Molecular observations of an enzyme reacting on its substrate is of prime interest to understand the correlation between protein function and its structural dynamics. The study by Eibinger and colleagues, in this issue of ACS Central Science, uses atomic force microscopy (AFM) to capture high-resolution snapshots, in real time at the minute time scale, of enzyme reaction on a solid substrate at near-physiological conditions. This study demonstrated the structural changes of the cellulosome, a protein complex, as it binds to and reacts on a cellulosic substrate.

Imaging techniques have expedited cellulosome discovery and development in the past few decades. In the very earliest studies that documented the discovery of the cellulosome, negative staining electron microscopy played a prominent role in revealing high-molecular-weight protein particles that contain multiple subunits and can be isolated from growth cultures of the cellulose-degrading bacterium Clostridium thermocellum. Further studies showed that cellulosomes contain repeated structures that bind to cellulose and completely hydrolyze it. Therefore, cellulosomes were described as multienzyme complexes. Molecular biology and genetic studies have further demonstrated that these multiple protein complexes are composed of a variety of enzymes, each bearing a single dockerin module and at least one nonenzymatic “scaffolding” component that contains multiple cohesin modules. The specific interaction between the dockerin and cohesin modules dictates the assembly of numerous enzymes onto the primary scaffolding subunit that, in turn, anchors onto the bacterial cell surface via a divergent type of cohesin–dockerin interaction. Thus, the
cellulosome represents a unique enzymatic system existing in selected species of *Clostridia* and other related bacteria, which demonstrates superior activities in degrading complex cellulotic substrates, such as plant cell walls (Figure 1). Broad mechanistic understanding of the dynamic structure of these protein complexes will support advanced biotechnological and nanotechnological engineering of novel types of cellulosome-based complexes. This would, for example, help to develop effective enzyme systems for biomass conversion that would enable economic production of lignocellulosic biofuels and biomaterials.

In the 1980s, imaging studies were instrumental in the discovery of the cellulosome. Following two decades of subsequent research, scientists proposed that the scaffold protein is highly flexible for integrating multiple enzymes into a complex, thereby promoting synergistic activity as a function of their structural proximity. Precise organization and assembly of cellulosomes on the bacterial cell surface was recently documented by using advanced imaging approaches, that is, super-resolution or stochastic optical reconstruction microscopy (STORM) and correlative light and electron microscopy (CLEM) (see Figure 2A). Crystallographic evidence, small-angle X-ray scattering (SAXS) techniques, and biochemical studies of recombinant cellulosomal components have indeed confirmed that the scaffold protein exhibits considerable freedom of motion and that its intermodular linker segments facilitate flexible accessibility of the catalytic domains to the substrate.

Atomic force microscopy (AFM) offers a unique tool to observe biomolecules at molecular resolution under near-physiological conditions. These studies provide real-time observation of cellulase binding to cellulose and in situ morphological changes of the substrate during enzyme reaction. At the minute time scale, AFM imaging revealed different mechanisms of action between free (uncomplexed) fungal cellulases versus bacterial cellulosome complexes, during the process of hydrolyzing cellulotic substrates. Reactions of free fungal cellulases on crystalline cellulose substrates were first imaged by AFM, where individual cellulases were shown to “run” processively along the hydrophobic surface of the cellulose crystal. Subsequently, bacterial cellulosome complexes were imaged by AFM on pretreated plant cell walls that contain lignin and hemicellulose in addition to cellulose. In the latter work, the cellulosomes remained localized on the cellulosic substrate in a “digging”-type mode, as opposed to the “running” mode exhibited by the free fungal cellulases. Thus, in a complex biomass like the plant cell wall, the relatively small fungal cellulases can penetrate into the fibrillar matrix of cell walls, whereas cellulosomes remain primarily on the surface due to their large size (Figure 2B,C).

Although early electron microscopy observations have demonstrated the dynamic conformational changes of cellulosomes from tightly packed particles to loosely elongated shapes upon binding to the cellulose surface, later studies primarily focused on structural changes of the cellulosic substrates. The current research further demonstrates the direct imaging of these dynamic protein assemblies with video recording, thereby highlighting, experimentally and in real time, the conformational changes of cellulosomes while binding to and digesting plant-derived cellulose nanocrystals.

