

# TECH SIGHT

## Biomaterials for Sensors, Fuel Cells, and Circuitry

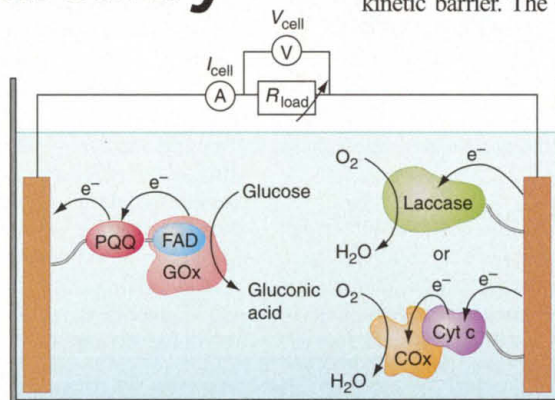
Itamar Willner

**B**ioelectronics is a rapidly progressing interdisciplinary research field that aims to integrate biomolecules and electronic elements into functional systems (1, 2). The ability to control the shape and structure of biomolecules, such as proteins and DNA, and the evolution-optimized chemical functions of biomaterials including binding, catalysis, ion-pumping, and self-assembly make biomolecules attractive building blocks for functional devices. Hybrid systems formed by the integration of biomolecules with electronic elements, such as electrodes, or transistors enable the electronic read-out detection of biomolecular functions, the transformation of biocatalyzed processes into electrical power, and the templating of nano-sized circuitry. Future applications of bioelectronic systems may include computation devices and prosthetic units.

The passage of electrons between biomolecules and electronic elements is the essence of all bioelectronic systems. Nevertheless, electronic units and biomolecules lack natural communication (3), so the structural design of biomolecular architectures on electronic supports is needed to facilitate communication between the components so that biological events are transduced into electronic signals.

A major focus in bioelectronics is the development of biosensors, devices that electronically transduce recognition events between biomolecules. These have potential uses ranging from clinical diagnostics and environmental analyses to detection of chemical and biological warfare, and forensic applications. The complex formation between an antigen and the antibody, the formation of nucleic acid–DNA or –RNA structure, and the interaction between an enzyme and its substrate represent specific biomolecular binding interactions. Redox enzymes are part of a broad class of biocatalysts that exchange electrons upon the oxidation or reduction of specific substrates. Although the electrical activation of redox enzymes by electrodes could theoretically be an approach for the development of amperometric biosensors to detect substrates, the redox-active centers in biocatalysts usually lack electron-transfer communication with the electrodes due to their electronic insulation by the protein matrix (3). Redox enzymes have been electronically connected with electrodes by incorporating biocatalysts into redox-active polymers (3), tethering of electroactive groups to the protein (4), and structurally aligning the redox enzyme on surface-immobilized electron-relay groups (5). In all of these systems, artificial redox groups mediate electron transfer between the biocatalyst and the electrode. Various electrochemical enzyme-electrodes that sense, e.g., glucose, nitrate, or lactate, have been developed.

The ability to connect redox proteins to electrodes allows bioelectrocatalytic electrodes to be used as active components in the tailoring of biofuel cells (6, 7). For example, oxidation of glucose by molecular oxygen is an energy-releasing process prohibited by a large kinetic barrier. The construction of an electrically contacted



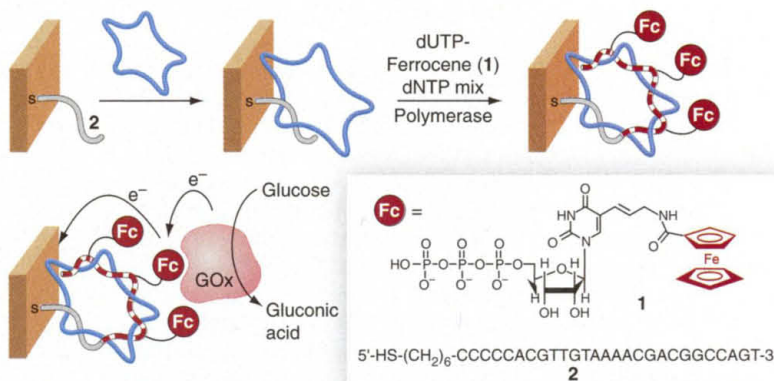
**Biofuel cell.** Bioelectronic elements generate electrical energy by the bioelectrocatalyzed oxidation of glucose. PQQ, pyrroloquinolone quinone; FAD, flavin-adenine dinucleotide.

glucose oxidase (GOx) enzyme electrode and the complementary organization of oxygen-reducing bioelectrocatalytic electrodes consisting of cytochrome c (Cyt c)–cytochrome oxidase (COx) or laccase create an anode and a cathode that generate electrical power through the bioelectrocatalytic oxidation of glucose (see figure, left). Such biofuel cells could yield a new generation of implanted devices (such as pacemakers and prosthetic elements) that generate their own electrical power from the glucose found in bodily fluids. Alternatively, the output of an implanted biofuel cell could act as an electronic signal for the monitoring of blood

glucose levels in diabetics (8). Substantial progress in the engineering of miniaturized biofuel cells has been reported recently (7).

