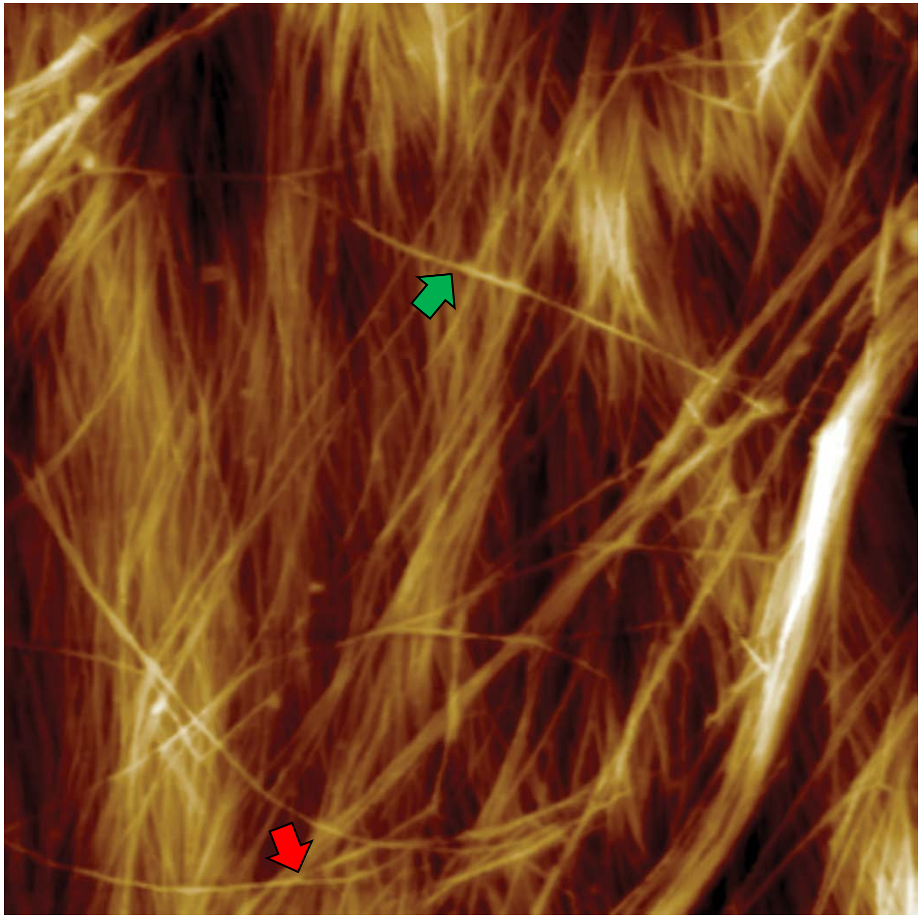
The microfibrils are very similar; minor changes in surface fibrils:

- Straightening
- Debundling

Dry



4 h in buffer



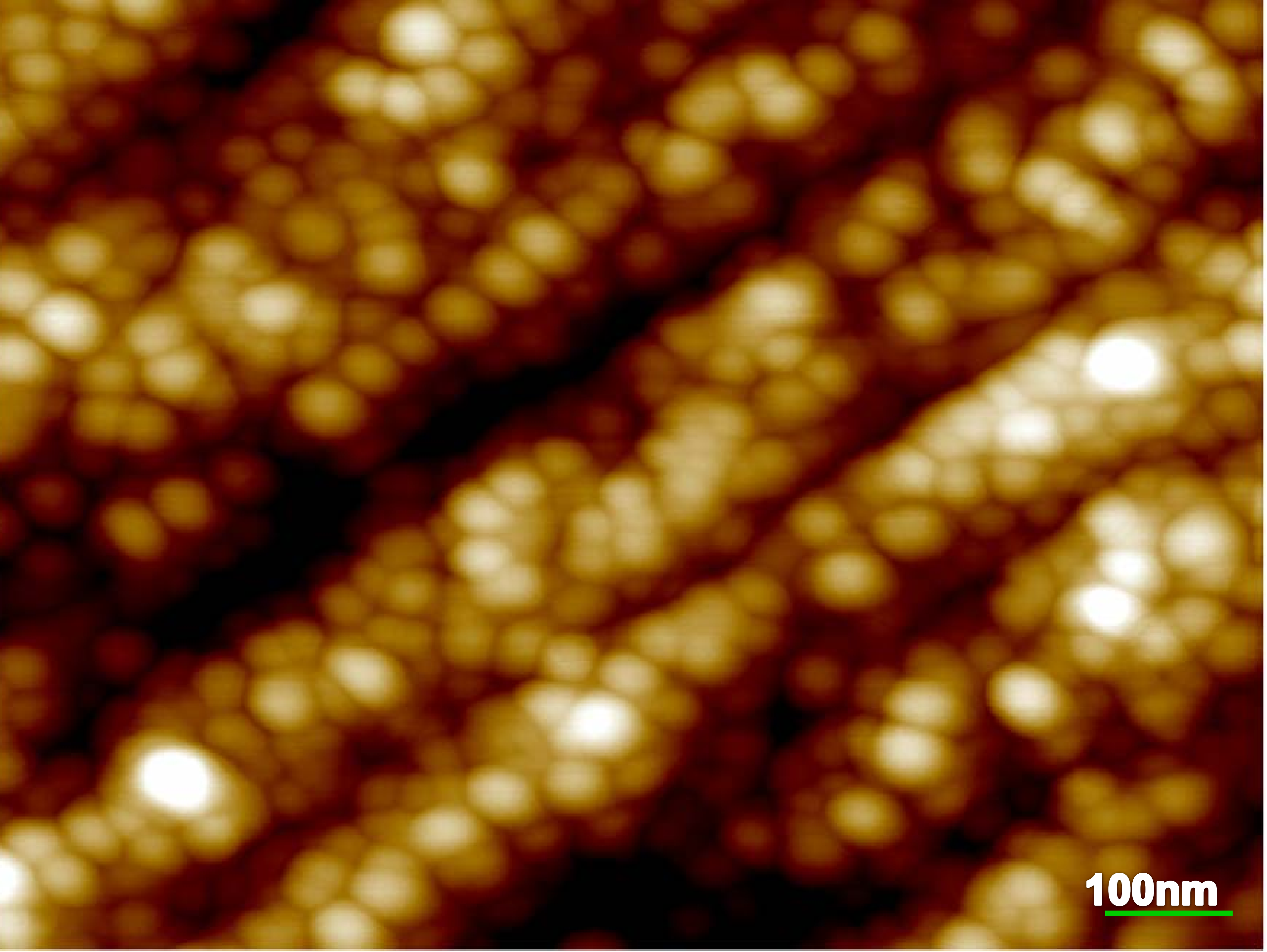
Cellulose nanocrystals – cross section shape