Therefore, AFM can provide the highly dynamic morphology of the sample surface at much higher resolution and under...
near-physiological conditions, but artifacts can become substan-
tial when imaging complex systems, such as protein
complexes and plant biomass. Recent developments to combine
single-molecule spectroscopy with fluorescently labeled
enzymes can be used in the future to track enzymes at the
nanometer scale of resolution. Additionally, AFM can be
used as built-in modules with vibrational spectroscopy to
image both morphology and chemistry, both correlatively
and simultaneously. As imaging technology becomes more
sophisticated, it will continue to refine our concepts of
cellulase and cellulosome action, and provide further insight
into their deployment for cost-effective renewable energy
and biotechnological applications in general.

As imaging technology becomes more sophisticated, it will
continue to refine our concepts of cellulase and cellulosome
action, and provide further insight into their deployment
for cost-effective renewable energy and biotechnological
applications in general.

Author Information

Corresponding Author
Edward A. Bayer — Department of Biomolecular Sciences, The
Weizmann Institute of Science, Rehovot 7610001, Israel;
Faculty of Natural Sciences, Life Sciences, Ben-Gurion University
of the Negev, Beer-Sheva 8410501, Israel; orcid.org/0000-
0001-7749-5150; Email: Ed.Bayer@weizmann.ac.il

Author
Shi-You Ding — Department of Plant Biology and Great Lakes
Bioenergy Research Center, Michigan State University, East
Lansing 48824-1312, Michigan, United States; orcid.org/
0000-0002-1102-1507

Complete contact information is available at:
https://pubs.acs.org/10.1021/acscentsci.0c00662

Notes
The authors declare no competing financial interest.

REFERENCES
(1) Eibinger, M.; Ganner, T.; Plank, H.; Nidetzky, B. A biological
nanomachine at work: watching the cellulosome degrade crystalline
cellulose. ACS Cent. Sci. 2020, 6, 739.
(2) Lamed, R.; Setter, E.; Bayer, E. A. Characterization of a cellulose-
binding, cellulase-containing complex in Clostridium thermocellum.
J. Bacteriol. 1983, 156, 828–836.
(3) Mayer, F.; Coughlan, M. P.; Mori, Y.; Ljungdahl, L. G.
Macromolecular Organization of the Cellulolytic Enzyme Complex of
Clostridium thermocellum as Revealed by Electron Microscopy. Appl.
Environ. Microbiol. 1987, 53 (12), 2785–92.
(4) Bayer, E. A.; Morag, E.; Lamed, R. The cellulosome — A
treasure-trove for biotechnology. Trends Biotechnol. 1994, 12, 379–
386.
(5) Artzi, L.; Dadosh, T.; Milrot, E.; Morais, S.; Levin-Zaidman, S.;
Morag, E.; Bayer, E. A. Co-localization and disposition of cellulosomes in
Clostridium clariflavum as revealed by correlative superresolution imaging.
mBio 2018, 9, No. e00012-18.
(6) Hammel, M.; Fierobe, H.-P.; Czjzek, M.; Karkal, V.; Smith, J. C.;
Bayer, E. A.; Finet, S.; Receveur-Bréchot, V. Structural basis of
cellulosome efficiency explored by small angle X-ray scattering. J. Biol.
Chem. 2005, 280, 38562–38568.
(7) 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. 2013, 23, 686–694.
(8) Igarashi, K.; Koivula, A.; Wada, M.; Kimura, S.; Penttila, M.;
Samejima, M. High speed atomic force microscopy visualizes
processive movement of Trichoderma reesei cellobiohydrolase I on
crystalline cellulose. J. Biol. Chem. 2009, 284 (52), 36186–90.
(9) Ding, S.-Y.; Liu, Y.-S.; Zeng, Y.; Himmel, M. E.; Bayer, E. A.;
et al. How does plant cell wall nanoscale architecture correlate with
enzymatic digestibility? Science 2012, 338, 1055–1060.
(10) Resch, M. G.; Donohoe, B. S.; Baker, J. O.; Decker, S. R.;
Bayer, E. A.; Beckham, G. T.; Himmel, M. E. Complementary
mechanisms of plant cell wall deconstruction by free and complexed
enzyme systems. Energy Environ. Sci. 2013, 6, 1858–1867.