To cells and tissues

From single molecules

To Biomolecular complexes

High-resolution imaging with the BioScope Catalyst across all biological size scales

Application Note #131

BioScope Catalyst: Accessing All Biological Size Scales with High-Resolution AFM Imaging

The BioScope™ Catalyst™ Atomic Force Microscope (AFM) operates under physiologically relevant conditions and integrates fully with optical microscopy techniques, providing life science researchers the ability to study biological species across a wide-range of size scales. With its superior engineering and mechanical stability, the Catalyst is ideally suited for obtaining high-resolution, three-dimensional images of individual biomolecules and small biomolecular complexes. From investigating nucleic acids and proteins to characterizing protein assemblies and membranes, high-resolution imaging conducted with the Catalyst offers scientists unique opportunities to research biomolecules and biomolecular processes at the single-molecule level.

Life Sciences Atomic Force Microscopy

Atomic force microscopy allows direct visualization of the three-dimensional structure of a sample surface with nanoscale resolution. Unlike many other high-resolution imaging techniques, AFM does not require staining or coating of the sample, leaving native surfaces unaltered. Together with the ability to operate in a fluid environment, this makes AFM an unparalleled technique for studying biomolecules and the dynamics of biological processes in situ and in real-time.

Biological research encompasses a wide-range of size scales and structural complexity, from single molecules to cells to tissues. As such, an instrument or technique capable of high-resolution imaging of single biomolecules, as well as larger biomolecular complexes, can provide biologists with unique research opportunities. The BioScope Catalyst AFM was specifically designed with this in mind. The system can be functionally integrated with various light microscopy techniques (brightfield, phase contrast, DIC, epi- and confocal fluorescence, etc.) to allow optically guided navigation of the AFM probe to regions of a sample for high-resolution AFM imaging and the production of correlated AFM and optical image datasets. With the industry's largest closed-loop, X-Y scan range of 150 microns, a Z range of ≥20 microns, and capability for environmental control, the Catalyst repeatedly has demonstrated its unmatched performance for combined AFM and optical microscopy studies of live cells (see figure 1).[1-4] However, the Catalyst is not just for live cell studies. The highest quality engineering and mechanical stability of the Catalyst AFM facilitates high-resolution imaging of smaller biomolecular species as well. Even when installed on an inverted optical microscope, the Catalyst's imaging performance is not compromised (see figure 2).
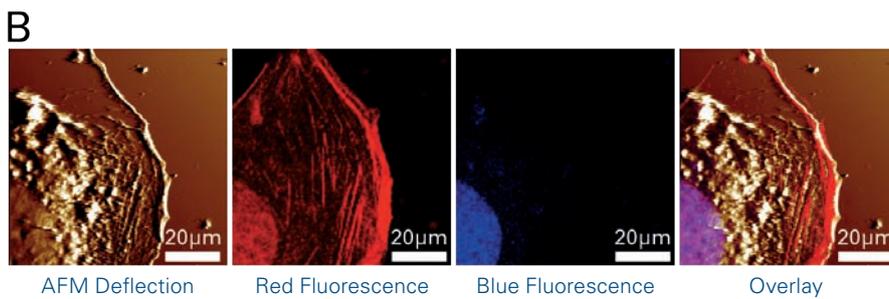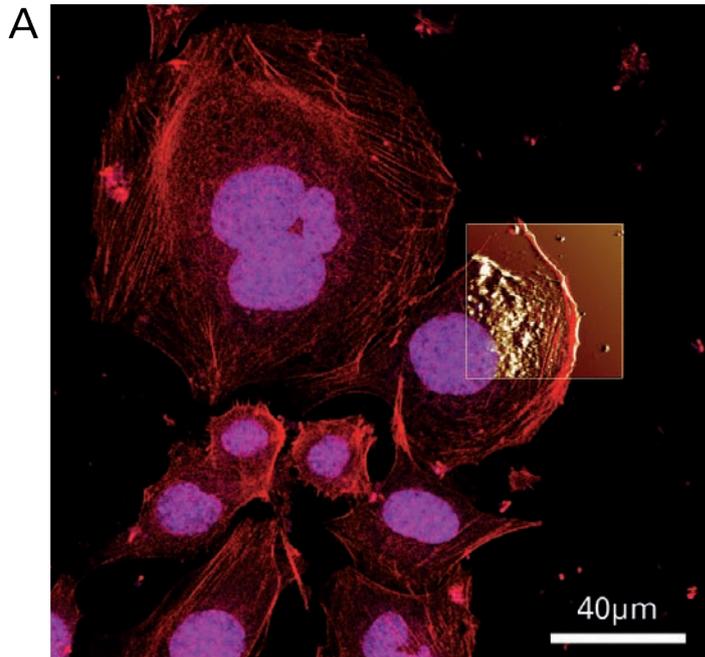
Figure 1. (A) Registered image overlay of a two-channel confocal laser scanning fluorescence microscopy image and AFM topography image of fibroblast cells labeled with Alexa Fluo 546 Phalloidin (red) & DAPI (blue). The BioScope Catalyst MIRO software enables registration of an optical field of view to the AFM scan area. Optical images can then be used to navigate the AFM probe to a region of interest to perform high resolution AFM imaging and/or highly sensitive force measurements. (B) Region of interest obtained from the image overlay showing correlated AFM and fluorescence data channels. Confocal fluorescence images were obtained with a Leica SP5 confocal system and using a 40x oil immersion objective. AFM images were obtained with a BioScope Catalyst operated in contact mode in buffer solution using MLCT AFM probes (k ~0.01N/m).

