New and Notable

Fingerprinting Single Molecules In Vivo

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The article by Wetherbee and colleagues on detecting the fingerprint of a modular protein fiber in the adhesive secretions of a diatom represents an important step forward toward an effective use of single molecule force spectroscopy in biology. Single-molecule biophysics is slowly revolutionizing cell biology by uncovering important information regarding protein function that cannot be obtained from standard bulk experiments. The recording of ionic currents through single ion channels provided the first and foremost example of single-molecule measurements to date. The study of single ion channels evolved from those incorporated into artificial lipid bilayers to the later characterization of ion channels in native membranes using the patch-clamp technique (1). In addition to its rapid technical development, the single ion channel recording field was marked by a rigorous effort to positively identify the nature of the putative ion channels being studied and avoid artifacts. The potential for artifacts was obvious given that all sorts of spurious currents interfered with the measurements, and the relatively low probability of recording a single ion channel for any length of time. Out of these efforts, clear procedures evolved to uniquely ''fingerprint'' the various types of ion channels being discovered. Ion channels could be uniquely distinguished by their ionic conductance, selectivity, kinetics, and sensitivity to a vast array of pharmacological agents (2). These standards ensured the reproducibility of the experiments being carried out, underpinning an explosive understanding of ion transport in membranes, which has impacted medicine broadly (3).

Since the advent of single ion channel recordings, many other types of single-molecule recordings have been developed (4–8). However, single-molecule sensitivity always challenges the investigator with a large background of spurious signals and interactions, unrelated to the process that we want to study. Although in the ion channel field the fingerprinting standards that evolved were clear and effective, it has proven difficult to develop a similar level of rigor for all types of single-molecule recordings. These difficulties quickly become apparent in the rapidly developing field of single protein mechanics, where a stretching force is applied to a protein by means of optical tweezers or an atomic force microscope (AFM) (9–11). By analogy with the ion channel example, it became essential to develop mechanical fingerprints that would work at the single-molecule level. Luckily, a well-defined mechanical fingerprint results from stretching tandem modular proteins. As a modular protein is extended, the sequential stretching and unfolding of its individual modules results in a characteristic ''saw-tooth pattern'' in the force-extension relationship. In a saw-tooth pattern, the distribution of peak force values is a measure of the protein's mechanical stability, whereas the regular spacing between peaks measures the increase in contour length, which counts the number of amino acids released by the unfolding event (12). Furthermore, the precise way in which the force rises up to each peak, can be analyzed using models of polymer elasticity, measuring the flexibility of the protein polymer through a parameter that is known in the literature as ''persistence length''. Detection of a regularly spaced saw-tooth pattern, combined with the measurement of these three parameters, have been shown to provide a well-defined single-molecule fingerprint that permits the identification of a protein with a level of rigor comparable to that of the ion channel field (13). However, all these studies were done with engineered or native tandem modular proteins of known sequence and well-defined identity. By contrast, Wetherbee and colleagues now apply these procedures to investigate the unknown molecular species present in the adhesive plaque of a benthic (grows attached to rocks and larger algae) diatom (*Toxarium undulatum)*. A simple procedure allowed fresh diatoms to adhere to a petri dish and the large adhesive pad of the diatoms could then be probed with an AFM while the live cell remained attached. They found that they could easily pick up micron-long molecules that upon stretching yielded highly repeatable saw-tooth patterns with a peak-to-peak spacing of ~34 nm. Furthermore, they found that once fully extended and unfolded, the same molecule could be relaxed and extended again for over 600 times while reproducing the same saw-tooth pattern shape, indicating rapid and efficient refolding of the molecular structure. Evidently, this suits the biology of the diatom that must remain attached despite the fluctuating shear of the surf. The biggest surprise came from their observation that the peak forces observed averaged ~800 pN, which was 3–10 times bigger than the typical peak force observed in titin, fibronectin, and other modular proteins. At the same time, the persistence length measured from these traces was unusually small, 0.026 nm, one-tenth of the persistence length measured in single modular proteins. The authors reconciled these observations by proposing that they are stretching native adhesive fibers composed of multiple modular proteins arranged in parallel that unfold and refold in register. This molecular arrangement is attractive because it explains the high unfolding force peaks (each peak results from a module

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unfolding in each fiber simultaneously, adding up the force) as well as the low persistence lengths observed (the apparent persistence length scales with the reciprocal of the number of parallel molecules). The possibilities ahead are very exciting. Their important predictions regarding the molecular structure of the adhesive polymer can be examined in future experiments. Furthermore, similarly to the expression cloning methods used to identify ion channel genes, the mechanical fingerprinting of molecular species with an AFM can be used to sort and identify the gene or genes encoding the molecule(s) composing the *T. undulatum* adhesive fibers.

REFERENCES

1. Neher, E., and B. Sakmann. 1976. Single-channel currents recorded from membrane of denervated frog muscle-fibers. *Nature.* 260:799–802.
2. Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer Press, Sunderland, MA.
3. Ackerman, M. J., and D. E. Clapham. 1997. Ion channels—basic science and clinical disease. *N. Engl. J. Med.* 336:1575–1586.
4. Bustamante, C., Z. Bryant, and S. B. Smith. 2003. Ten years of tension: single-molecule DNA mechanics. *Nature.* 421:423–427.
5. Asbury, C. L., A. N. Fehr, and S. M. Block. 2003. Kinesin moves by an asymmetric hand-over-hand mechanism. *Science.* 302: 2130–2134.
6. Yildiz, A., and P. R. Selvin. 2005. Fluorescence imaging with one manometer accuracy: application to molecular motors. *Acc. Chem. Res.* 38:574–582.
7. Forkey, J. N., M. E. Quinlan, M. A. Shaw, J. E. Corrie, and Y. E. Goldman. 2003. Three-dimensional structural dynamics of myosin V by single-molecule fluorescence polarization. *Nature.* 422:399–404.
8. Howard, J. 1997. Molecular motors: structural adaptations to cellular functions. *Nature.* 389:561–567.
9. Kellermayer, M. S. Z., S. B. Smith, H. L. Granzier, and C. Bustamante. 1997. Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science.* 276:1112–1116.
10. Tskhovrebova, L., J. Trinick, J. A. Sleep, and R. M. Simmons. 1997. Elasticity and unfolding of single molecules of the giant muscle protein titin. *Nature.* 387:308–312.
11. Rief, M., M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub. 1997. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science.* 276:1109–1112.
12. Carrion-Vazquez, M., A. F. Oberhauser, S. B. Fowler, P. E. Marszalek, S. E. Broedel, J. Clarke, and J. M. Fernandez. 1999. Mechanical and chemical unfolding of a single protein: A comparison. *Proc. Natl. Acad. Sci. USA.* 96:3694–3699.
13. Li, H. B., W. A. Linke, A. F. Oberhauser, M. Carrion-Vazquez, J. G. Kerkvliet, H. Lu, P. E. Marszalek, and J. M. Fernandez. 2002. Reverse engineering of the giant muscle protein titin. *Nature.* 418:998–1002.