NANOBIOTECHNOLOGY

Single-molecule circuits

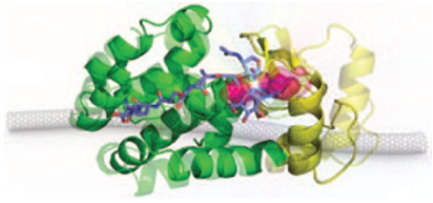
An electronic circuit enables analysis of dynamic processivity of single molecules.

In basic electronic circuits like those that students tinker with in physics class, the transistor is a small three-pronged device, and one of its functions is to amplify a signal. The transistor, developed over half a century ago, has revolutionized the electronics industry; continuing improvements make possible the ever-smaller phones, computers and other devices we rely on.

Extending this work into the realm of biomolecules, physicist Philip Collins and biochemist Gregory Weiss at University of California Irvine joined forces to create a molecular circuit that amplifies and reports a protein's motions. They reasoned that a nanotube would be small enough to act as a wire. "IBM began looking at nanotube transistors 15 years ago," explains Collins, "but there aren't any nanotube transistors in commercial products today because they are just too sensitive to things all around them." When the goal is to detect tiny molecular motions, however, this sensitivity is a boon.

Building on previous work, the teams of Collins and Weiss conjugated a protein to a linker that was noncovalently attached to the nanotube (Choi *et al.*, 2012a). They selected this strategy to only minimally disrupt the nanotube, which carries the current within the device—the so-called field-effect transistor (FET). The premise of the device is that when protein conformation changes, movement of charged residues in proximity to the nanotube would change the current.

The resulting data are inherently noisy, so in this proof-of-principle work the researchers chose the well-studied T4 lysozyme to establish the method. They submerged the nanotube FET with its single lysozyme into a saline buffer. When the enzyme's substrate, peptidoglycan, was present in the buffer, the enzyme would begin oscillating. The protein motions caused matching changes in the current flowing through the device, which the researchers could easily record and analyze using statistical methods. Two types of oscillations, slow and fast, corresponded to lysozyme's known hinge movement determined using fluorescence-based techniques: a slow catalytic motion and a nonproductive high-frequency motion.



Motions of a single lysozyme are monitored by an electronic circuit. Image courtesy of P. Collins.

Without the need for a fluorescent label, FET-based studies avoid limitations associated with labeling, fluorophore blinking and so on. An important advantage of the FET over fluorescence-based techniques is its continuous signal, which enabled an analysis of lysozyme processivity. "We can watch a single molecule for minutes if not hours," notes Collins and adds that another important characteristic of the FET is a high bandwidth, of almost a megahertz—approaching a million events per second. "For any molecule that is functioning at a fast rate, your technique needs to have the resolution to be able to see that."

In another set of experiments, the researchers watched the lysozyme 'eat' two different substrates (Choi *et al.*, 2012b). Faced with a linear peptidoglycan, the enzyme spent most of the time processing it, but addition of a cross-linked peptidoglycan resulted in many more oscillations indicative of nonproductive motion. "You can really see the difference between a lysozyme chewing on one type versus another," comments Collins.

Having optimized the technique with lysozyme, the team has since used these FETs to study a few different proteins, including a kinase. "We are getting the sense that this technique is generalizable and can be used to watch molecule activity across a broad range," Collins adds. The FETs can now be manufactured commercially, and with the current work showing how to make sense of the noise, they are poised to find their way into a biophysicist's toolbox as well as diagnostic equipment.

Irene Kaganman

RESEARCH PAPERS

Choi, Y. *et al.* Single-molecule lysozyme dynamics monitored by an electronic circuit. *Science* **335**, 319–324 (2012a).

Choi, Y. *et al.* Single-molecule dynamics of lysozyme processing distinguishes linear and cross-linked peptidoglycan substrates. *J. Am. Chem. Soc.* **134**, 2032–2035 (2012b).