Nucleic Acids: DNA

DNA (deoxyribonucleic acid) is the fundamental molecule of life. As such, much biological research has been focused on studying its structure and its interactions with other biomolecules. Ultimately, understanding how the genetic code is stored by and expressed from DNA is critical to gaining insights into genetically related diseases and the development of treatment methods or the prevention of these disease states. Much of the information on DNA structure and function has been resolved using a combination of molecular biology, x-ray diffraction and electron microscopy techniques. However, increasingly the AFM has been an attractive approach to DNA studies with its ability to study intermolecular interactions and processes in real-time under near physiological conditions.[5-9]

For AFM imaging, DNA is typically deposited onto a freshly cleaved mica surface, using either (1) divalent cations (e.g., Ni++ or Mg++) to passivate the negatively charged mica surface,[9-13] or (2) chemically modifying the mica surface itself with a positively charged silane (e.g., APS-mica)[14-16], to facilitate adsorption of the negatively charged DNA strands. Using either of these immobilization techniques, the structure of the DNA molecules are easily resolved in the AFM images, while proteins or enzymes can be directly introduced into the imaging solution and their interactions with the DNA observed in real-time (see figure 3).[17-21]

The Catalyst's small-volume flow cell provides an enclosed fluid environment for observing the structure and dynamics of biomolecules over an extended period of time. Inlet
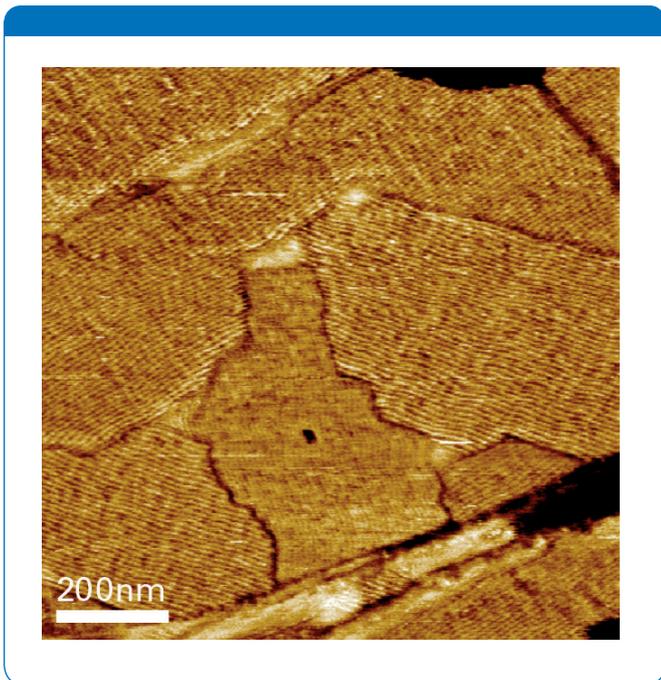
Figure 2. A 1µm Phase Image of a C60H122 alkane. The C60H122 is spincast onto an HOPG substrate with the resulting ultra-thin alkane layer exhibiting a lamellar structure ~7.5nm in width and ~0.4nm in height. Images were acquired on a BioScope Catalyst AFM operated in TappingMode using FESP AFM probes (k ~3N/m).
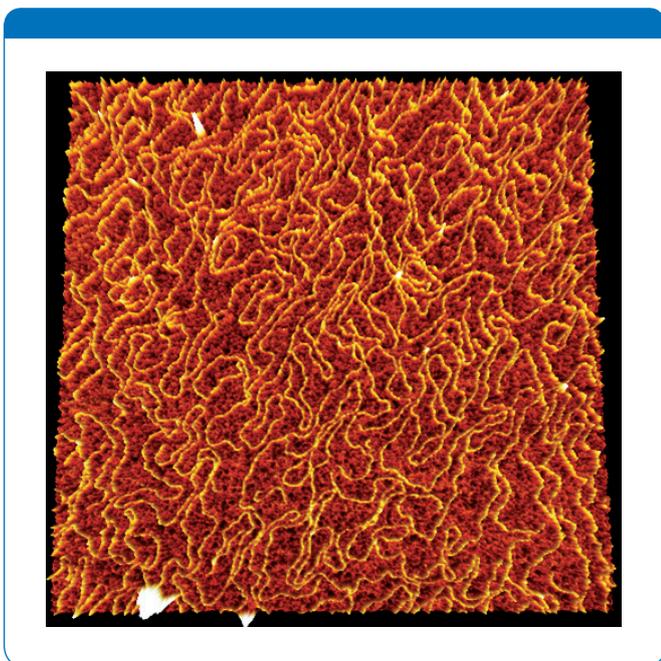
Figure 3. Three-dimensional topography image of pUC plasmid DNA adsorbed onto a mica substrate. The individual DNA strands are clearly visible against the mica background. Images were acquired on a BioScope Catalyst AFM operated in PeakForce Tapping in buffer solution using ScanAsyst Fluid+ AFM probes (k ~0.7N/m). Image XY-Scale = 2µm.