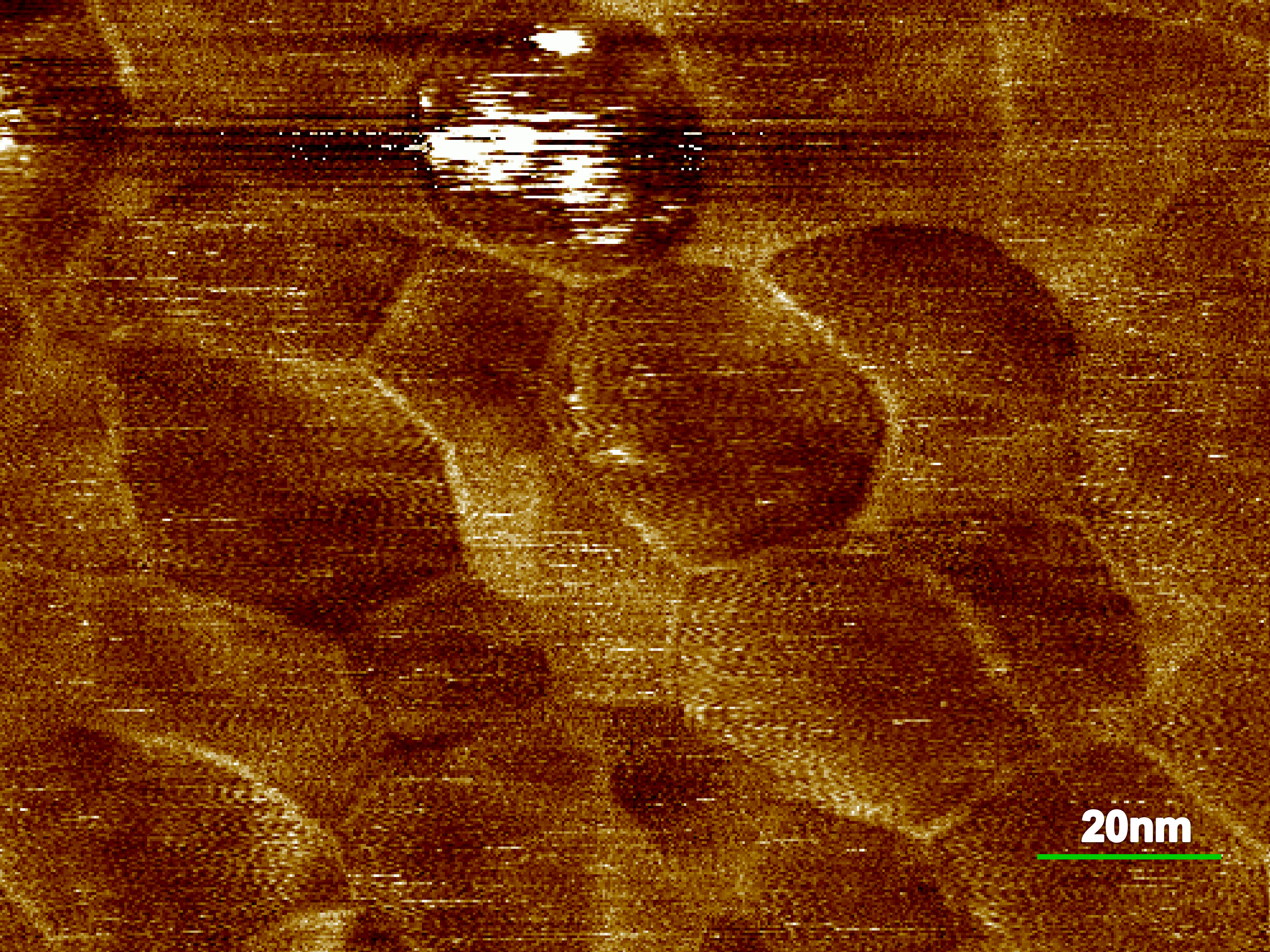
U.S. DEPARTMENT OF ENERGY

Valonia ventricosa

Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

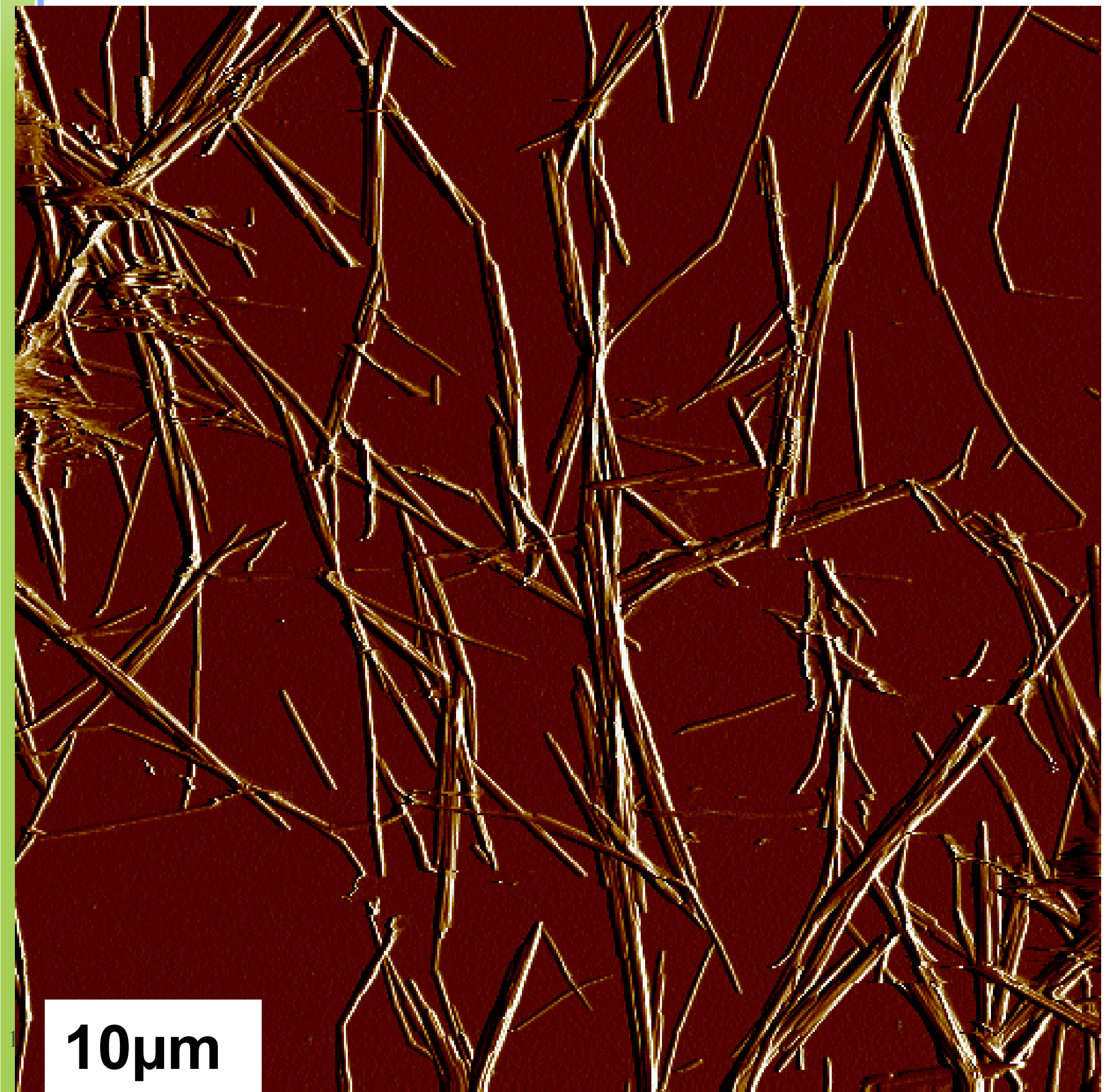
BESC
BioEnergy Science Center


100nm


20nm

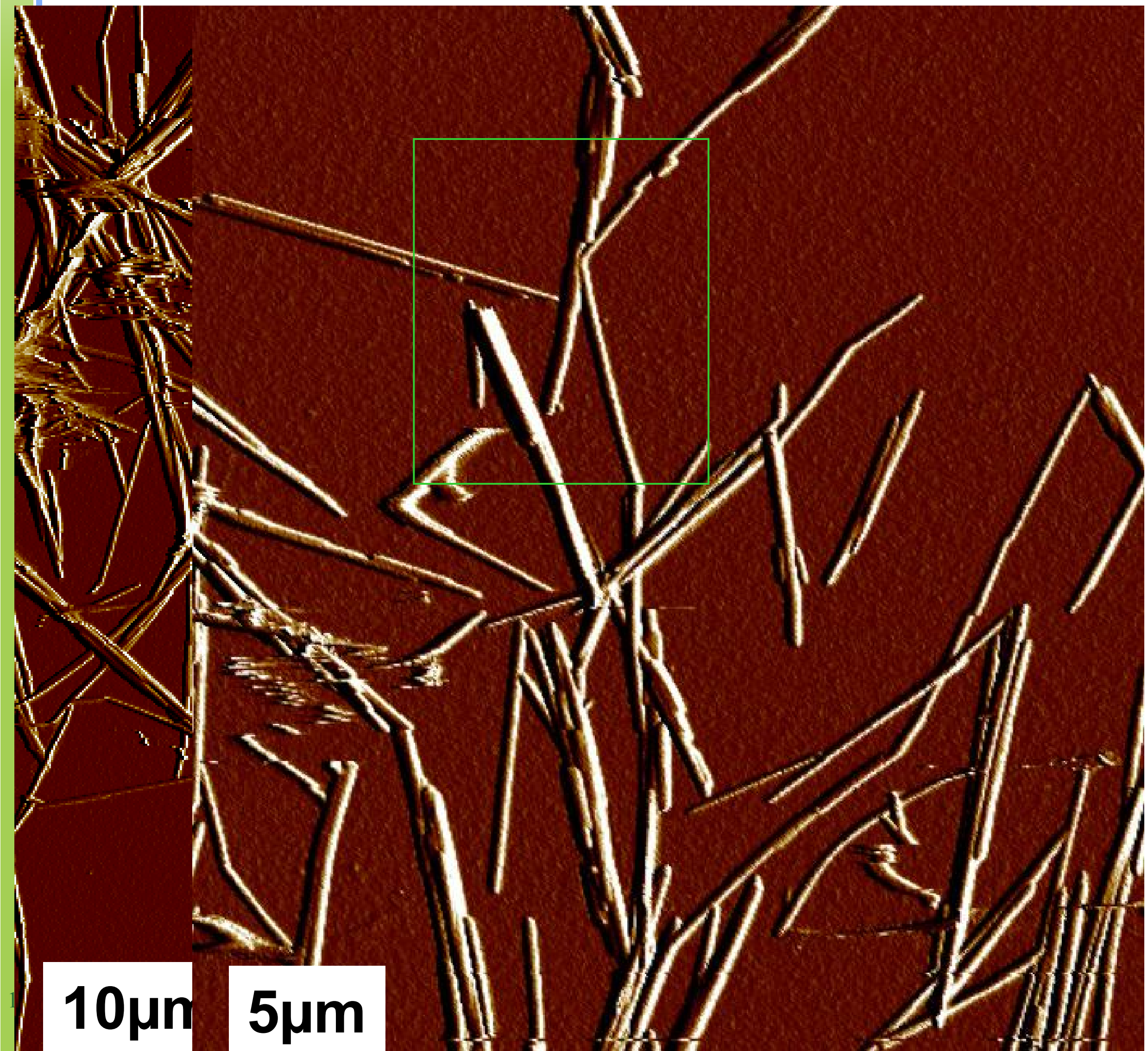
Cellulose nanocrystals

Real-time imaging of cellulose surface from μm to nm scales



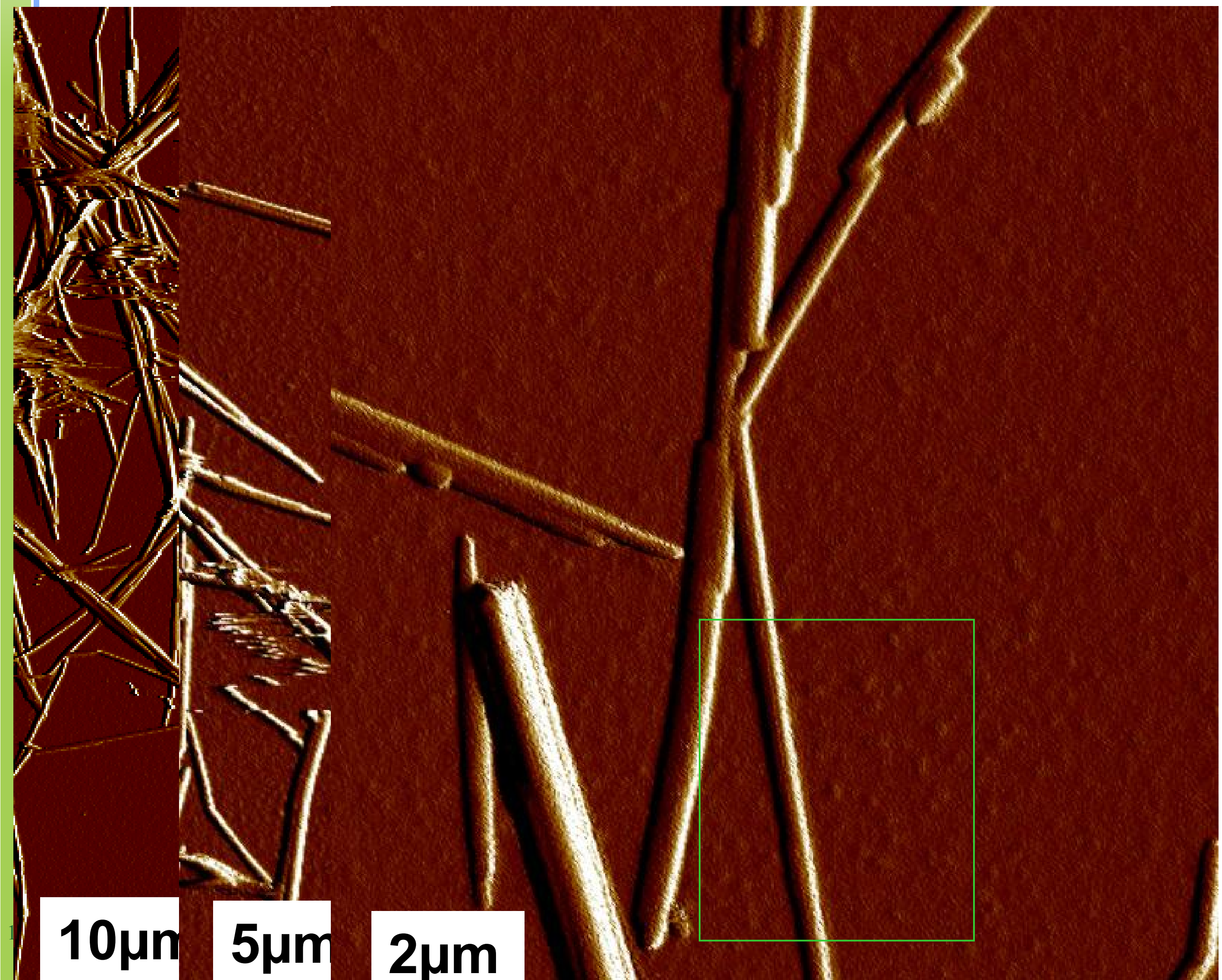
Cellulose nanocrystals

Real-time imaging of cellulose surface from μm to nm scales



Cellulose nanocrystals

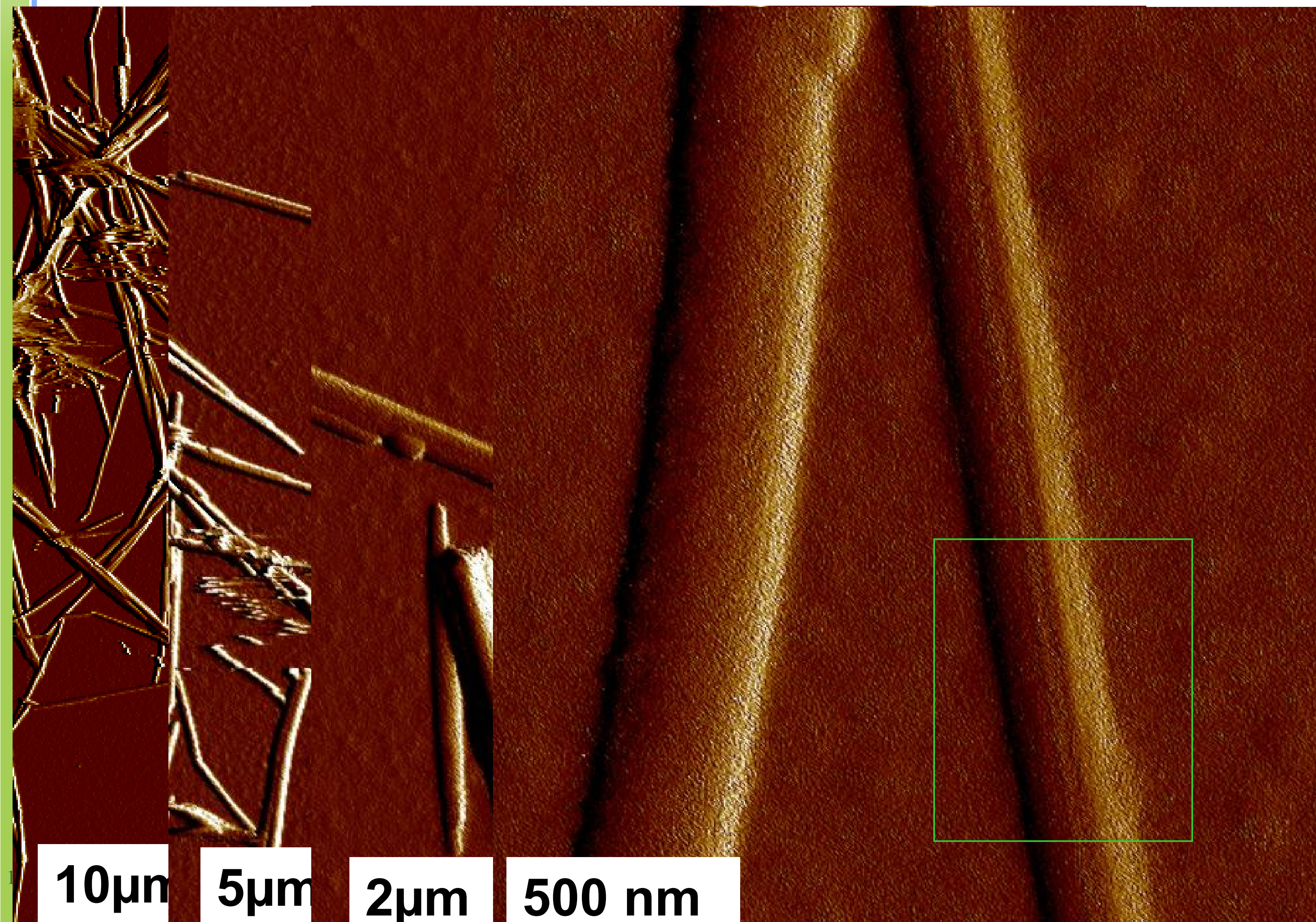
Real-time imaging of cellulose surface from µm to nm scales



Cellulose nanocrystals

U.S. DEPARTMENT OF ENERGY

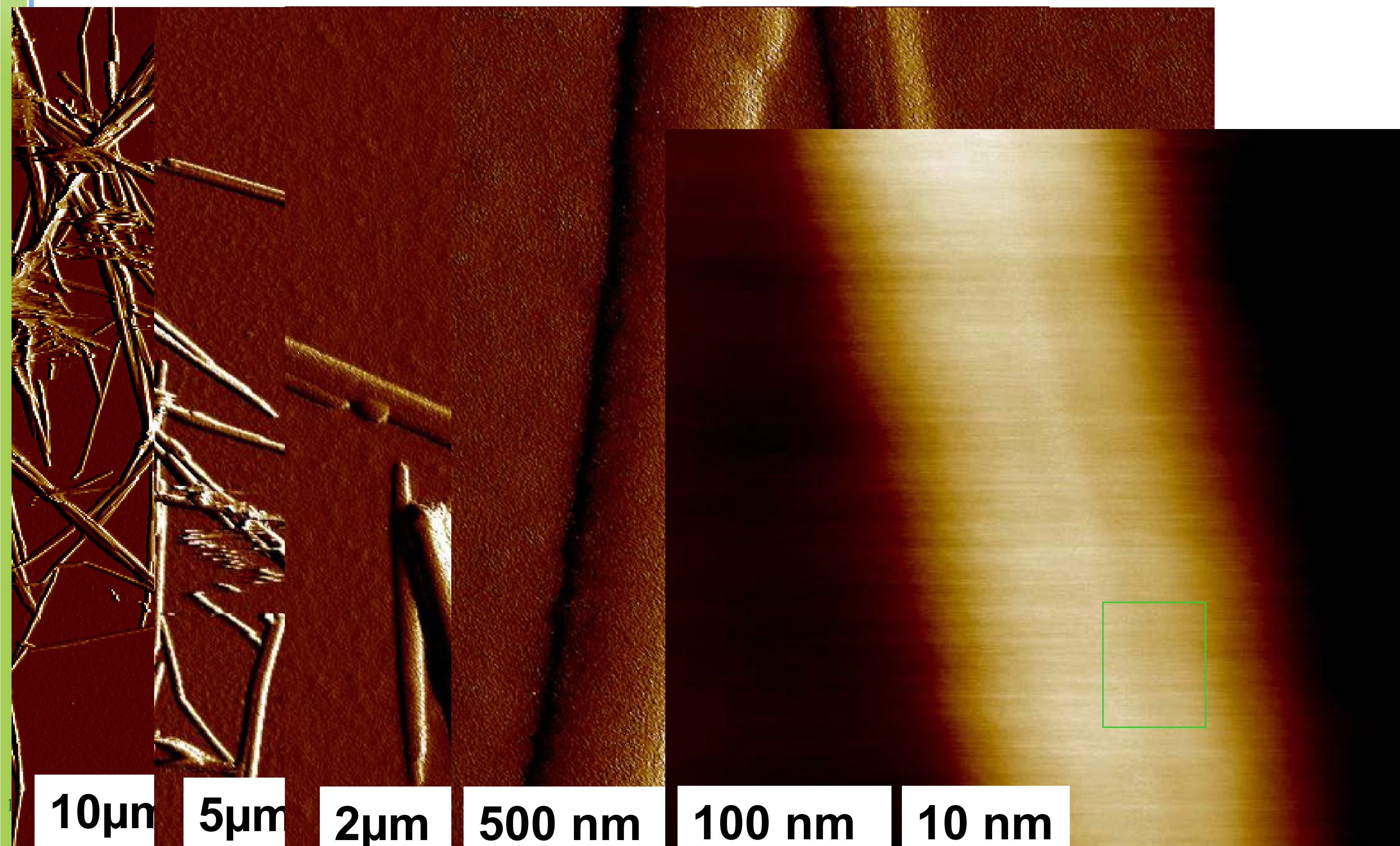
