

UNZIPPING A MEMBRANE

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The atomic force microscope (AFM) is well known for its outstanding spatial resolution, but it is becoming increasingly useful as the instrument for force spectroscopy. In the force spectroscopy mode, the AFM can measure tiny tension forces, in the piconewton (pN) range. Daniel Müller, Wolfgang Baumeister, and Andreas Engel have used the AFM in both the imaging and force spectroscopy modes to pull proteins out of membranes in a controlled fashion.²

Müller *et al.* used *Deinococcus radiodurans*, a bacterium best known for its high resistance to radiation (as its Genus name implies), as their test subject. They extracted a highly regular membrane from the bacterium, the hexagonally packed intermediate (HPI) layer. They mounted the HPI on mica, so that the hydrophilic outer surface of the HPI adsorbed strongly to the mica, exposing the hydrophobic inner surface to the silicon nitride AFM stylus. With the AFM in the contact imaging mode, they could clearly visualize what looked like a closely-packed group of circular pies, each pie cut into six equal pieces called protomers. At the center of the pie was a pore, whose diameter was controlled by the arrangement of the protomers.

After imaging the HPI with the AFM, Müller *et al.* brought the AFM stylus into contact with the specimen for one second, allowing the stylus to contact the protomers on the inner layer of the HPI. When the stylus stuck to a protomer on the inner surface, and the stylus was withdrawn, interesting measurements were made. In most cases, the force spectrograph showed a deflection of the stylus indicating a bond until the stylus was about 10 nm from the surface where the stylus sprang back indicating the bond had ruptured. To use our analogy, a piece of pie was picked up, but was dropped back into the pie before we got very far. In the remaining cases, three different spectro-

graphs were seen. In the first case, a force peak of about 90 pN was seen after retracting the stylus about 15 nm, indicating that a molecular bridge was extended from the stylus to the surface. This is like a small thread was present that pulled our piece of pie back into the pie. In the second case, a force peak closer to 300 pN was measured, about three times higher than the first case when the protomer detached from the stylus and was pulled back into the hexagon by the molecular bridge. After this measurement, the follow up image made with the AFM showed a protomer was missing (follow up images after the other measurements did not show any changes). The thread connected to our piece of pie had been broken, and the piece was extracted from the pie. But the best is yet to come.

The third type of force spectrograph is the most amazing. There were up to six equally spaced peaks in the 200-300 pN range, with the peak-to-peak distance corresponding to about 7.3 nm. So we have picked up a piece of pie to find each piece of pie threaded together, occasionally allowing us to pick up the entire pie, piece by piece. Müller *et al.* referred to this as "controlled unzipping." This demonstrates the ability of the AFM to not only measure intermolecular forces, but to also directly visualize and correlate the resulting structural changes.

With current techniques it is difficult to study the structure of membrane proteins. The novel approach demonstrated by Müller *et al.* greatly increases our ability to examine the structural features of membrane proteins *in situ*. It will not be long until the single molecule AFM technique demonstrated in this article can monitor the conformational changes of ion channels and receptors during their physiological activity. ■

1. The authors gratefully acknowledge Dr. Andreas Engel for reviewing this article.
2. Müller, D.J., W. Baumeister, and A. Engel, Controlled unzipping of a bacterial surface layer with atomic force microscopy, *Proc. Nat. Acad. Sci. USA* 96:13170-13174, 1999.

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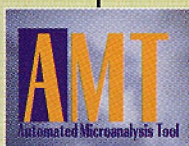
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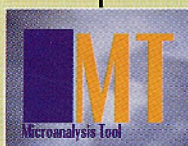
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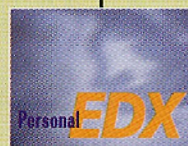
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The RMC Electron Microscopy product line has been acquired by Boeckeler Instruments, Inc., a local Tucson high technology company, from Ventana Medical Systems.

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MME recently conducted a mail-back study of 5000 materials scientists involved in semiconductor and industrial materials applications. With 2.5% responding (well within the expected 2-3% response rate), and an error range of approximately +/- 6%, Microscopy Today was tied for second place (within error rate) with the prestigious R&D as the publication on which these scientists relied most heavily when evaluating equipment for purchase.