and outlet ports allow for convenient fluid exchange while the 60 microliter total volume of the flow cell minimizes the amount of fluid and sample needed. This is often an important consideration when using limited or expensive reagents, such as DNA and proteins/enzymes.

The recent release of PeakForce Tapping™ and ScanAsyst™ operating modes has further enhanced the ease of high-resolution imaging in fluid with Catalyst. ScanAsyst's self-optimization imaging capabilities, its ability to maintain very low imaging forces (<100 piconewtons), as well as its removal of the need to determine cantilever resonance (cantilever tuning), combine to enable researchers to routinely achieve high-resolution images of their samples with consistent results. Figure 3 shows a height image of Lambda DNA obtained using PeakForce Tapping Mode on the BioScope Catalyst. The use of very sharp probes (radius of curvature ~2 nanometers) coupled with the ability to maintain optimal surface tracking over extended periods of time in fluid, not only provides routine images of DNA strands with typically ≤6 nanometers diameter but also opens up opportunities for the observation of time-dependent dynamics without loss of resolution.

Proteins

While DNA is the molecule of life, the genetic code that it makes up allows for the synthesis of numerous protein molecules necessary to sustain life. Proteins not only exist as single molecules, they also assemble with other proteins and molecules to form complexes that play important roles in the regulation of biological processes. The direct observation of proteins and their self-assembly by AFM can offer unique insights into biomolecular structure-function relationships.

Viruses are infectious agents that spread by infecting host bacterial or eukaryotic cells. Outside of a cell, a virus exists as a protein shell, also known as a capsid, which encapsulates the viral DNA or RNA (ribonucleic acid). This structure acts to protect the virus until it can find a host. Once a virus has successfully infected a host cell, this DNA/RNA is released and the virus uses the cell's own DNA/RNA replicating mechanisms to create more virus particles. These new viruses then infect other host cells and the infection spreads.
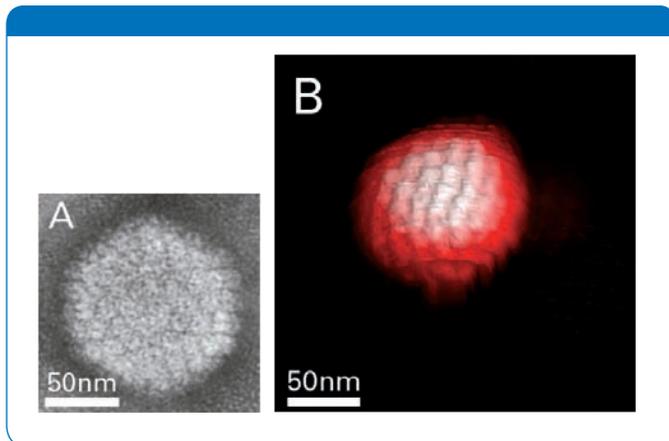
Figure 4. (A) Transmission electron micrograph of a Herpes Simplex Virus capsid. Image courtesy of Wouter Roos, Vrije Universiteit, Amsterdam, Netherlands (Reprinted with permission. Source: Roos et al., Proc. Natl. Acad. Sci. USA, 2009, Vol. 106, 9673-78) (B) A 250nm AFM Topography image of a single herpes simplex virus capsid. The arrangement of protein molecules as 3-dimensional subunits on the surface of the capsid, known as capsomeres, is clearly visible in the AFM image. AFM images were obtained on the BioScope Catalyst operated in PeakForce Tapping mode in buffer conditions and using ScanAsyst Fluid+ AFM probes (k ~0.7N/m). Sample courtesy of Wouter Roos and Gijs Wuite, Vrije Universiteit, Amsterdam, Netherlands.