Immunosensors that electronically transduce antigen-antibody recognition processes represent an important class of bioelectronic devices. Such devices represent major advances over conventional enzyme-linked immunosorbent assays (ELISAs) because they are more sensitive and enable precise quantification of the antigen or antibody. Several approaches for constructing electronic immunosensors have been suggested, including the application of redox-labeled antigens that compete with the analyzed antigen for the sensing antibody interface (9), the use of antibody-enzyme conjugates that generate a redox-active product (10), and the detection of the weight changes of a piezoelectric crystal that occur as a consequence of antigen-antibody complex formation on its surface (11).

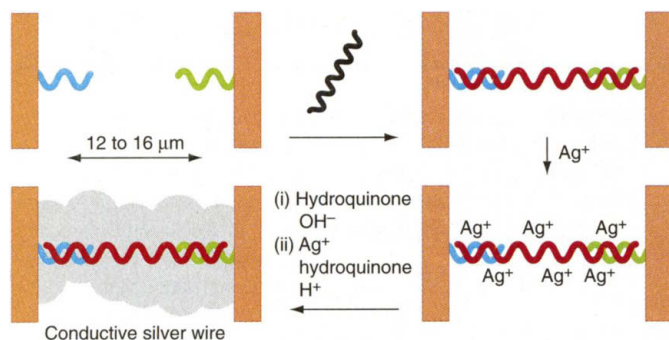
Electroactive molecular components or redox enzymes coupled to nucleic acid–DNA or –RNA complexes associated with electrodes



**Amplified DNA sensing.** By the generation of a redox-active replica and activating the bioelectrocatalyzed oxidation of glucose, DNA amplification can be detected. Fc, ferrocene unit; dNTP, deoxynucleotide triphosphate mixture.

The author is at the Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel. E-mail: willnea@vms.huji.ac.il





**DNA template.** Versatility of DNA allows the precise generation of an Ag nanowire.

may be used for electronic gene analysis. Such DNA detection schemes are useful for the detection of genetic disorders and viral and microbial pathogens and for tissue matching, forensic applications, and the development of new therapeutic drugs. The amperometric detection of DNA has been achieved by the intercalating of a redox-active ferrocenylanthracene diimide molecule with a double-stranded complex (12). Using a related approach, redox-active doxorubicin was intercalated into the double-stranded complex formed between a nucleic acid probe and a target DNA on an electrode surface (13). The electrochemical reduction of the intercalator mediates the electrocatalyzed reduction of O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub>, activating chemiluminescence in the presence of luminol and horseradish peroxidase. The generation of H<sub>2</sub>O<sub>2</sub> and the subsequent emission of light represent optobioelectronic paths for the photonic transduction of the DNA recognition event. The amplified amperometric transduction of DNA sensing with a bioelectronic system was demonstrated (14) by the generation of a redox-active DNA strand complementary to the analyzed DNA, which activates a secondary bioelectrocatalytic process (see figure, previous page, bottom). The complex generated between the 7249-base M13φ viral DNA and a 27-base primer, (2), associated with a gold electrode was replicated in the presence of polymerase and a mixture of nucleotides that includes ferrocene-modified deoxyuridine triphosphate (dUTP), (1). The resulting ferrocene-labeled replica was then coupled to GOx and, upon electrochemical oxidation of the ferrocene units, the GOx-biocatalyzed oxidation of glucose was activated. Because the enzyme exhibits a high turnover rate, the electrical output from this process represents an amplified electrical signal of the primary recognition event between the M13φ viral DNA and the sensing nucleic acid associated with the electrode. Other enzymes and labels were coupled to the primer–DNA recognition complex for biocatalytic amplification paths (15, 16). Also, different methods for the electronic detection of base mismatches in DNA mutants have been developed (15, 17). Bioelectronic detection schemes for DNA have also been developed by the application of metal or semiconductor nanoparticle–DNA hybrid systems (18, 19). For example, nucleic acid–functionalized Au nanoparticles were used as catalytic labels for the formation of double-stranded DNA complexes in an insulating gap separating two microelectrodes. The catalytic deposition of Ag on the Au nanoparticles bridged the electrodes with a conductive wire that enabled the electrical monitoring of the formation of the DNA recognition complex (20). Similarly, the catalyzed deposition of metals on nucleic acid Au nanoparticle labels linked to DNA recognition complexes associated with piezoelectric crystals was used for the detection of amplified DNA (18).