Real-time imaging of cellulose surface from µm to nm scales



BESC
oEnergy Science Center

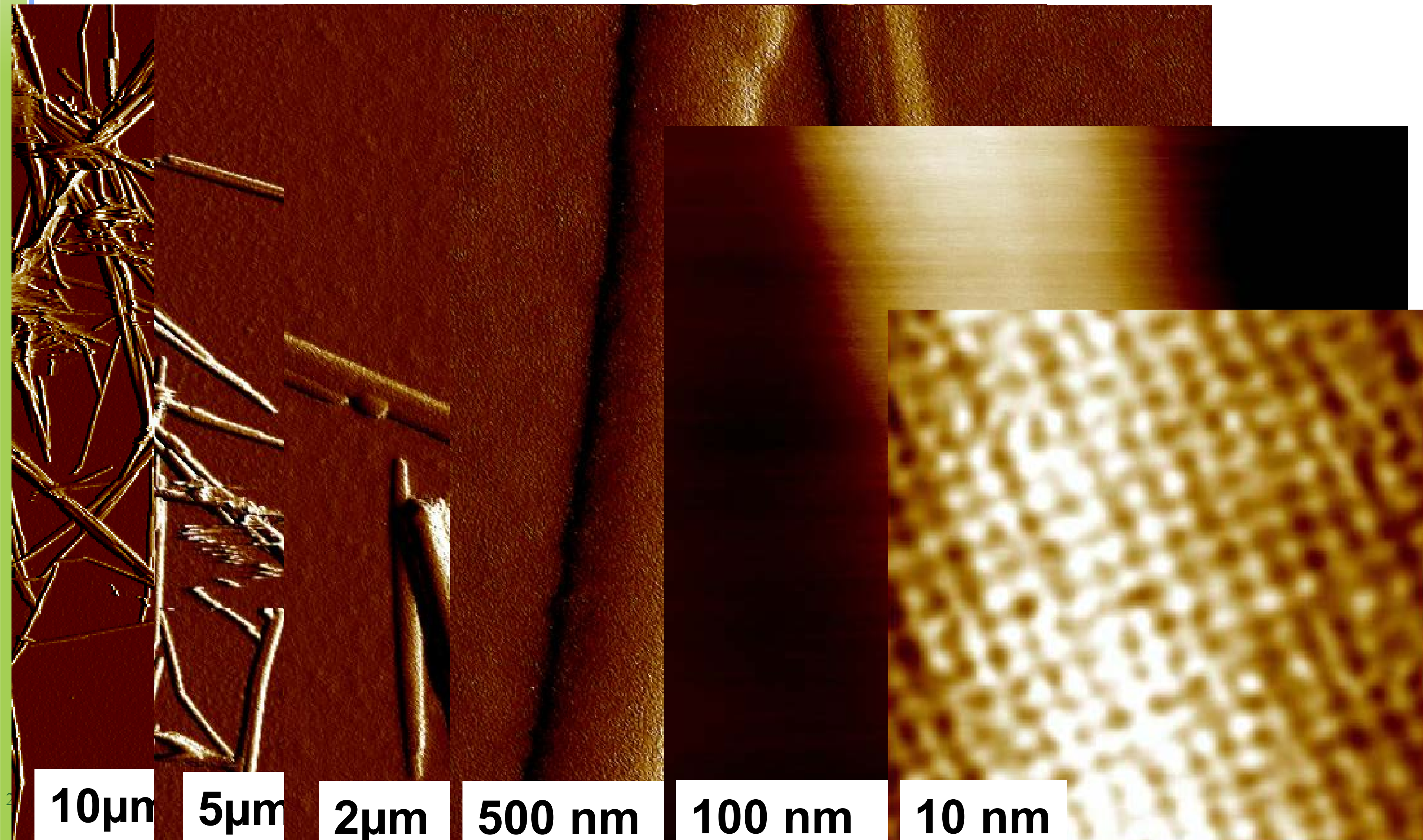
Cellulose nanocrystals

Real-time imaging of cellulose surface from μm to nm scales



Cellulose nanocrystals

Real-time imaging of cellulose surface from µm to nm scales



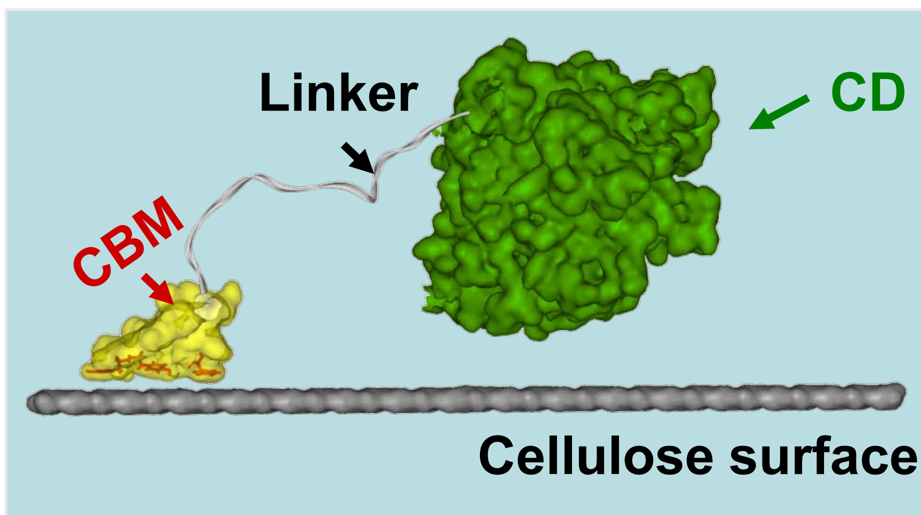
Cellulase Systems

U.S. DEPARTMENT OF ENERGY

A typical cellulase contains:

Two modules and a linker between them:

- Carbohydrate-binding module (**CBM**) binds to the substrate
- Catalytic domain (**CD**) hydrolyzes/depolymerizes carbohydrate polymers



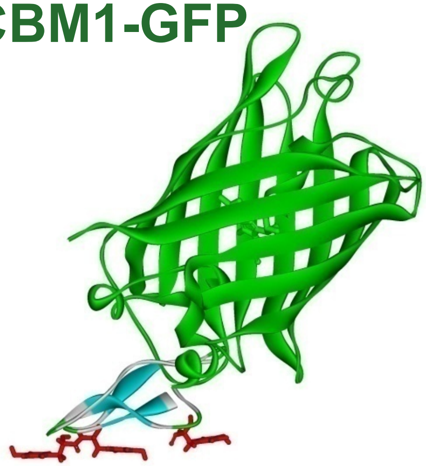
Ding et al., 1999, 2000, 2001 J Bacteriol.; Ding et al., 2009, Curr. Opini. Biotech.