Viruses can cause any number of diseases. The key to treating and preventing these diseases is in understanding the different processes of how viruses infect host cells. An important aspect of this is the identification and classification of viruses based on similar structure or function. For example, viruses are classified into families based on their capsid structure, which is determined by the arrangement of the protein molecules that form the capsid architecture. Researchers have visualized the structure of viral capsids using a variety of techniques, including x-ray crystallography, nuclear magnetic resonance (NMR) imaging, and transmission and scanning electron microscopy (see figure 4A). While these techniques provide valuable structural information, they often require complex sample preparation with coating, staining or crystallization of the virus particles. AFM imaging, however, requires minimal sample preparation and the imaging is performed on the unaltered, native capsid surface.[22-24] Figure 4B shows the structure of a single Herpes Simplex Virus particle obtained with the BioScope Catalyst. The arrangement of protein molecules as three-dimensional subunits on the surface of the capsid, also known as capsomeres, is clearly visible in the AFM image.
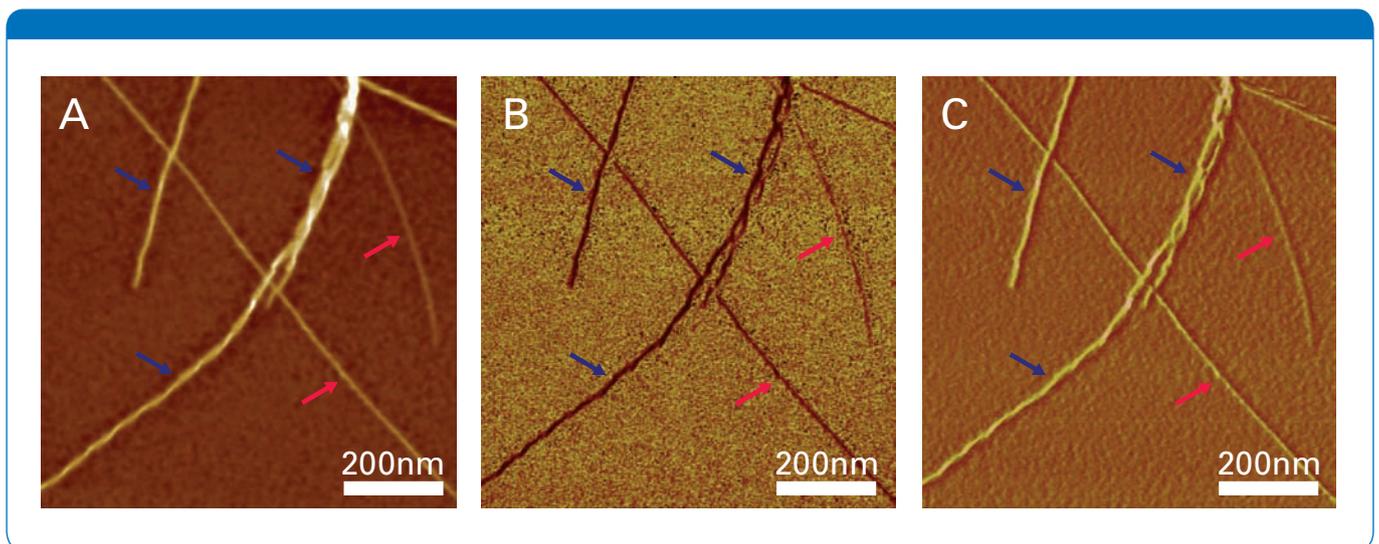
Figure 5. PeakForce QNM images of amyloid fibers adsorbed onto a freshly cleaved mica surface. (A) Topography images reveal some of the fibers to have a twisted structure (blue arrows) while others do not (red arrows). (B) The Modulus data and the (C) Deformation data channels are obtained simultaneously to the topography image. These images indicate the amyloid to have a lower modulus (darker color scale) and correspondingly, a higher degree of deformation (lighter color scale) than the underlying mica substrate. It is also observed that the twisted amyloid fibers have a slightly lower modulus value as compared to those fibers that are not twisted (i.e. twisted fibers appear slight darker than the straight fibers in the modulus image). Images were obtained on a BioScope Catalyst operated in PeakForce Tapping mode using ScanAsyst AFM probes (k ~0.4N/m). Sample courtesy of Xingfei Zhou, Ningbo University, China.

Figure 6. Setup of the BioScope Catalyst Perfusion Stage Incubator (PSI). (1) The PSI supports standard glass bottom petri dishes for compatibility with high NA objectives. (2) The flow diffuser directs liquid flow in laminar fashion across the sample area, ensuring even fluid exchange and isolating noise from the liquid inlet and outlet. (3) The perfusion clamp stabilizes the petri dish and contained stainless steel inlet and outlet tubes that are in thermal contact with the heating stage to preheat the incoming liquid and gas. (4) A silicone baffle seals between the petri dish and probe holder, reducing evaporation and allowing control of the gas space above the liquid. (5) The specialized PSI probe holder contains a temperature sensor for local monitoring of temperature. The right-side image shows the PSI fully assembled together with the sample heating stage on the BioScope Catalyst AFM.

While self-assembly and association with other molecules is most often the desired behavior of proteins required for the progression of biological signals and processes, there are often instances where this is not the case. Abnormal protein assembly and aggregation of proteins in the brain is associated with the development of various neurodegenerative diseases, such as Alzheimer's, Parkinson's, and prion diseases. In Alzheimer's disease, this non-native self-assembly of the typically soluble amyloid-beta (A-β) protein results in the formation of plaques, called amyloid. As these amyloid plaques accumulate in the brain they become toxic, eventually leading to cell degeneration and the symptomatic progression of Alzheimer's disease.