Since ASM International, publishers of Advanced Materials and Processes, provided the list, the data contained an expected bias toward that publication.

Key rankings were as follows:

- Advanced Materials & Processes: 45%
- R&D: 27%
- Microscopy Today: 21%
- Microscopy & Microanalysis (MSA Journal): 11%
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- Barbara Foster, President,
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 - ✓ April 16/20: Electronic Imaging
 - ✓ April 23/27: Scanning Electron Microscopy
 - ✓ May 30/June 1: Fluorescence Microscopy
 - ✓ Sept. 5/7: Microtomy
 - ✓ Sept. 10/14: Advanced FTIR Microscopy
 - ✓ Sept. 24/26: Caking of Crystals
 - ✓ Nov. 5/9: Particle Isolation, Manipulation and mounting for Additional Analysis
 - ✓ Nov. 12/16: Scanning Electron Microscopy
- For further information, contact Ms. Nancy Daerr: (312)842-7100, Fax: (312)842-1078, eMail: ndaerr@mcri.org

✓ November 27/December '00: **Scanning Electron Microscopy** (McCrone Research Institute) Chicago, IL, Nancy Daerr, (312)842-7100, ndaerr@mcri.org

✓ December 9/13 '00: **40th Annual Meeting American Society for Cell Biology** San Francisco, CA. www.ascb.org/ascb

✓ January 20/26 '01: **Multiphoton Microscopy in the Biomedical Sciences** (SPIE) San Jose, CA. http://spie.org/web/meetings/calls/pw01/conf/BO31.html

✓ February 25/28 '01: **Contact Mechanics in Adhesion Science** (The Adhesion Society) Williamsburg, VA, www.adhesionsociety.org/

✓ March 4/9 '01: **PITTCON 2001**, New Orleans, LA. www.pittcon.com

✓ April 1/4 '01: **Focus On Microscopy 2001**, University of Amsterdam, The Netherlands. www.focusonmicroscopy.org/

✓ May 5/7 '01: **SCANNING 2001: (FAMS)** New York City. Mary K. Sullivan : (201)818-1010, scanning@fams.org

✓ May 6/10 '01: **Modern Developments and Applications in Microbeam Analysis (EMAS)**, Tampere, Finland, www.emas.ac.uk/

✓ May 13/16 '01: **Food Structure & Functionality Symposium 2001**, Minneapolis, MN. eMail: meeting@AOCS.org

✓ May 21/25 '01: **FE-SEM and Image Analysis Courses** Montreal, Canada. Dr. Pierre Hovington: (450)652-8125, eMail: hovington.pierre@ireq.ca

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 - ✓ June 10, '01: Introduction to SEM and EDS
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- For further information, contact Ms. Sharon Coe at: tel.: (610)758-5133, fax: (610)758-4244, eMail: sharon.coe@lehigh.edu
- ✓ June 18/21 '01: **17th Annual Short Course on Molecular Microspectroscopy** (Miami University) Oxford OH. (513)529-2874
 - ✓ July 2/6 '01: **16th International Conference on X-ray Optics and Microanalysis (ICXOM XVI)**, Vienna, Austria, www.icxom.at
 - ✓ Sept. 20/25 '01: **5th Multinational Congress On Electron Microscopy**. Lecce, Italy. Dr. Massimo Catalano, email: massimo.catalano@ime.le.cnr.it
 - ✓ Sept.30/Oct.4 '01: **2001 Eastern Analytical Symposium**, Atlantic City, NJ, http://www.eas.org
 - ✓ August 4/9 '02: **Microscopy & Microanalysis 2002 (MSA/MAS)** Quebec City, Canada. http://msc.rsvs.ulaval.ca
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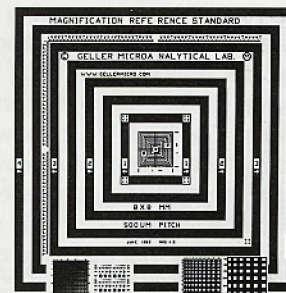
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