MICROSCOPY TODAY

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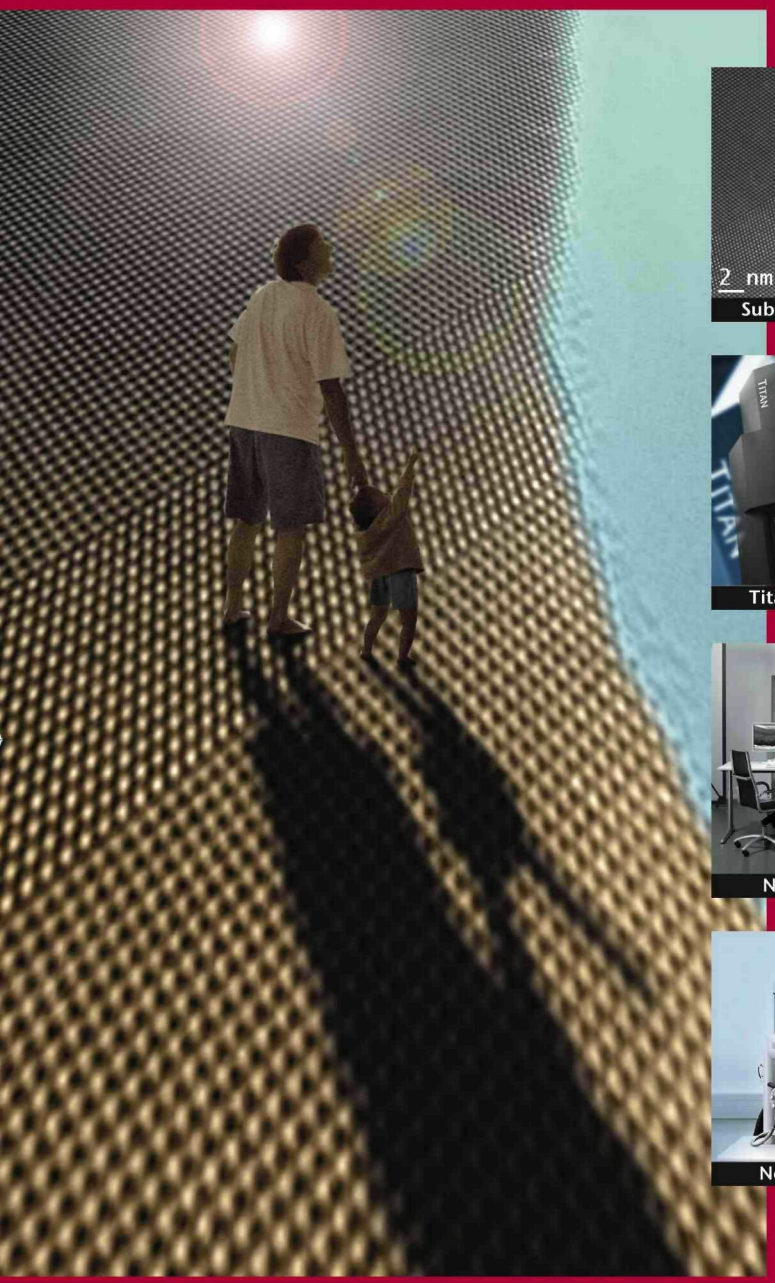
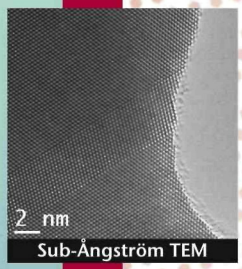
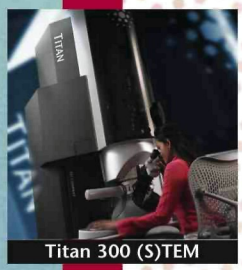
Explore, Discover, Build.



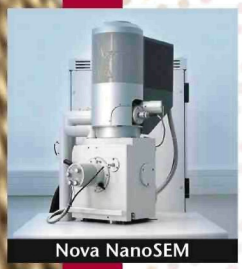


Sub-Ångström TEM



Titan 300 (S)TEM


Nova NanaLab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Getting the Green Light to Measure Small Forces

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Green fluorescent protein (GFP) and related molecules have been important signal molecules for cell biologists because they emit a bright signal to mark the position of tagged structures as well as changes in calcium and proton concentrations. Related molecules even emit in different colors. Now Hendrik Dietz and Matthias Rief from the Technical University of Munich have given us a detailed look at the "energy landscape" of GFP with the promise that it may also be useful indicator for forces.[2] In this context, energy landscape refers to a three-dimensional map of the energy levels of the molecule as it is transitioned from its native state to an unfolded state.

Dietz and Rief used the atomic force microscope (AFM) to conduct force spectroscopy studies of single molecules. In order to grab on the N- and C-termini they selected a certain type of GFP and inserted it into two well-characterized proteins of modular quartary structure, one representing a region of titin, a molecule from human heart muscle and the other an actin-crosslinker protein from *Dictyostelium discoidem*. The modified chimeric molecules showed typical GFP fluorescence. One terminus of such a molecule was then attached to a gold substrate, the other to the gold-coated cantilever tip. As the molecule was stretched, force-extension traces were generated.

Analysis of these experiments indicates that the α-helix at the N-terminal plays a dominant role in the thermodynamic stability of GFP, whereas mechanical stability is governed by the activation barrier for unfolding of the GFP-barrel. After the N-terminal α-helix has detached from the intact β-stranded barrel core and unwound, a β-strand is peeled off of the barrel (like prying loose a stave); and this then results in unfolding of the rest of the molecule.

Whereas measurement and analysis of the force curves at high bandwidth and with cantilevers of high resonance frequencies lead to the detection of the above two intermediate states with short life spans, measurements at even higher bandwidths may show additional intermediates that exist at the microsecond timescale.

An interesting observation made by Dietz and Rief is that the unfolding path through the energy landscape followed a zigzag course. This was because the protein responded to the detachment of the α-helix by turning and reorienting along a new N-terminal-to-C-terminal direction. This happened again at the second intermediate stage.

Dietz and Rief pointed out that it will be important to correlate the precise connection between the stages of unfolding of GFP with the breakdown of the ability of the molecule to fluoresce. They pointed to the future by suggesting that the tailoring of GFP mutants with altered stability has the potential to provide a group of molecular force sensors. When this is achieved, biologists will have a powerful tool with which to measure forces within cells. This elegant study by Dietz and Rief provides that important first step toward this goal! ■

1. The author gratefully acknowledges Drs. Hendrik Dietz and Matthias Rief for reviewing this article.
2. Dietz, H. and M. Rief, Exploring the energy landscape of GFP by single-molecule mechanical experiments, Proc. Nat. Acad. Sci. 101:16192-16197, 2004.

INDEX OF ARTICLES

ABOUT THE COVER

This issue's cover shows the lift-off of Space Shuttle Columbia on its ill-fated research mission, STS-107. Sixteen days later it re-entered the Earth's atmosphere and was destroyed, killing the seven astronauts aboard. Sandeep Shah and Greg Jerman, from the NASA Marshall Space Flight Center, Materials Diagnostic Team, have written an article, page 8, that shows how the application of microscopy and related techniques were employed in the analytical determination of the location of the fatal damage that resulted is this tragedy.