The goal of Alzheimer's research is not only to understand the pathogenesis of the disease, but also to provide insights into treating and preventing Alzheimer's and other related neurodegenerative diseases. While the formation and structure of A-β fibers has been extensively studied using a variety of analytical techniques, findings must

often be interpreted carefully based on the type of sample preparation required for the technique used (coating, dehydration, etc.), which may influence the results. AFM not only allows researchers to conduct studies in fluid under near-physiological conditions, it also allows them to investigate and determine, in situ, the conditions under which soluble, monomeric A-β proteins may be prone to fiber formation.[25-27] Figure 5 shows AFM images obtained using PeakForce QNM™ (Quantitative Nano-Mechanical) imaging on the BioScope Catalyst of A-β fibers deposited onto a mica surface. Interestingly, AFM imaging revealed some of the fibers to have a twisted structure while others did not. PeakForce QNM imaging of the fibers not only provides details as to the structure of the fibers (topography, see figure 5A), it can also reveal important information on their nanomechanical properties. PeakForce QNM images include individual data channels, such as modulus, adhesion, deformation, and dissipation, that correlate directly to the simultaneously obtained topography data channel. Using

a calibrated AFM probe, researchers can easily obtain quantitative measurements of these mechanical properties as a function of spatial location across the surface of their sample. Not only are these data channels quantitative, they are acquired at the typical pixel density resolution required for high-resolution imaging. Figure 5B reveals the fibers to have a lower modulus as compared to the underlying mica surface. When comparing the two different fiber structures, the twisted fibers also appear to have a slightly lower modulus than the straight fibers. Figure 5C also shows the amyloid fibers to undergo larger deformation relative to the mica surface, which is reflected in the softer modulus measurements of the fibers.

Researchers are often interested in observing the interaction and effects of these amyloid proteins on live cells.[28-29] The BioScope Catalyst Perfusion Stage Incubator (PSI) provides the necessary environmental control to enable long duration live cell experiments required for this type of study. The PSI, integrated with the Catalyst heating stage, maintains ideal cell culture conditions, including temperature and media/fluid and gas perfusion (see figure 6). The PSI is fully integrated with the Catalyst system, allowing for uncompromised optical imaging using all the system's imaging modes (brightfield, phase contrast, DIC, epifluorescence, CLSM, etc). Using the combination of the

Catalyst AFM, the PSI accessory, and MIRO (Microscopy Image Registration and Overlay) software, which allows optical images to be registered to and overlaid with AFM images and to use these optical images to navigate the AFM probe, researchers can perform true correlated optical and AFM imaging or AFM force measurements to examine any changes in cell structure or mechanical properties in response to amyloid fiber formation.

Membranes and Membrane Proteins

Cell membranes surround cells, physically separating the intracellular components from the extracellular environment. Cell membranes are involved in many important functions, including anchoring the cytoskeleton and providing shape to the cell, as well as facilitating the transport of nutrients and materials needed for survival in- and outside of the cell. The movement of substances across the cell membrane is facilitated by various protein molecules embedded within or associated with the membrane. This movement may be triggered as a result of a response to some chemical or ion gradient, or in response to the binding of a molecule to the extracellular portion of a membrane protein, causing a conformational change in the protein structure and the opening of a channel in the membrane to the inside of the cell.
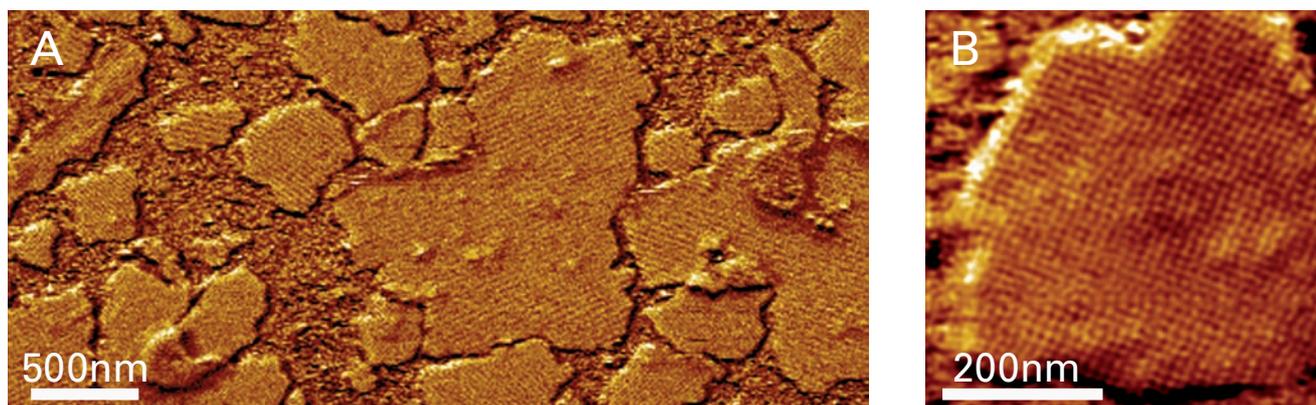
Figure 7. (A) AFM phase image of bacterial S-layers from E. coli. The bacterial membranes were excised from the cells and the membrane patches immobilized onto a freshly cleaved mica surface. The lattice pattern formed by the S-layer proteins is clearly evident in the phase image and is observed to have a periodicity of ~18nm. (B) High-resolution image of the S-layer lattice periodicity observed on a single membrane patch. Images were obtained on a BioScope Catalyst operated in TappingMode in buffer conditions using SNL AFM probes (k ~0.32N/m). Sample courtesy of Hans Oberleithner, Institute for Physiology II, University of Muenster, Germany.