BESC BioEnergy Science Center

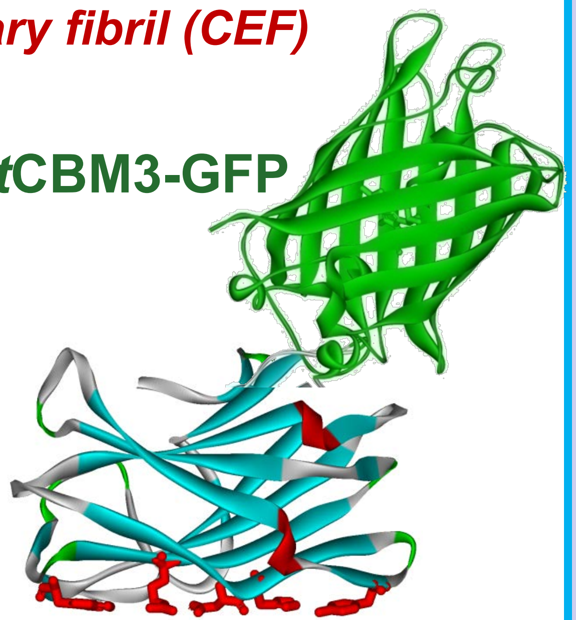
Carbohydrate-Binding Modules (CBM)

- ***Trichoderma reesei*** **CBM1**
  - *Tr*CBM1 was cloned from Cel7A (CBH I) and expressed with GFP
- ***Clostridium thermocellum*** **CBM3**
  - *Ct*CBM3 was cloned from CipA (Scaffoldin) and expressed with GFP
  - ***Both CBMs bind to the planar face of the cellulose elementary fibril (CEF)***

***Tr*CBM1-GFP**

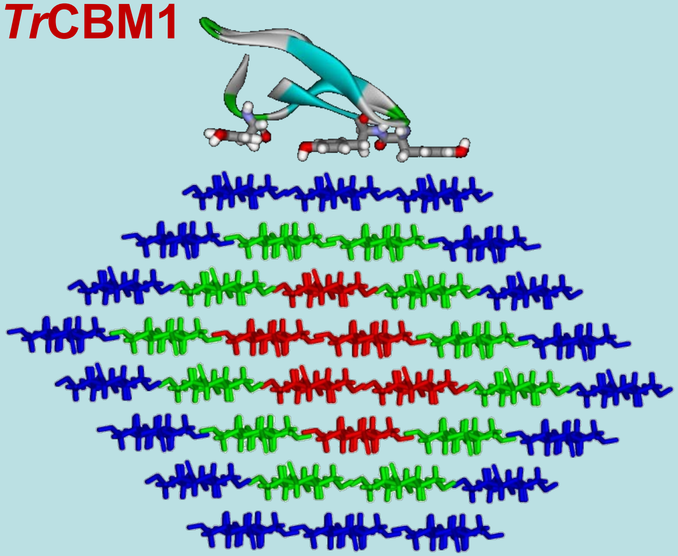


***Ct*CBM3-GFP**



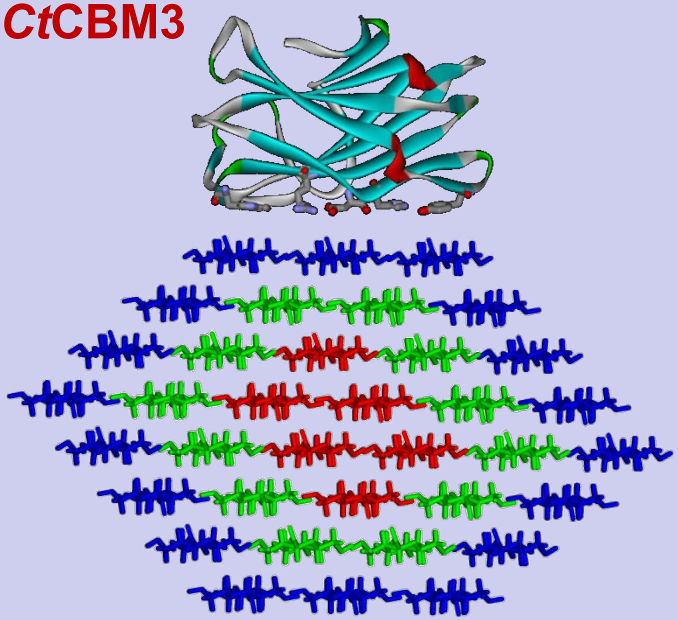
Ding et al., 2005 Industr. Biotech., 2006 BioTechniques, Xu et al., 2008 Cellulose, Liu et al., 2009 Cellulose, Liu et al., 2010 SPIE ***, Dagel et al., 2010 J. phys. Chem., Liu et al., 2011 J. Bio. Chem.***

***Tr*CBM1**



Cellulose elementary fibril (CEF)

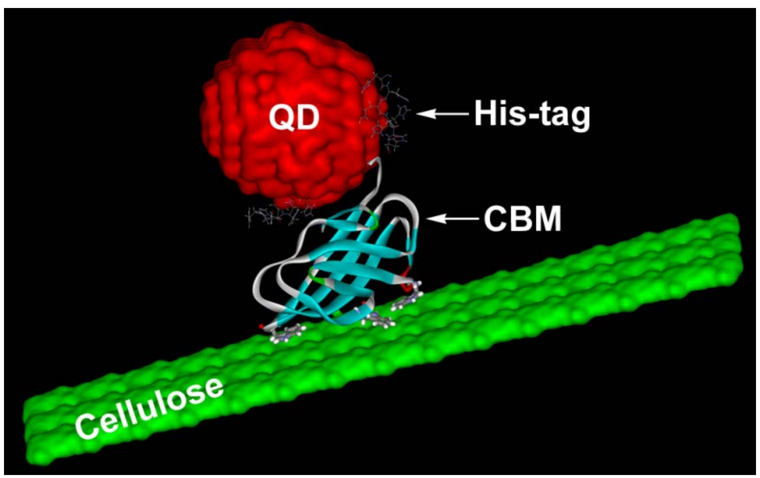
***Ct*CBM3**

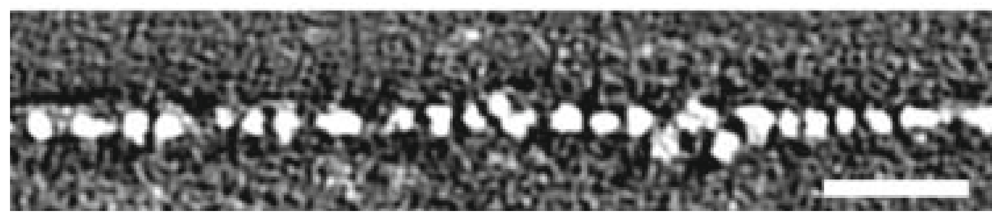


Cellulose elementary fibril (CEF)

Cellulases Bind Only to the Hydrophobic Faces

Atomic force micrograph of cellulose crystal shows the hydrophobic surface, the *Valonia* cellulose crystal is about 20-40 nm in diameters, the hydrophobic faces (110) is approximately 3 nm



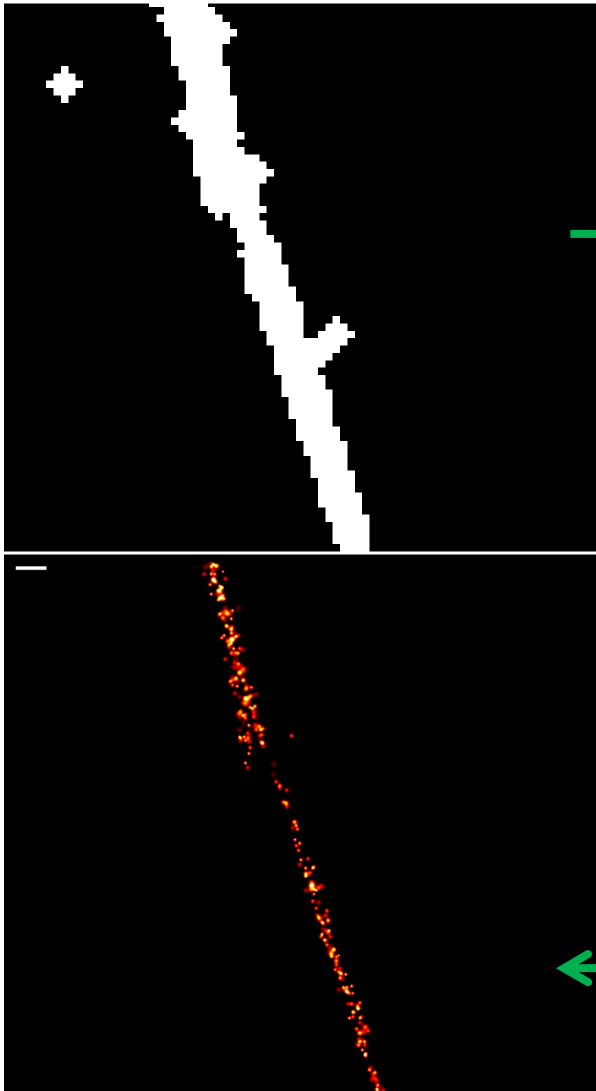


Scanning transmission electron microscopy of cellulose crystal labeled by CBM-QDs shows CBM binds only to the hydrophobic faces, linearly – indicating the (110) face allow only one CBM to bind

Xu & Ding et al., 2009, Cellulose; Liu &Ding et al., 2009, Cellulose; Liu & Ding et al., 2010 SPIE Proc.

Photo-Activated Localization Microscopy

- *Where do the CBMs bind to?*



(1) Fit the diffraction-limited spot to a 2D Gaussian: centroid (x_0,y_0) , width *s*