DNA is an attractive template for the generation of electronic nanocircuitry. The ability to synthesize DNA of predesigned length, shape, and base order and the availability of enzymes acting as biocatalytic tools for the ligation, scission, or polymerization of nucleic

acids provide versatile tools to manipulate the DNA templates. The association of metal ions with DNA, the intercalation of molecules into DNA, and the association of catalytic metal nanoparticles or photocatalytic semiconductor nanoparticles with DNA suggest ways to introduce electronic functionalities to DNA frameworks. The metallization of ions linked to the DNA (21) or the incorporation of Au nanoparticle–functionalized intercalators into DNA (22) are primary efforts to assemble DNA-based circuitry. For example, two separated microelectrodes modified with two different nucleic acids were interconnected by a complementary DNA strand (see figure, left). The association of Ag<sup>+</sup> with the double-stranded DNA followed by the reduction of the ions and catalytic metal deposition generated a conductive nanowire (21). Related studies have demonstrated the programmed linkage of semiconductor nanoparticles to double-stranded DNA, thus establishing concepts for designing future nanoarchitectures of electronic circuitry. Other biomaterials, such as de novo proteins, engineered antibodies, and nucleic acid–protein complexes, could provide additional templates for nanostructured constructs (23).

Three facets of bioelectronics—biomaterial-based electronic sensors, biofuel cells, and biomaterial-based electronic circuitry—continue to progress rapidly in research, and some of its applications have been developed commercially. Other topics, such as hybrid systems of neural network and electronic elements (24), biomaterial-based computers (25), and biomaterial-based micromachinery devices (26) represent other opportunities in bioelectronics that show promise for future applications. Despite this outlook, however, challenges in bioelectronics remain, including the development of implantable fuel cells, biomaterial-based micromachines (e.g., prosthetic units), and the fabrication of functional electronic circuitry. Recent advances in nanotechnology, the availability of quantum-size nanoparticles and nanotubes of unique electronic and catalytic properties, and nanoscopic microscopy tools for manipulating surfaces could open nanobioelectronics as a new research field. The electronic detection of biorecognition events of single molecules, the optical or electronic read-out of biological processes by functional nanoparticles, the high-throughput analysis of numerous genes or protein functions on dense sensing arrays, the construction of biomaterial-based electronic devices of nanodimensions, and the tailoring of ultra-small self-powered devices for the controlled release of therapeutic drugs may be possible as well. Blurring the boundaries between chemistry, biology, physics, and material science, bioelectronics and the interdisciplinary nature of its research make it a sure source of exciting findings in the years to come.

## References

1. I. Willner, B. Willner, *Trends Biotechnol.* **19**, 222 (2001).
2. I. Willner, E. Katz, *Angew. Chem. Int. Ed.* **29**, 1180 (2000).
3. A. Heller, *J. Phys. Chem.* **96**, 3579 (1992).
4. W. Schuhmann et al., *J. Am. Chem. Soc.* **113**, 1394 (1991).
5. I. Willner et al., *J. Am. Chem. Soc.* **118**, 10321 (1996).
6. E. Katz et al., *J. Electroanal. Chem.* **479**, 64 (1999).
7. T. Chen et al., *J. Am. Chem. Soc.* **123**, 8630 (2001).
8. E. Katz, A. F. Bückmann, I. Willner, *J. Am. Chem. Soc.* **123**, 10752 (2001).
9. K. Di Gleria, H. A. O. Hill, C. J. McNeil, *Anal. Chem.* **58**, 1203 (1986).
10. J. Rishpon, I. Rosen, *Biosensors* **4**, 61 (1989).
11. B. König, M. Grätzel, *Anal. Chem. Acta* **280**, 37 (1993).
12. S. Takenaka et al., *Anal. Chem.* **72**, 1334 (2000).
13. F. Patolsky, E. Katz, I. Willner, *Angew. Chem. Int. Ed.* **41**, 3398 (2002).
14. F. Patolsky, Y. Weizmann, I. Willner, *J. Am. Chem. Soc.* **124**, 770 (2002).
15. D. J. Caruana, A. Heller, *J. Am. Chem. Soc.* **121**, 768 (1999).
16. F. Patolsky et al., *Angew. Chem. Int. Ed.* **40**, 2261 (2001).
17. F. Patolsky, A. Lichtenstein, I. Willner, *Nature Biotechnol.* **19**, 253 (2001).
18. Y. Weizmann, F. Patolsky, I. Willner, *The Analyst* **126**, 1502 (2001).
19. I. Willner, F. Patolsky, J. Wasserman, *Angew. Chem. Int. Ed.* **40**, 1861 (2001).
20. S.-J. Park, T. A. Taton, C. A. Mirkin, *Science* **295**, 1503 (2002).
21. E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, *Nature* **391**, 775 (1998).
22. F. Patolsky et al., *Angew. Chem. Int. Ed.* **41**, 2323 (2002).
23. C. M. Niemeyer, *Angew. Chem. Int. Ed.* **40**, 4128 (2001).
24. G. Zeck, P. Fromherz, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10457 (2001).
25. L. Wang et al., *J. Am. Chem. Soc.* **122**, 7435 (2000).
26. J. Fritz et al., *Science* **288**, 316 (2000).