



more distantly related *Bt* toxins that almost certainly bind to other receptors<sup>11</sup>. Nematodes with mutant Gal-T and resistance to Cry5B had 19-fold cross-resistance to a related *Bt* toxin, Cry14A (ref. 2), but remained sensitive to an unrelated *Bt* toxin, Cry6A (ref. 10).

Reduced binding of *Bt* toxins to target sites in the gut is the general category of resistance mechanisms described above for both *Heliothis* and *C. elegans*, but even this broad explanation does not cover all cases of *Bt* resistance in insects. Eleven-fold resistance to Cry1Ab in Indianmeal moth, a stored-grain pest, is associated with loss of a proteinase<sup>12</sup>. In susceptible insects, this enzyme cleaves Cry1Ab, converting it to a toxic form from a nonlethal precursor. In several other examples, including some strains of diamondback moth and *Heliothis*, reduced binding of *Bt* toxin has been excluded as the primary mechanism of resistance, but alternative mechanisms have not been identified<sup>9</sup>.

Discovery of a gene underlying *Bt* resistance in a key insect pest begins a new era in which molecular biology will play an increasingly important role in understanding, tracking, and managing insect resis-

tance to *Bt* crops. Researchers will race to apply the novel information to address a host of previously intractable questions about resistance and the mode of action of *Bt* toxins. In this giddy rush, perhaps the parallel discovery from a lowly nematode will help keep scientists humble and remind us of the elegance and diversity with which evolution finds answers.

1. Gahan, L.J. *et al. Science* **293**, 857–860 (2001).
2. Griffiths, J.S. *et al. Science* **293**, 860–864 (2001).
3. Charles, J.-F. *et al. (eds) Entomopathogenic bacteria from laboratory to field application.* (Kluwer Academic, Dordrecht, The Netherlands; 2000).
4. Tabashnik, B.E. *Annu. Rev. Entomol.* **39**, 47–79 (1994).
5. US Environmental Protection Agency. *The Environmental Protection Agency's white paper on Bt plant-pesticide resistance management.* (EPA Publication 739-S-98-001, 1998). [www.epa.gov/pesticides/biopesticides/white\\_bt.pdf](http://www.epa.gov/pesticides/biopesticides/white_bt.pdf).
6. Luo, K. *et al. Insect Biochem. Mol. Biol.* **27**, 735–743 (1997).
7. Vadlamudi, R.K. *et al. J. Biol. Chem.* **270**, 5490–5494 (1995).
8. Gould, F. *et al. Proc. Natl. Acad. Sci. USA* **94**, 3519–3523 (1997).
9. Tabashnik, B.E. *et al. Phil. Trans. R. Soc. Lond. B* **353**, 1751–1756 (1998).
10. Marroquin, L.D. *et al. Genetics* **155**, 1693–1699 (2000).
11. Gould, F. *et al. J. Econ. Entomol.* **88**, 1545–1559 (1995).
12. Herrero, S. *et al. Appl. Environ. Microbiol.* **67**, 1085–1089 (1997).

static interaction of the charge carriers with an intermediate control (gate) electrode. In a CHEMFET (or variants thereof), this control is affected by a chemical species of interest<sup>6</sup>. Such sensors are the focus of considerable interest in laboratories around the world (e.g., see ref. 7). The primary utility of FET devices, in general, lies in the decoupling of the gate signal from the source/drain current (i.e., the gate voltage modulates the transconductance of the source/drain without the gate voltage itself being affected). In a “good” FET, no current flows between the gate electrode and the source/drain.

The sensors described by Cui *et al.* follow the same basic structure. An oxidized silicon wafer acts as a platform for a flat semiconducting nanowire; 10–20 nm diameter silicon wires a few microns in length, boron-doped for p-type (hole) conductivity are connected on each end to electrodes fanning out to electrical contacts. The electrodes are ~2–4 μm in size and in spacing. A micromolded elastomeric channel (~100 μm in width) is placed atop the electrode/nanowire device, allowing controlled exposure of the sensor surface to various fluids both for functionalization and solution assays. The overall dimensions of the active device portion of these sensors were ~2 μm wide by 6–12 μm long. This basic structure was then functionalized in different ways to demonstrate four different prototype sensors.