Studying the structure of membrane proteins has presented researchers with quite a challenge. The majority of techniques available have been developed and applied to soluble proteins. However, unlike soluble proteins, membrane proteins contain hydrophobic domains that span the membrane. As a result, the solubilization of the protein from the cell membrane required for most analytical techniques, often leads to changes in the protein's native structure in an attempt to shield the hydrophobic domain(s) from the surrounding aqueous environment. It is this instability outside the membrane that has limited the structural and functional studies of membrane proteins. AFM imaging, however, not only allows for examination of membrane proteins within their native membrane environment, studies can also be conducted in fluid, under near-physiological conditions, providing researchers the unique opportunity to observe membrane protein dynamics. While AFM is capable of imaging live cells, high-resolution imaging of the structure of cell membranes and the organization of membrane-associating proteins is typically conducted on either excised cell membrane patches deposited onto an atomically flat substrate (e.g., mica) or supported planar lipid bilayers in which the proteins of interest have been reconstituted.[36,37]

Figure 7a is an image of bacterial membrane patches adsorbed onto a mica surface. High-resolution imaging of individual membrane patches (figure 7b) reveals the organization of membrane-associated proteins as a two-dimensional crystalline lattice structure, known as an S-layer. S-layer proteins are associated with the surface of the cell membrane. While the S-layer plays a role in various functions of the cell, as the outermost layer of bacteria and archaea, its essential function is the mechanical and chemical protection of the cell.[38] In the AFM image, the lateral spacing of the individual protein molecules in the S-layer is ~18 nanometers. The small lattice periodicities of S-layers have been shown to be attractive for biomimetic or nanotechnology applications, such as the immobilization matrices for the templating of biomolecular arrays or nanoparticles required for nanoelectronics and nonlinear optics.[39,40]

Conclusions

AFM provides many advantages for high-resolution studies of single biomolecules. With a high signal-to-noise ratio, the elimination of the need for coating, staining, or crystallization of a sample, and the ability to operate in fluid, today's high-performance life science AFM facilitates the study of biomolecules under near-physiological conditions in real-time and in situ. From single molecules to live cells, the BioScope Catalyst provides high-resolution imaging of biological samples across all biomolecular size scales. The mechanical stability of the Catalyst when integrated with an inverted optical microscope allows researchers to take advantage of many of the features of this AFM, including PeakForce Tapping, ScanAsyst, and PeakForce QNM imaging modes, the small volume flow cell, MIRO software capabilities, as well as the environmental control afforded by the sample stage heater and Perfusion Stage Incubator (PSI), which together open the door to a wide-range of unique high-resolution imaging experiments.

References

1. A. Berquand, A. Holloschi, and P. Kioschis, "Characterizing the Effect of Anticytoskeletal Drugs on Living Cells Using MIRO Software and the BioScope Catalyst AFM," Bruker Application Note AN125 (2010).

2. F. Lafont and A. Berquand, "Atomic Force Microscopy with BioScope II: Detecting Specific Ligand-Receptor Interactions on Live Cancer Cells in situ," Bruker Application Note AN111 (2008).

3. A. Berquand, A. Holloschi, S. Ritz, M. Hafner, and P. Kioschis, "AFM and Neurodegenerative Diseases (Part I): Correlating Atomic Force Microscopy (AFM) and Fluorescence Microscopy to Detect Changes in Cell Morphology Caused by Protein Aggregates of Mutant Huntingtin," Bruker Application Note AN117 (2008).

4. A. Berquand, S. Ritz, A. Holloschi, M. Hafner, and P. Kioschis, "AFM and Neurodegenerative Diseases (Part II): Correlating Atomic Force Microscopy (AFM) and Fluorescence Microscopy to Study the Interaction between Dopamine and the D1-Receptor in SH-SY5Y Cells," Bruker Application Note AN118 (2008).

5. A.L. Weisenhorn, H.E. Gaub, and H.G. Hansma, "Imaging Single-Stranded DNA, Antigen-Antibody Reaction and Polymerizes Langmuir-Blodgett Films with an Atomic Force Microscope," Scanning Microscopy 4: 511 -16 (1990).

