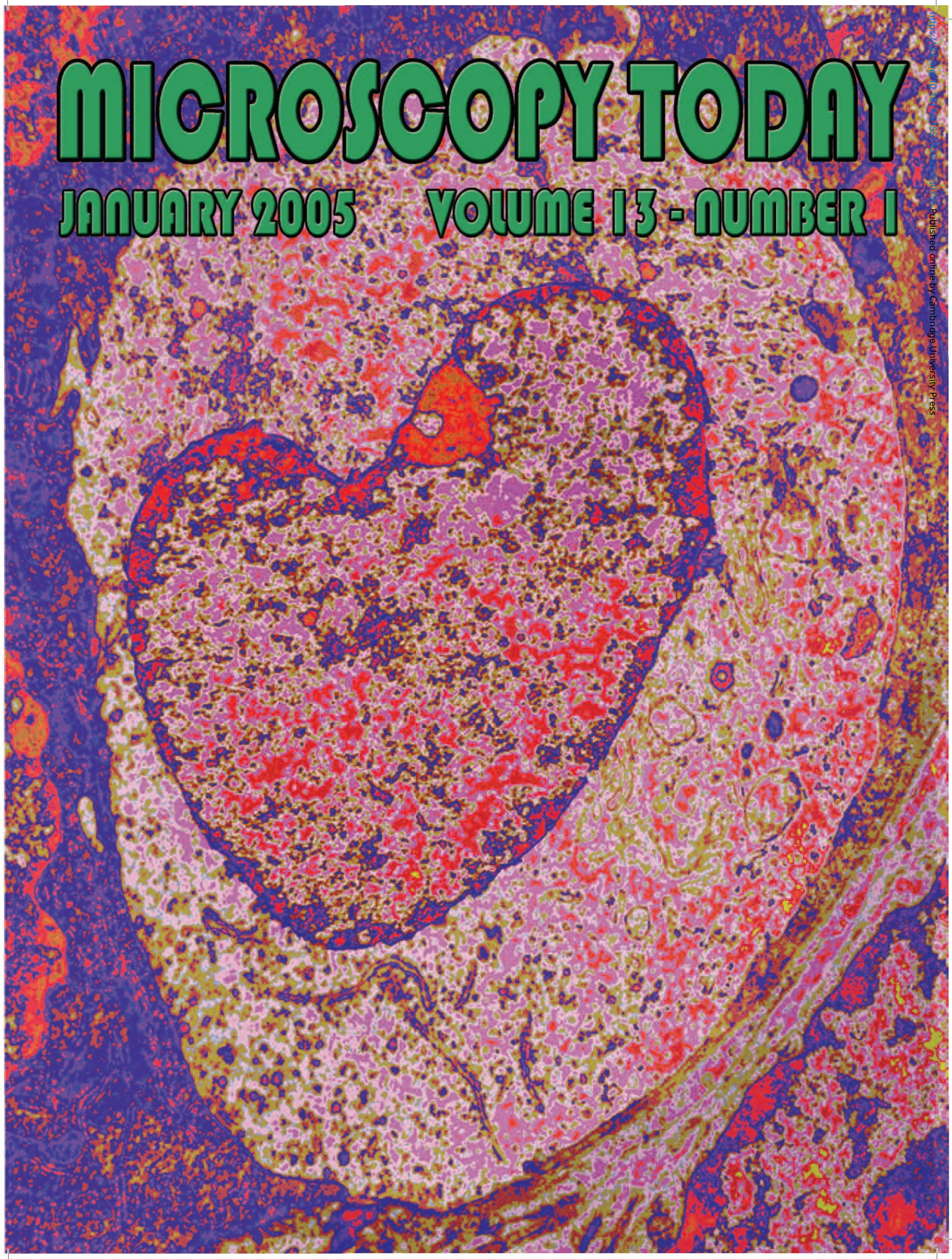
MICROSCOPY TODAY

JANUARY 2005 VOLUME 13 - NUMBER 1



Unfolding and Folding Proteins

Stephen W. Carmichael[1]
Mayo Clinic
stephen.carmichael@mayo.edu

The atomic force microscope (AFM) is being utilized in even more clever ways to reveal details of molecular structure and function. As an example, Julio Fernandez and Hongbin Li have used the AFM in the force-clamp mode to monitor the folding trajectory of a single protein.[2] They have demonstrated in a unique way in which a single protein behaves when put under tension, and then what happens when the tension is relieved.

Fernandez and Li linked several molecules of ubiquitin, a small protein, together to form a polyubiquitin chain. With one end of the chain attached to a substrate, the tip of a soft cantilever of the AFM attached to a single protein, and from one to nine ubiquitin molecules were picked up. Using an improved piezoelectric actuator, a force of 120 picoNewtons (pN) could be suddenly applied as tension on the chain. A stepwise increase in the length of the chain could be measured, each step corresponding to the unfolding of a single ubiquitin molecule.

Then it got interesting. The tension on the chain was suddenly decreased to 15 pN. There was an immediate shortening of the chain, corresponding to the elastic recoil of the unfolded chain. This was followed by a complex series of about three more stages. Interestingly, in the 81 events that were analyzed, the patterns of these latter stages were similar, but never identical. This suggests that there are multiple folding pathways for this protein, that is, it doesn't always fold the same way twice. When the tension was restored to 120 pN, stepwise unfolding was observed again.

When the polyubiquitin chain was fully unfolded, each residue of each molecule was exposed to the saline solution in which the experiment was conducted. Under these conditions, the unfolded chain can be considered to be a polymer coil in a poor solvent. It is well known that under these conditions the chain tends to collapse in a 'coil-globule' phase transition. This is similar, but not identical, to the folding trajectories observed in this study, suggesting that protein folding differs from polymer collapse. Fernandez and Li interpreted their findings, as more likely, a more complex phenomenon in which the collapsing chain rapidly forms bonds that limit the degrees of freedom of the collapsing chain, guiding the trajectory to the native state of the proteins. In other words, the folding of ubiquitin did not consist of transitions between discrete states, but rather occurred through a series of continuous changes, finally resulting in a configuration with the lowest energy.

The direct observation of the folding trajectory of a small protein, as demonstrated by Fernandez and Li, opens the way for detailed studies of protein-folding pathways. By studying these trajectories under different physical-chemical conditions, and using genetically engineered proteins, we may begin to identify specific physical phenomena underlying protein folding! ■

Footnotes:

1 The author gratefully acknowledges Dr. Julio Fernandez for reviewing this article.

2 Fernandez, J.M., and H. Li, Force-clamp spectroscopy monitors the folding trajectory of a single protein, Science 303:1647-1678, 2004.

INDEX OF ARTICLES

ABOUT THE COVER

The nucleus of this undifferentiated gastric epithelial cell from the glandular mucosa of a mouse with a gene targeted deletion of one of the NHE (*Slc9a*) exchangers inspired the whimsical use of PhotoShop to accentuate its ultrastructure and serendipitous heart shape for Valentine's Day. The morphometry of similar parietal cells has improved understanding of the subtle ultrastructural consequences of the loss of a single ion transport genes *in vivo*. See the article by Miller on page 12.