$$PSF(x,y) = A\exp\left[-\frac{\left[(x-x_o)^2+(y-y_o)^2\right]}{2s^2}\right]$$

(2) Calculate the uncertainty in the centroid position, $\sigma_{x,y}$

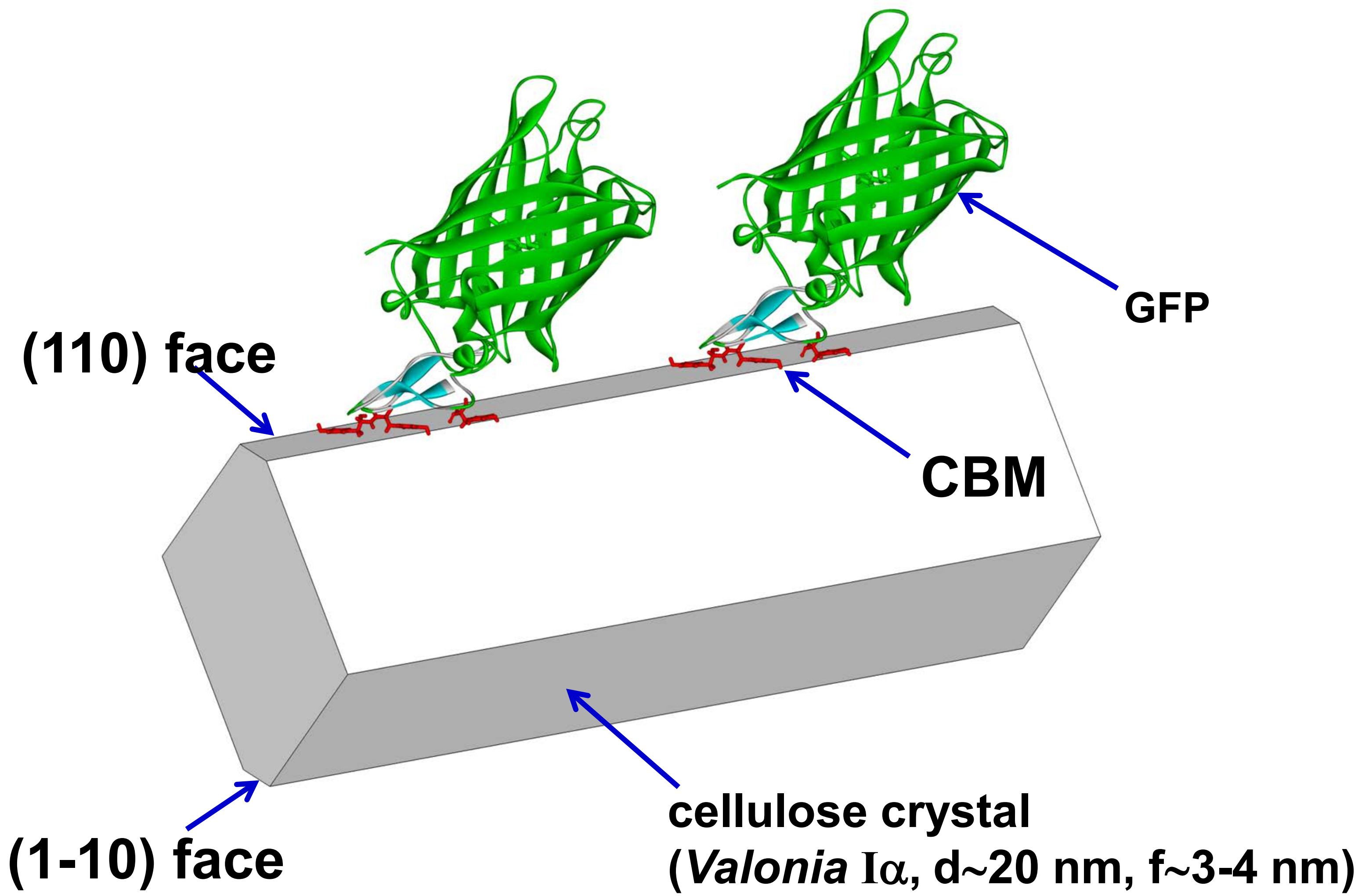
$$\sigma_{x,y}{}^2 = \frac{s^2+\frac{a^2}{12}}{N} + \frac{8\pi s^4 b^2}{a^2 N^2}$$

- x_o ,y_o ≡ centroid
- *a* ≡ effective pixel size
- *N* ≡ total photons
- *s* ≡ PSF width
- *b* ≡ background noise/pixel

(3) Replace the width *s* with the localized width $\sigma_{x,y}$

$$PSF(x,y) = A\exp\left[-\frac{\left[(x-x_o)^2+(y-y_o)^2\right]}{2\sigma^2}\right]$$

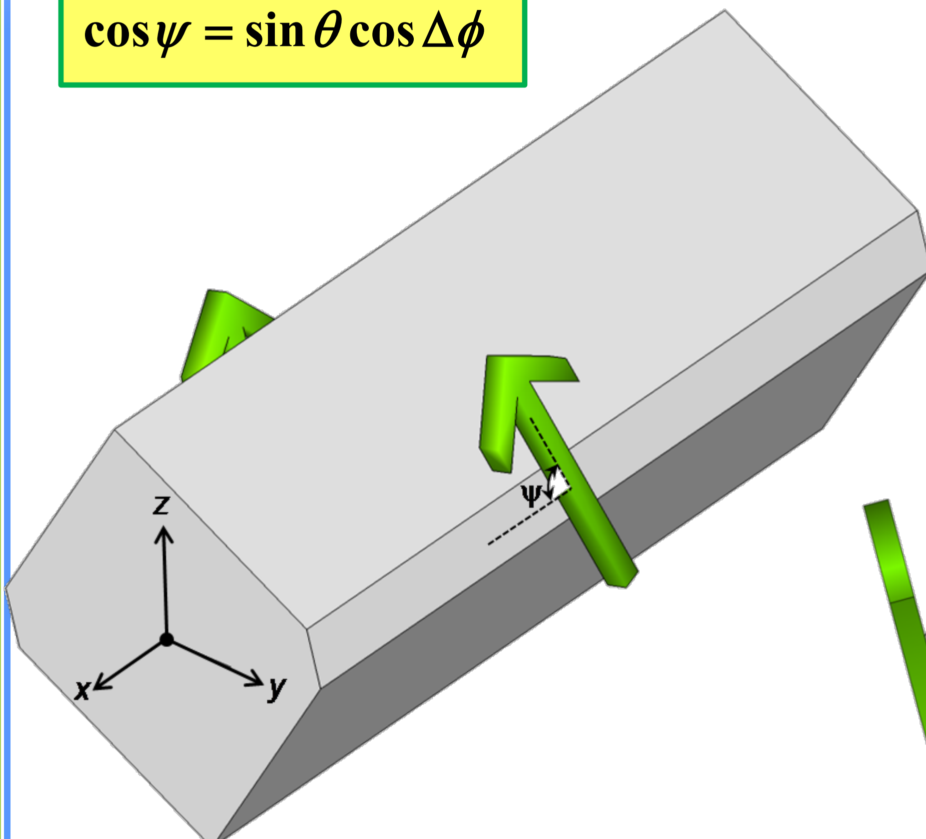
Label CBM with Green-Fluorescence-Protein (GFP)

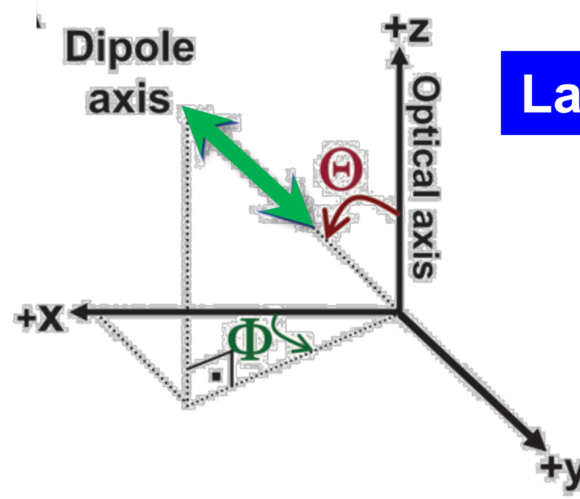


DOPI Coordinate Transformation

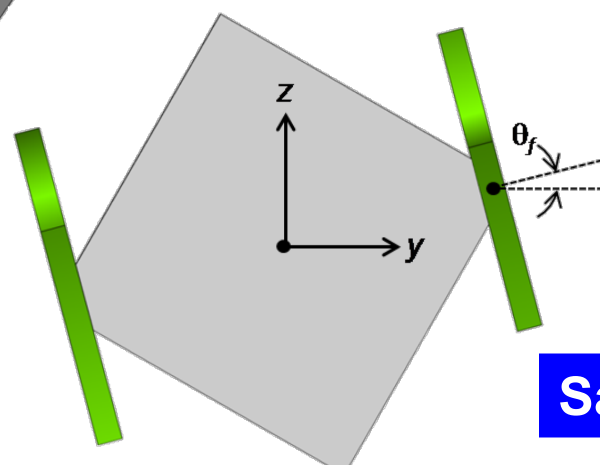
$$\tan\theta_f = \sin\Delta\phi \tan\theta$$

$$\cos\psi = \sin\theta \cos\Delta\phi$$





Lab Frame

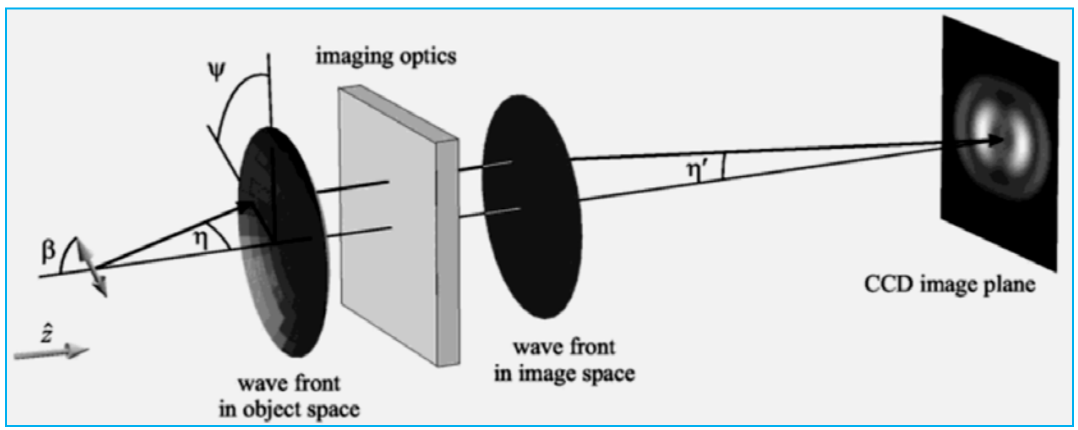


Sample Frame

Dipole orientation referenced to cellulose crystal: (*Left*) Azimuthal angle ψ describes the angle at which the dipole crosses the fiber axis (*x*-axis). (*Right*) Polar angle denotes the tilt of the dipole relative to the *z*-axis of the lab frame.

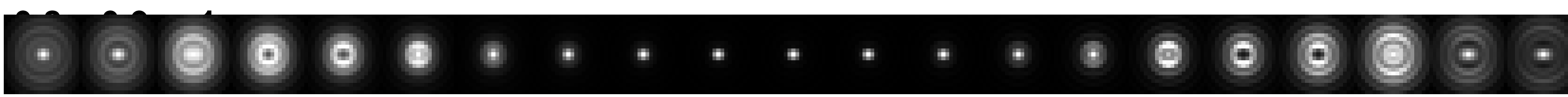
Defocused Orientation and Position Imaging (DOPI)

- Exploit the anistropy of dipole radiation outside of the focal plane to obtain 3D orientation
- Angular distribution changes dramatically due to the self-interaction of the emitting dipole with the back-reflected EM field
- *Extract* each defocused spot from the image
- *Compute* correlation coefficients between the spot and all the (θ,ϕ) theoretical orientations
- *Assign* the maximum value as the correct orientation
- *Visualize* (3D, coordinate transformations)



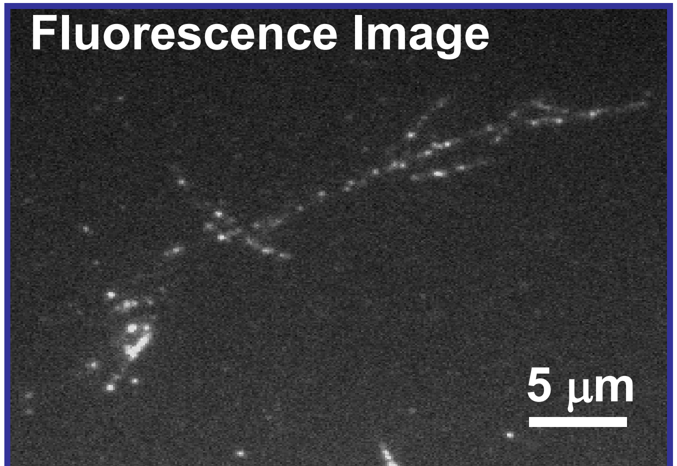
Defocus (μm)

-1 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7

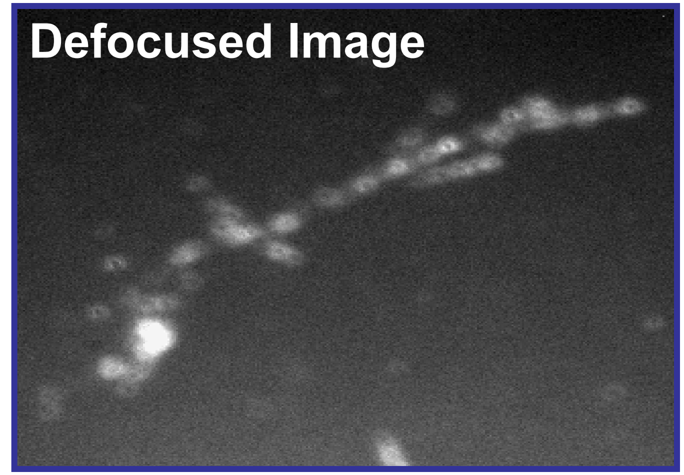


DOPI of GFP-DBM bound on cellulose crystal

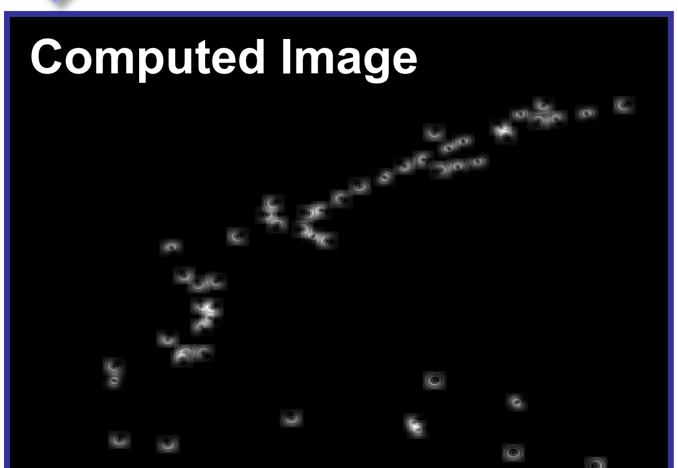
- How do the CBMs bind ?