6. H.G. Hansma, J. Vesenka, C. Siegerist, G. Kelderman, H. Morett, R.L. Sinsheimer, V. Elings, C. Bustamante, and P.K. Hansma, "Reproducible Imaging and Dissection of Plasmid DNA Under Liquid with the Atomic Force Microscope," Science 256: 1180 -84 (1992).

7. Y. Lyubchenko, L. Shlyakhtenko, R. Harrington, P. Oden, and S. Lindsay, "Atomic Force Microscopy of Long DNA: Imaging in Air and Under Water," Proc. Natl. Acad. Sci. USA 90: 2137 -40 (1993).

8. H.G. Hansma, D.E. Laney, M. Bezanilla, and R.L. Sinsheimer, "Applications for Atomic Force Microscopy of DNA," Biophys. J. 68: 1672 -77 (1995).

9. D. Pastre, O. Pietrement, S. Fusil, F. Landousy, J. Jeusset, M-O. David, L. Hamon, E. Le Cam, and A. Zozime, "Adsoprtion of DNA to Mica Mediated by Divalent Counterions: A Theoretical and Experimental Study," Biophys. J. 85: 2507 -18 (2003).

10. J. Vesenka, M. Guthold, C. L. Tang, D. Keller, E. Delain, and C. Bustamante, "Substrate Preparation for Reliable Imaging of DNA Molecules with the Scanning Force Microscope," Ultramicroscopy 42 -44: 1243 -49 (1992).

11. M. Bezanilla, B. Drake, E. Nudler, M. Kashlev, P.K. Hansma, and H.G. Hansma, "Motion and Enzymatic Degradation of DNA in the Atomic Force Microscope," Biophys. J. 67: 2454 -59 (1994).

12. N.H. Thomson, S. Kasas, B.L. Smith, H.G. Hansma, and P.K. Hansma, "Reversible Binding of DNA to Mica for AFM Imaging," Langmuir 12: 5905 -08 (1996).

13. Y. Jiao, D.I, Cherny, G. Heim, T.M. Jovin, and T.E. Schaffer, "Dynamic Interactions of p53 with DNA in Solution by Time-Lapse Atomic Force Microscopy," J. Mol. Biol. 314: 233 -43 (2001).

14. Y.L. Lyubchenko, A.A. Gall, L.S. Shlyakhtenko, R.E. Harringtion, P.I. Oden, B.L. Jacobs, and S.M. Lindsay, "Atomic Force Microscopy Imaging of Double Stranded DNA and RNA," J. Biomolec. Struc, Dyn. 9: 589 -606 (1992).

15. Y.L. Lyubchenko, B.L. Jacobs, and S. M. Lindsay, "Atomic Force Microscopy of Reovirus dsRNA: A Routine Technique for Length Measurements," Nucl. Acids Res. 20: 3983 -86 (1992).

16. Y.L. Lyubchenko and S.M. Lindsay, "DNA, RNA, and Nucleoprotein Complexes Immobilized on AP-Mica and Imaged with AFM," Procedures in Scanning Probe Microscopy, Ed. R.J. Colton., J. Wiley & Sons, Ltd: 493 -96 (1998).

17. S.J.T. van Noort, K.O. van der Werf, A.P.M. Eker, C. Wyman, B.G. de Grooth, N.F. Van Hulst, and J. Greve, "Direct Visualization of Dynamic Protein-DNA Interactions with a Dedicated Atomic Force Microscope," Biophys. J. 74: 2840 -49 (1998).

18. M. Guthold, X. Zhu, C. Rivettis, G.Yang, N.H. Thomson, S. Kasas, H.G. Hansma, B. Smith, P.K. Hansma, and C. Bustamante, "Direct Observation of One-Dimensional Diffusion and Transciption by Escherichia coli RNA Polymerase," Biophys. J. 77: 2284 -94 (1999).

19. F. Landousy and E. Le Cam, "Probing DNA-Protein Interactions with Atomic Force Microscopy," Bruker Application Note AN89 (2006).

20. Y.L. Lyubchenko and, L.S. Shlyakhtenko, "AFM for Analysis of Structure and Dynamics of DNA and Protein-DNA Complexes," Methods 47: 206 -13 (2009).

21. D. Pastre, L. Hamon, I. Sorel, E. Le Cam, P.A. Curmi, and O. Pietrement, "Specific DNA-Protein Interactions on Mica Investigated by Atomic Force Microscopy," Langmuir 26: 2618 -23 (2010).

22. Y.G. Kuznetsov, A.J. Malkin, R.W. Lucas, M. Plomp, and A. McPherson, "Imaging of Viruses by Atomic Force Microscopy," J. Gen. Virol. 82: 2025 -34 (2001).

23. A.J. Malkin, A. McPherson, and P.D. Gershon, "Structure of Intracellular Vaccinia Virus Visualized by In Situ Atomic Force Microscopy," J. Virol. 77: 6332 -40 (2003).

