Probing Individual Proteins in Unsupported Membranes
Stephen W. Carmichael

Proteins in biologic membranes perform a large variety of essential functions. The fact that about one third of all genes code for membrane proteins, and that the majority of drugs target these proteins, attest to that fact. However, until now, proteins have been studied under artificial conditions, such as after being crystallized, frozen, or adsorbed to a substrate. Rui Pedro Gonçalves, Guillaume Agnus, Pierre Sens, Christine Houssin, Bernard Bartenlian, and Simon Scheuring have devised a novel setup with the atomic force microscope (AFM) to allow proteins to be probed while they are in unsupported membranes. Their method is similar in principle to methods where a small area of a membrane is sampled, such as when a piece of membrane is examined by patch clamping. This provided “viewing holes” in the range of 90 nm to 250 nm. With the AFM, the membranes could be imaged and manipulated while an aqueous buffer medium at ambient pressure and temperature.

Gonçalves et al. chose the surface membrane of Corynebacterium glutamicum as their model for imaging studies because this membrane has been so well characterized in many different ways. Patches of membranes could be clearly seen to be spanning holes on the nano-patterned Si(001) support, yet proteins within patches were considerably smaller than the holes. Using a specific AFM mode, they visualized the subunit architecture of the proteins. Such structural details as a pore about 15Å in diameter, corresponding to a channel pore, could be clearly seen. In addition to imaging, force measurements could also be made on the unsupported membranes. Specifically, they could analyze forces as the AFM tip made contact with the membrane and when it pierced the membrane. Measuring forces between these two events provided interesting information on the lateral interaction force between proteins within the membrane that suggested fairly weak protein-protein interactions reinforcing the importance of cooperative interactions.

Using Halobactrium salinarium purple membranes, known to contain proton pumps, Gonçalves et al. isolated wells of about 10 attoliters beneath suspended membranes. These wells were primed with the pH-sensitive fluorescent probe pyranine. Using quantitative fluorescence microscopy, they were able to demonstrate a significant increase in pH under appropriate circumstances. This was clear evidence that the pumping of protons was being directly observed.

This novel technique is an extension of several methods for imaging and manipulating individual membrane proteins as reviewed by Gonçalves and Scheuring. The key to their new method is that a membrane is suspended over a very small well. As techniques to manipulate fluids on a nano-scale are devised to mimic events occurring within cells, new methods can be developed to measure these events. Also, as AFMs become faster and more sensitive at acquiring images, these observations can be made at low physiologic levels and with better spatial and temporal resolution. We will be able to, in effect, see and measure what membrane proteins realistically do within cells with more precision than has ever been possible!

1. The author gratefully acknowledges Dr. Simon Scheuring for reviewing this article.

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ABOUT THE COVER

This composite image was taken by the navigation camera during the close approach phase of Stardust’s Jan 2, 2004 flyby of comet Wild 2. Several large depressed regions can be seen. Comet Wild 2 is about five kilometers (3.1 miles) in diameter. To create this image, a short exposure image showing tremendous surface detail was overlain on a long exposure image taken just 10 seconds later showing jets. Together, the images show an intensely active surface, jetting dust and gas streams into space and leaving a trail millions of kilometers long. See the article by Rietmeijer starting on page 6. Image credit: NASA/IPL-Caltech.

Interested readers may want to Google “wild 2 comet pictures” to find many more images of Wild 2, including stereo pairs.

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2007
✓ Microscopy and Microanalysis 2007
  August 5-9, 2007, Fort Lauderdale, FL
  mm2007.microscopy.org
✓ Cryo Technique and Immunogold labelling Workshop
  August 12-16, 2007, Emory University, Atlanta, GA
  hyi@emory.edu
  September 3-7, 2007, Glasgow, Scotland
  www.iop.org/Conferences... (search ‘forthcoming’ then date)
✓ EUROMAT 2007
  September 10-13, 2007, Nürnberg, Germany
  www.euromat2007.fems.org/
✓ Course: Live-cell imaging, FRET, FRAP and Spectral Unmixing
  September 18-21, 2007, Univ. of York, UK
  www.york.ac.uk/depts/biol/tf/imaging_course.htm
✓ FEMMS 2007 - Frontiers of E.M. in Material Science
  September 23-27, 2007, Sonoma, CA
  femms2007.llnl.gov/index.html
✓ Optical Microscopy and Imaging in the Biomedical Sciences
  October 9-18, 2007, MBL, Woods Hole, MA
  www.mbl.edu/education/courses/special_topics/om.html
✓ AVS 54th Symposium and Exhibition,
  (In-situ e.m. symp.)
  October 14-19, 2007, Seattle, WA
  www.avstoday.org
✓ Neuroscience 2007
  November 3-7, 2007, San Diego, CA
  www.sfn.org/index.cfm
✓ The American Society for Cell Biology
  December 1-5, 2007, Washington, DC
  www.ascb.org

2008
✓ ACCM-20 & IIUMAS IV
  February 10-15, 2008, Perth, Australia
  microscopey.org.au/ACCM20/
✓ PITTCON 2008
  March 6-9, 2008, New Orleans, LA
  www.pittcon.org
✓ American Soc. for Biochemistry and Molecular Biology
  April 3-9, 2008, San Diego, CA
  www.asmb.org
✓ Scanning 2008
  April 14-16, 2008, Washington, DC
  www.fams.org
✓ Lehigh Microscopy School
  June 3-15, 2008, Bethlehem, PA
  www.lehighmicroscopy.org
✓ Microscopy and Microanalysis 2008
  August 3-7, 2008, Albuquerque, NM
  www.msa.microscopy.com
✓ 14th Electron Microscopy Congress, EMC 2008
  September 1-5, 2008, Aachen, Germany
  www.eurmic soc.org/emc2008.html

2009
✓ Microscopy and Microanalysis 2009
  August 3-6, 2009, Baltimore, MD
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Please check the “Calendar of Meetings and Courses” in the MSA journal “Microscopy and Microanalysis” for more details and a much larger listing of meetings and courses.

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