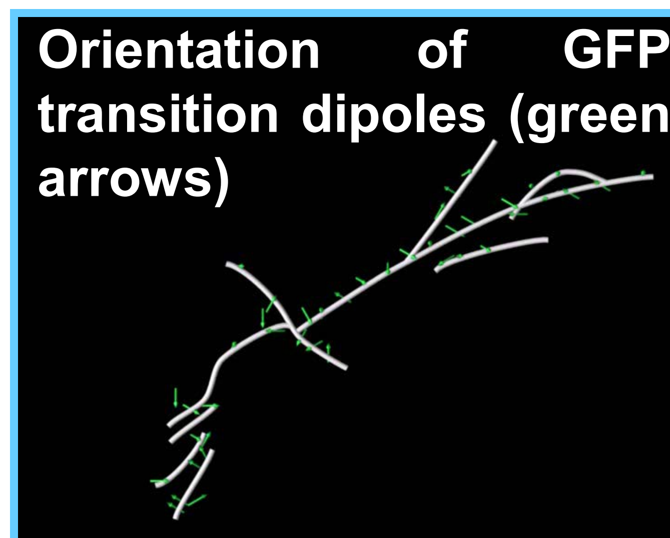


+0.6 μm defocus



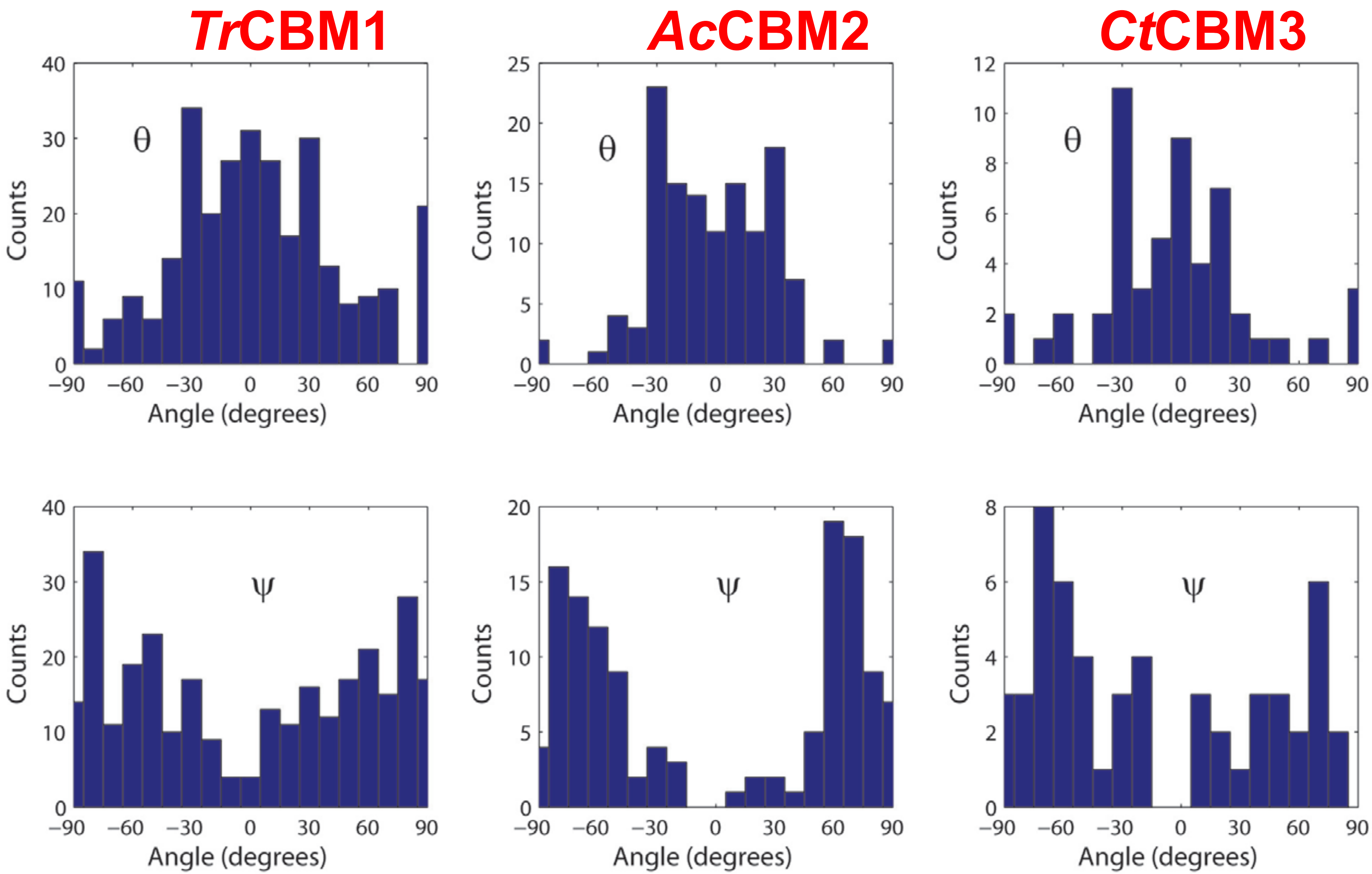
centroid positions



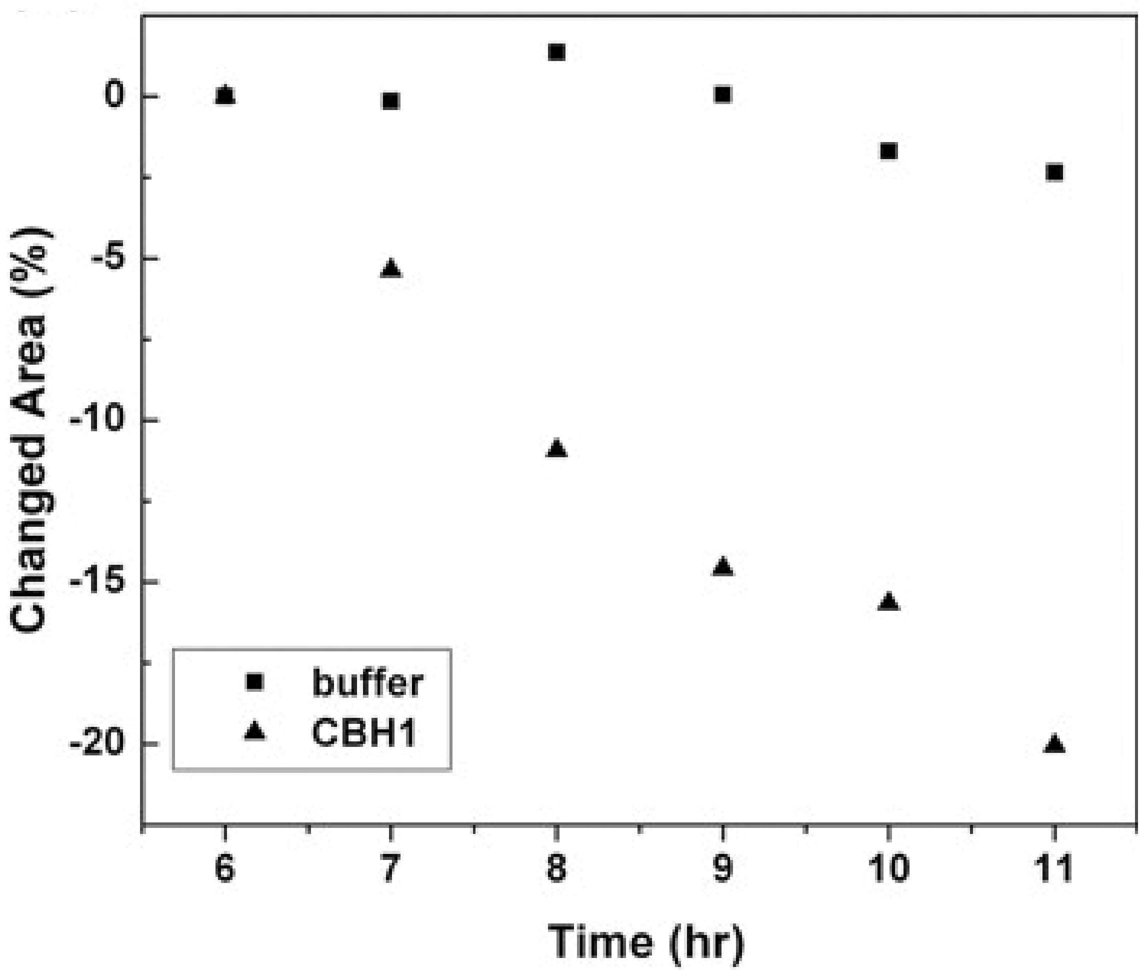


Dagel et al., 2011, J. Phys. Chem. B

CBMs Bind to Cellulose with Defined Orientation

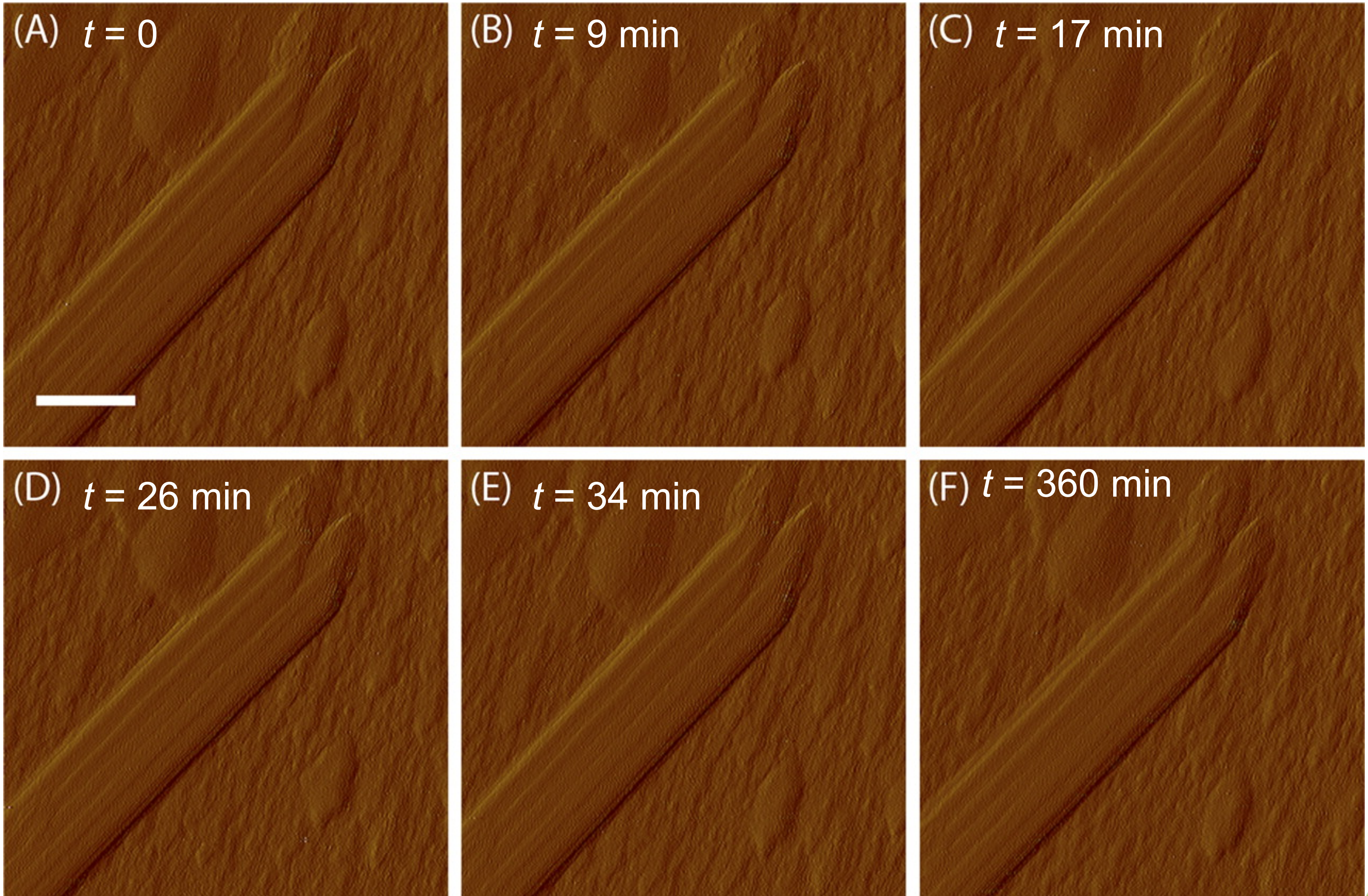


Model of cellulase hydrolysis



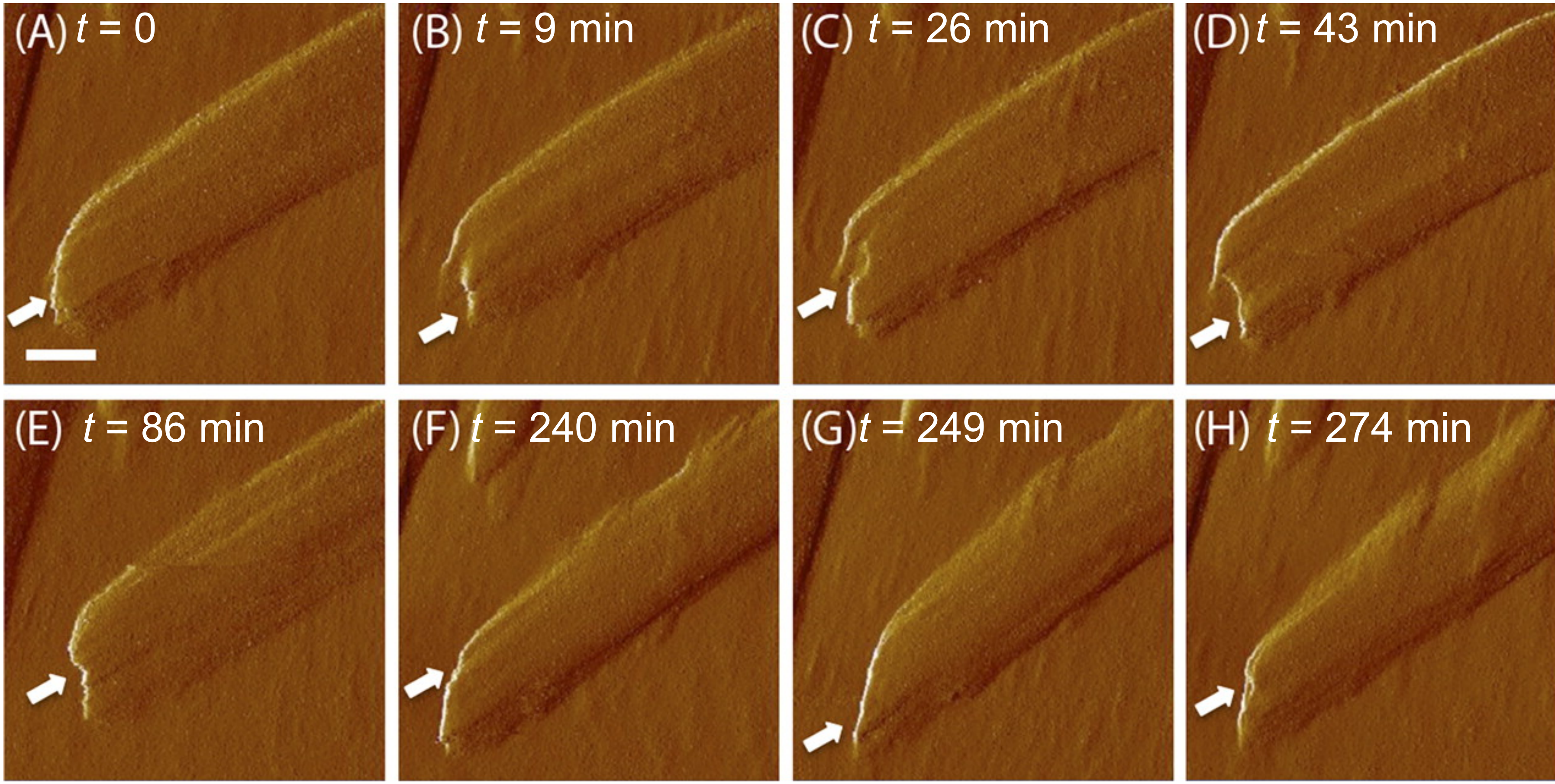
Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

Cellulose Crystal in Buffer



Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

Changes of the End of Cellulose

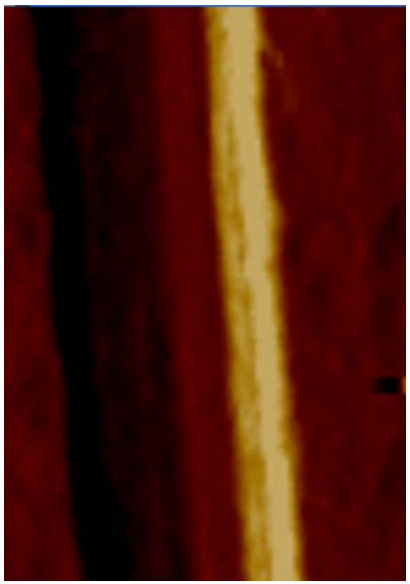


At successive time points, the crystal ends appeared smooth (A), sawtoothed (B), and then nicked (C), indicating that CBH I erodes cellulose from the end of the crystal irregularly.