24. W.H. Roos, K. Radtke, E. Kniesmeijer, H. Geertsema, B. Sodeik, and G.J.L. Wuite, "Scaffold Expulsion and Genome Packaging Trigger Stabilization of Herpes Simplex Virus Capsids," Proc. Natl. Acad. Sci. USA 106: 9673 -78 (2009).

25. T. Kowalewski and D. Holtzman, "In Situ Atomic Force Microscopy Study of Alzheimer's b-Amyloid Peptide on Different Substrates: New Insights into Mechanism of b-Sheet Formation," Proc. Natl. Acad. Sci. USA 96: 3688 -93 (1999).

26. H.K.L. Blackley, G.H.W. Sanders, M.C. Davies, C.J. Roberts, S.J.B. Tendler, and M.J. Wilkinson, "In-situ Atomic Force Microscopy Study of b-Amyloid Fibrillization," J. Mol Biol. 298: 833 -40 (2000).

27. C.M. Yip and J. McLaurin, "Amyloid-b Peptide Assembly: A Critical Step in Fibrillogenesis and Membrane Disruption," Biophys. J. 80: 1359 -71 (2001).

28. I. Peters, U. Igbavboa, T. Schutt, S. Haidari, U. Hartig, S. Bottner, E. Copanaki, T. Deller, D. Kogel, W.G. Wood, W.E. Muller, and G.P. Eckert, "The interaction of beta-amyloid protein with cellular membranes stimulates its own production," Biochim. Biophys. Acta. 1788: 964 -72 (2009).

29. D.A. Bateman and A. Chakrabartty, "Cell Surface Binding and Internalization of A Modulated by Degree of Aggregation", Int. J. Alzheimers Dis., In Press (2011).

30. C. Moller, M. Allen, V. Elings, A. Engel, and D. J. Mueller, "Tapping-Mode Atomic Force Microscopy Produces Faithful High-Resolution Images of Protein Surfaces," Biophys. J. 77: 1150 -58 (1999).

31. S. Scheuring, F. Reiss-Husson, A. Engel, J-L. Rigaud, and J-L. Ranck, "High-Resolution AFM Topographs of Rubrivivax gelatinosus Light-Harvesting Complex LH2," EMBO J. 20: 3029 -35 (2001).

32. A. Slade, J. Luh, S. Ho, and C.M. Yip, "Single Molecule Imaging of Supported Planar Lipid Bilayer-Reconstituted Human Insulin Receptors by In Situ Scanning Probe Microscopy," J. Struc. Biol. 137: 283 -91 (2002).

33. A. Philippsen, W. Im, A. Engel, T. Schirmer, B. Roux, D.J. Mueller, "Imaging the Electrostatic Potential of Transmembrane Channels: Atomic Probe Microscopy of OmpF Porin," Biophys. J. 82: 1667 -76 (2002).

34. D.J. Mueller and A. Engel, "Atomic Force Microscopy and Spectroscopy of Native Membrane Proteins," Nature Protocols 2: 2191 -97 (2007).

35. B. Seantier, M. Dezi, F. Gubellini, A. Berquand, C. Godefroy, P. Dosset, D. Levy, and P-E. Milhiet, "Transfer on Hydrophobic Substrates and AFM Imaging of Membrane Proteins Reconstituted in Planar Lipid Bilayers," J. Mol. Recog. In Press (2011).

36. D.J. Mueller, J.B. Heymann, F. Oesterhelt, C. Moller, H. Gaub, G. Buldt, and A. Engel, "Atomic Force Microscopy of Native Purple Membrane," Biochim. Biophys. Acta 1460: 27 -38 (2000).

37. D. Fotiadis and A. Engel, "High-Resolution Imaging of Bacteriorhodopsin by Atomic Force Microscopy," Methods Mol. Biol. 242: 291 -303 (2004).

38. G. Seltmann and O. Holst, "Chapter 6: Components Outside the Cell Wall," The Bacterial Cell Wall, Springer-Verlag (2002).

39. U.B. Sleytr and T.J. Beveridge, "Bacterial S-layers," Trends Microbiol. 7: 253 -60 (1999).

40. U.B. Sleytr, C. Huber, N. Ilk, D. Pum, B. Schuster, and E.M. Egelseer, "S-Layers as a Tool Kit for Nanobiotechnological Applications," FEMS Microbiol. Lett. 267: 131 -44 (2007).

Authors

Andrea L. Slade, Bruker Nano Surfaces Division, (andrea.slade@bruker-nano.com)

Alexandre Berquand, Bruker Nano Surfaces Division (alexandre.berquand@bruker-nano.com)

Peter DeWolf, Bruker Nano Surfaces Division (peter.dewolf@bruker-nano.com)

Bruker Nano Surfaces Division

Santa Barbara, CA · USA
+1.805.967.1400/800.873.9750
productinfo@bruker-nano.com

www.bruker.com