To illustrate protonation/deprotonation sensitivity, the device was functionalized with 3-aminopropyltriethoxysilane (APTES). Conductance as a function of time and pH was measured at integer pH values from 2 to 9, and discrete increases in the conductance of the device were observed, consistent with the protonation/deprotonation of the APTES. The conductance versus pH response appeared linear over the range measured, whereas nonfunctionalized devices gave a nonlinear conductance response over the full pH range.

To demonstrate selective bioactivity sensing, devices were functionalized with biotin, which (at the appropriate pH) irreversibly binds negatively charged streptavidin molecules. Exposure to a 250 nM streptavidin solution increased the conductance by 50 nS (~3%). A subsequent wash with pure buffer solution resulted in no conductance change. Control experiments were consistent; the introduction of streptavidin with a nonfunctionalized device, and a functionalized device where biotin was blocked with four equivalents of d-biotin, yielded no conductance changes.

A reversible reaction was tested with biotin-functionalized devices (see Fig. 1).

## Hotwiring biosensors

### Nanowire sensors decorated with specific capture molecules can detect minute quantities of biological and chemical species.

James F. Klemic, Eric Stern, and Mark A. Reed

Imagine a microchip biosensor with an enormous number of highly specific and sensitive antibody/antigen assay reactions, interfaced to the fast parallel electronic signal processing capability of an integrated circuit (IC). Recent developments suggest that such a device may eventually be realized. In a recent issue of *Science*, Cui *et al.*<sup>1</sup> report the conductance behavior of nanofabricated doped silicon wires functionalized with organic molecules and biomolecules. Results indicate these functionalized nanowires may provide a new type of platform for sensitive and selective chemical and biological sensors. What is particularly exciting about these results is

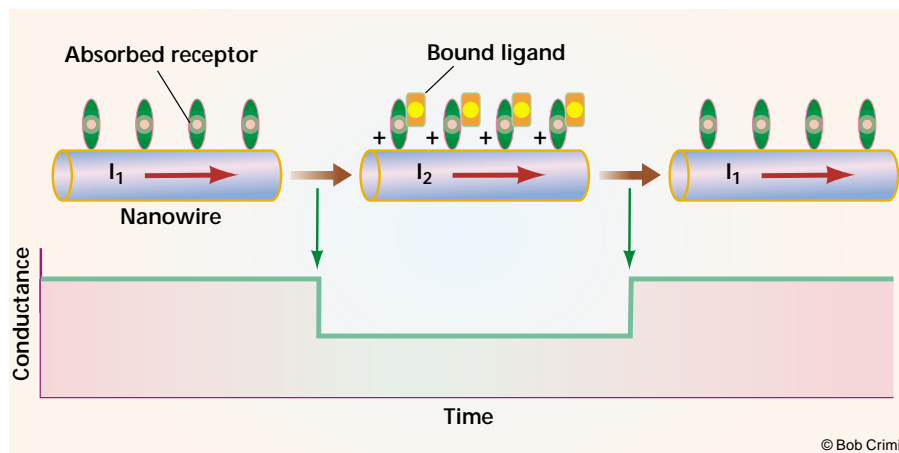
the potential for realizing a nanoscale interface that bridges solid-state electronics and true molecular-scale processes, not only for detection and selectivity but also for eventual use in extra- and intercellular measurements<sup>2</sup>.

Advances in nanofabrication techniques allow one to build essentially arbitrary materials in novel shapes and configurations. Of particular interest is the realization of nanoscale structures using semiconducting materials similar to those in IC electronics<sup>3,4</sup>. These tiny structures hint at the possibility of coupling the sophisticated electronic functionality of today's ICs with smaller scale, possibly even molecular-scale, processes.