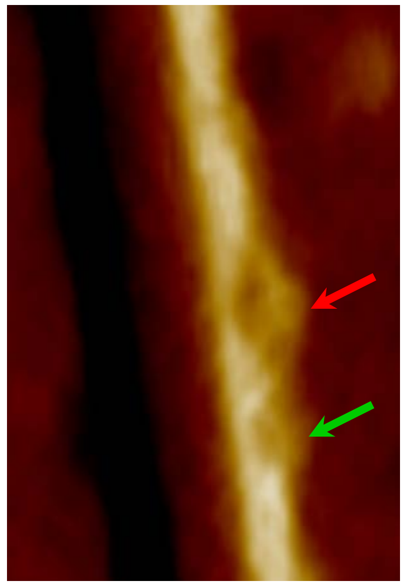
Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

Real-Time Imaging of Cellulose Hydrolysis

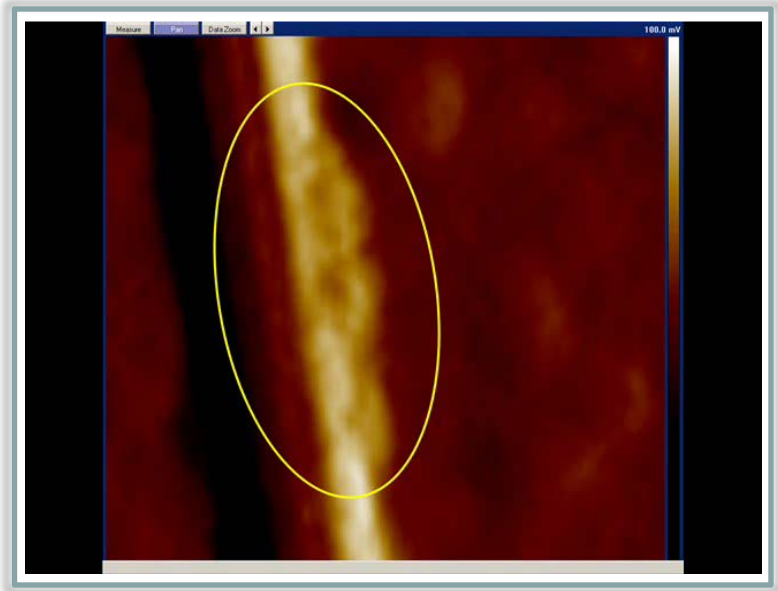
U.S. DEPARTMENT OF ENERGY



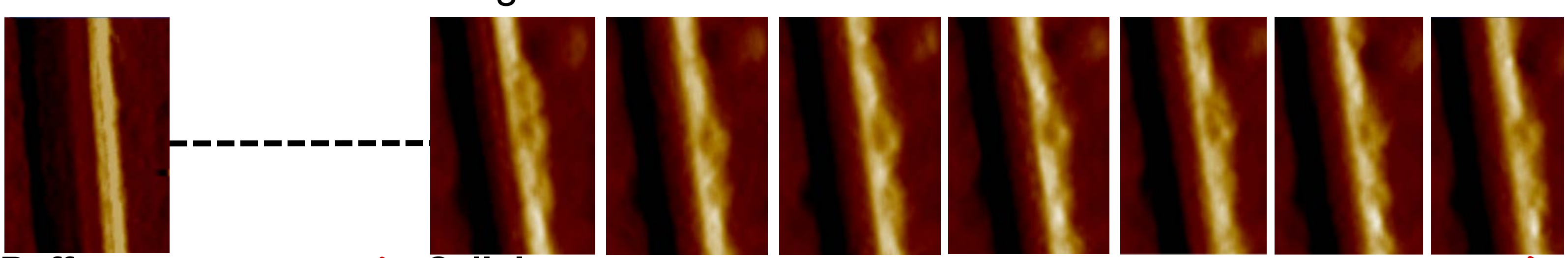
Valonia cellulose crystal in acetate buffer



The same *Valonia* cellulose crystal after adding CBH I



Real-time AFM of cellulose hydrolysis by cellulase



Buffer → **Cellulase** →

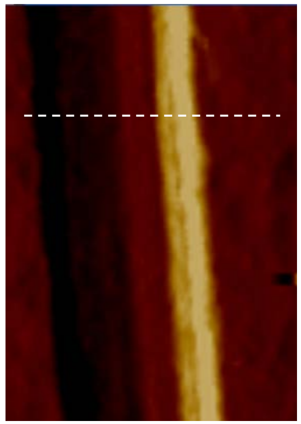
Time 0 **11 hours** **22 hours**

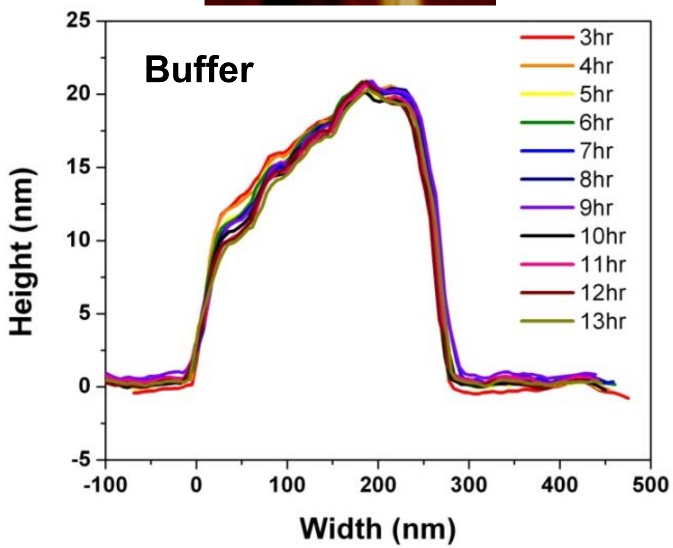
Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

BESC BioEnergy Science Center

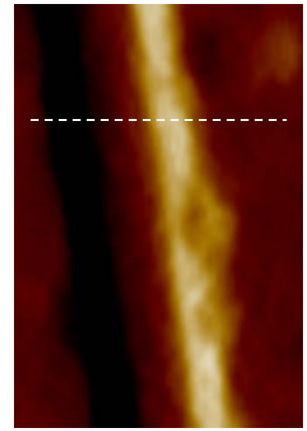
Crystal shape changes

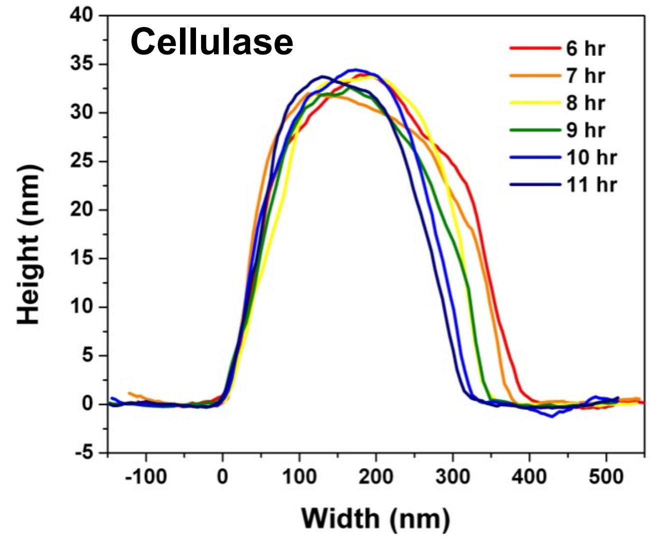
Buffer





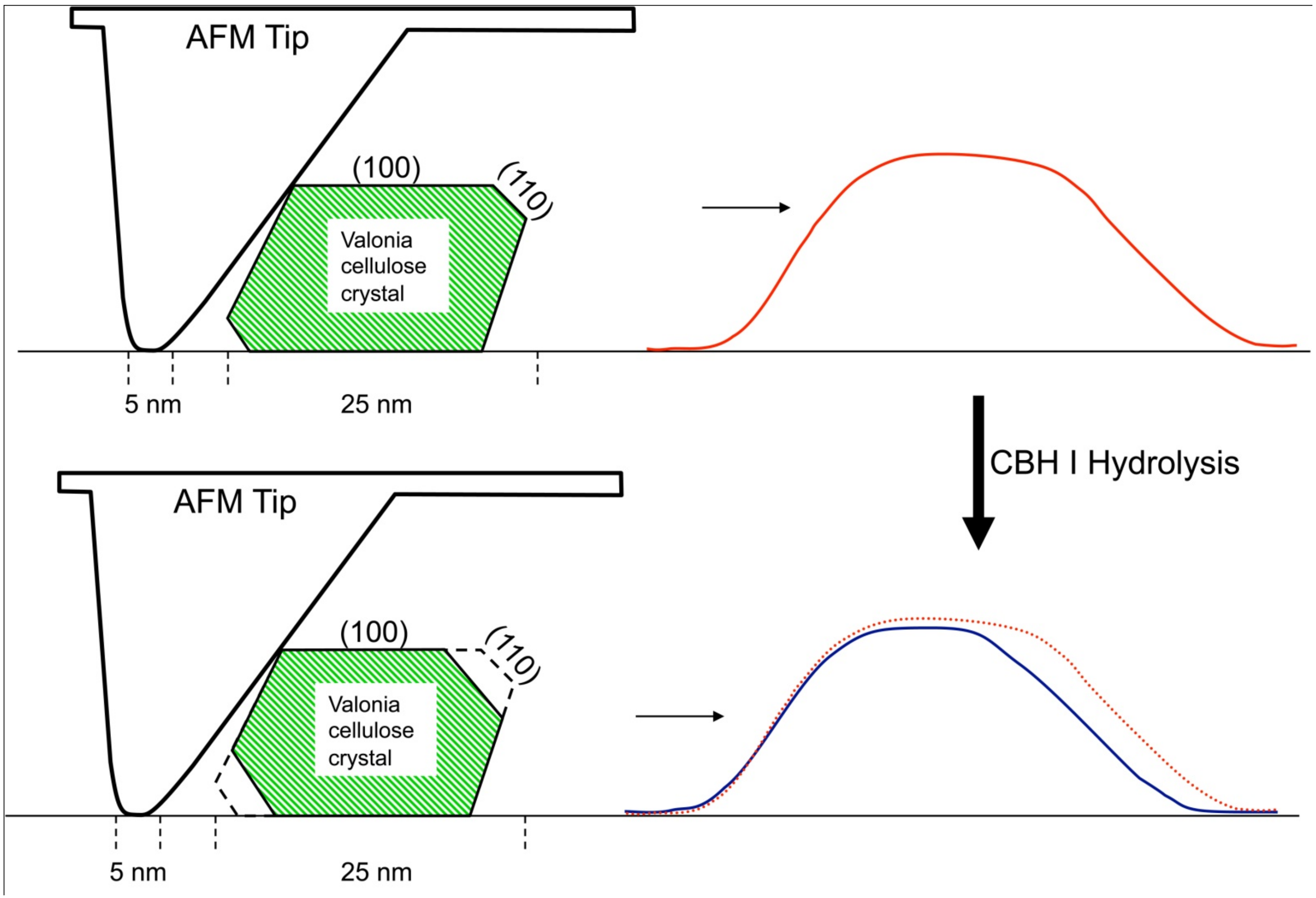
Cellulase





Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

Model of cellulase hydrolysis

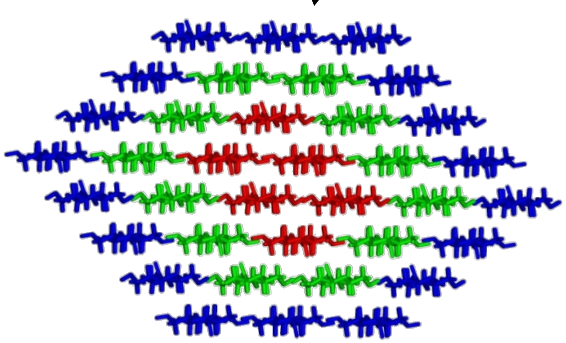


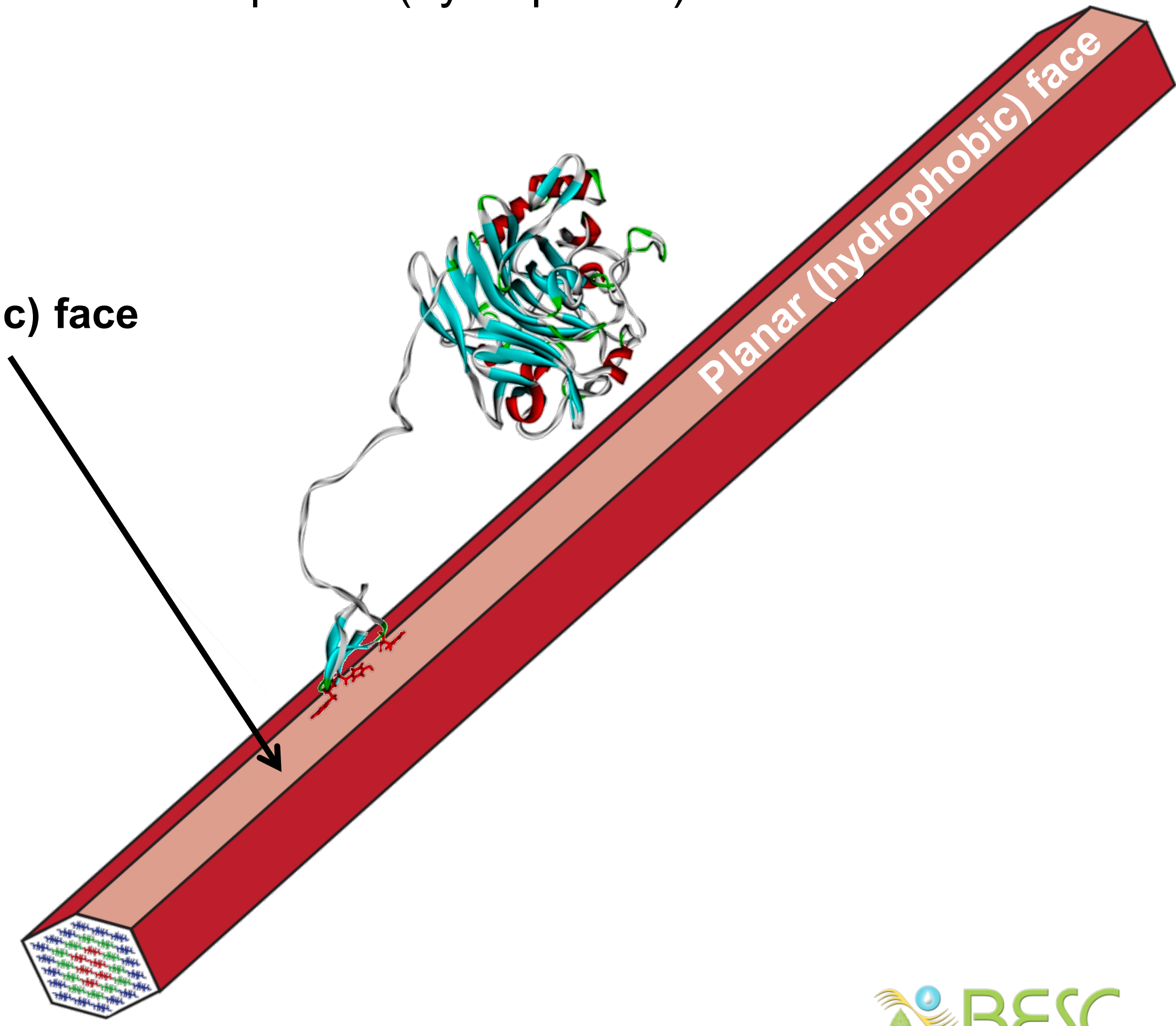
Liu & Ding et al., 2010 SPIE Proc.; Liu & Ding et al., 2011, J. Biol. Chem.

Summary

Cellulases bind to and hydrolyze from the planar (hydrophobic) faces of cellulose elementary fibril

Planar (hydrophobic) face





Cellulose elementary fibril

Liu et al., 2011, J. Bio. Chem.

Collaborators and funding

U.S. DEPARTMENT OF ENERGY

- NREL Team

- ***Yu-San (Angela) Liu***
  - Single Molecule

- ***Yining Zeng***
  - Chemical Imaging

- *John Baker, Melvin Tucker, Mark Davis, Mike Himmel, Tom Haas, Scott Luo, Hui Wei, Qi Xu*

Current Collaborators

- ***Prof. Ed Bayer, Weizmann Institute of Science***
- *Prof. Arthur J. Ragauskas, Georgia Institute of Technology*
- *Prof. Raphael Lamed, Tel Aviv University*
  Prof. Maureen McCann, Purdue University
- ***Prof. Steve Smith, South Dakota School of Mines***
- *Prof. Jeff Squier, Colorado School of Mines*
- *Prof. Junji Sugiyama, RISH, Kyoto University*
- *Prof. Joe Wall, Brookhaven National Laboratory*
- ***Prof. Sunney Xie, Harvard University***
- *Prof. Richard Dixon and Fang Chen, The Samuel Robert Noble Foundation*

Office of Science
U.S. DEPARTMENT OF ENERGY

The U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER) through the BioEnergy Science Center (BESC), a DOE Bioenergy Research Center, and grant ER65258 for instrumentation and data analysis. The US DOE, EERE, the Office of the Biomass Program supports partially for instrumentation.

BESC
BioEnergy Science Center