The concept of a chemically sensing electronic device, specifically a chemically modified field effect transistor (CHEMFET) has been long known<sup>5</sup>. Field-effect transistors (FETs) operate by modulating the flow of electrons between the input (source) and output (drain) by the electro-

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**Figure 1.** Reversible nanoscale biosensing. A silicon nanowire functionalized with biotin (absorbed receptor). Introduction of m-antibiotin (bound ligand) caused a drop in conductance across the nanowire. Rinsing with buffer returned the conductance to its initial level.

Upon the introduction of monoclonal anti-biotin (m-antibiotin) there was an  $\sim 120$  nS drop in conductance ( $\sim 12\%$ ); the device returned to approximately the original conductance value after washing with pure buffer. Nonfunctionalized devices had no conductance change upon introduction of m-antibiotin. Introduction of bovine immunoglobulin G (IgG), which is not specific for biotin, also resulted in no conductance change, while a subsequent addition of m-antibiotin produced a conductance drop.

Finally,  $\text{Ca}^{2+}$  sensing was demonstrated by functionalizing the device with calmodulin. An  $\sim 40$  nS drop in conductance ( $\sim 5\%$ ) was observed upon the introduction of a  $25 \mu\text{M}$   $\text{Ca}^{2+}$  solution. When a pure buffer solution was subsequently introduced, conductance returned to its approximate original value.

Although the basic nanowire electrode device appears similar to standard monolithic IC devices (i.e. those made on a single substrate), it has important differences. The nanowires are grown off-chip and then dispersed onto a wafer; contact leads are then individually aligned to the randomly placed nanowires. The large-scale integration of this technique is limited by the hybrid and serial (versus monolithic and parallel) fabrication of devices, with concurrent issues of yield and uniformity. Additionally, the present functionalization process is not restricted to just the nanowire, but also the oxide surfaces of the wafer around the nanowire and between the electrodes. Although one should not underestimate these as well as other fabrication challenges that would need addressing before practical device integration, they should not be viewed as fundamental limitations or detract from the very exciting and intriguing preliminary test device results.

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The truly exciting aspect of the work is the ability to potentially achieve unprecedented sensitivity and selectivity by using the inherently small active volume of the nanowires. The observed conductance results are consistent with, and attributed to, FET behavior (C. Lieber, personal communication), which could allow tuning of the carrier density in the nanowires to be sensitive to even single-electron events<sup>8</sup>. As yet, these reported conductance variations have not been unambiguously shown to be a field effect; if alternative mechanisms (e.g., leakage current through the functionalized layer) contribute, much of the favorable scaling effect of these devices is lost. However, one can feel confident, given the advanced understanding of engineering semiconductor nanostructures, that such limitations—even if they do exist—will be short-lived. The field of nanoscale engineering shows promise as an important tool in the understanding of biosystems on a level never before achievable, and this paper is one of the first in what will assuredly become a trend.

1. Cui, Y. *et al. Science* **293**, 1289–1292 (2001).
2. Klemic, K.G. *et al. Biophys. J.* **80**, 337a (2001). Abstract presented at the 2001 annual meeting of the Biophysical Society, Boston, MA.
3. Reed, M.A. & Kirk, W.P. (eds) *Nanostructure physics and fabrication*. (Academic Press, San Diego, CA; 1989).
4. Nalwa, H. (ed.) *Handbook of nanostructured materials and nanotechnology*. (Academic Press, San Diego, CA; 2000).
5. Bergveld, P. *IEEE Trans. Biomed. Eng.* **BME-17**, 70–71 (1970).
6. Morrison, S.R. Chemical sensors. In *Semiconductor sensors*. (ed. Sze, S.M.) 404–408 (John Wiley and Sons, New York, NY; 1994).
7. Wu, D.G. *et al. Chem. Eur. J.* **7**, 1743–1749 (2001).
8. Ralls, K.S. *et al. Phys. Rev. Lett.* **52**, 228–231 (1984).