

Bioelectronics

From Theory to Applications

Edited by

Itamar Willner and Eugenio Katz



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Bioelectronics

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Preface

The integration of biomolecules with electronic elements to form functional devices attracts substantial recent research efforts. The entire field was named with the general buzzword, “bioelectronics”. Exciting advances in the area include the integration of enzymes, antigen/antibodies, DNA, or bioreceptors with electronic units to yield specific biosensors for clinical diagnosis, detection of pathogens, environmental and food analysis, and homeland security applications. Another general scientific effort is directed to the coupling of neurons with electronic elements to assemble neuroelectronic junctions and neuronal networks that are anticipated to act as “brain computers” and information processing devices. Other merging research efforts include the development of biofuel cells, and biomolecule-based motors and devices. Progress in the rapidly developing area of nanotechnology introduced new concepts and scientific paradigms to bioelectronics. Conjugation of biomolecules and metallic or semiconducting nanoparticles yields hybrid materials with unique electronic and photonic properties that provide fascinating scientific and technological opportunities. New nanostructured sensors, electronic nanocircuitry based on biomolecular templates, nanostructured devices and nanoscale drug delivery systems are a few viable examples where bioelectronics “meet” nanotechnology.

The various topics covered highlight key aspects and the future perspectives of bioelectronics. The book discusses theoretical limitations in the electronic coupling of biomolecules with electronic elements, the chemical strategies to immobilize biomolecules such as proteins or DNA on electronic transducers, and to apply the systems as biosensors. The junction between bioelectronics and nanotechnology is introduced by exemplifying the microscopic imaging of biomolecular assemblies on surfaces at the single molecule level, the use of biomolecules as a mold to synthesize functional nano-objects and devices, and the use of biomolecule-nanoparticle hybrid systems as functional biosensing elements. The assembly of neuronal networks as information processors, and the use of biomolecules as information storage and computing systems are further topics that are discussed in detail.

The different topics addressed in this book will be of interest to the interdisciplinary community active in the area of bioelectronics. It is hoped that the collection of the different chapters will provide chemists, biologists, physicists, material scientists and engineers with a comprehensive perspective of the field. Furthermore, the book is aimed

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to attract young scientists and introduce them to the field while providing newcomers with an enormous collection of literature references. We, indeed, hope that the book will spark the imagination of scientists to further develop the topic.

Finally, we would like to thank all scientists that contributed to this effort and made possible the publication of this book.

Jerusalem, January 2005

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1

Bioelectronics – An Introduction*Itamar Willner and Eugenii Katz*

The integration of biomolecules with electronic elements to yield functional devices attracts substantial research efforts because of the basic fundamental scientific questions and the potential practical applications of the systems. The research field gained the buzzword “bioelectronics” aimed at highlighting that the world of electronics could be combined with biology and biotechnology [1–3]. Mother Nature has in course of evolution processed the most effective catalysts (enzymes), and biomolecules of optimal recognition and binding capabilities that lead to highly selective and specific biopolymer complexes (antigen–antibody, hormone-receptor, or duplex DNA complexes). Similarly, biology provides the fastest and most complex computing and imaging systems where optical information is processed and stored in the form of three-dimensional memorable images (vision process). The tremendous biochemical and biotechnological progress in tailoring new biomaterials by genetic engineering or bioengineering provides unique and novel means to synthesize new enzymes and protein receptors, and to engineer monoclonal antibodies or aptamers for nonbiological substrates (such as explosives or pesticides) and DNA-based enzymes. All these materials provide a broad platform of functional units for their integration with electronic elements. The latter electronic elements may involve, for example, electrodes, field-effect transistor devices, piezoelectric crystal, magnetoresistance recording media, scanning tunneling microscopy (STM) tips and others. The bioelectronic devices, Figure 1.1, may operate in dual directions: In one configuration, the biological event alters the interfacial properties of the electronic element, thus enabling the readout of the bioreaction by monitoring the performance of the electronic unit such as the readout of the potential, impedance, charge transport, or surface resistance of electrodes or field-effect transistors, or by following the resonance frequencies of piezoelectric crystals. The second configuration of bioelectronic systems uses the electronic units to activate the biomaterials toward desired functions.

The major activities in the field of bioelectronics relate to the development of biosensors that transduce biorecognition or biocatalytic processes in the form of electronic signals [4–6]. Other research efforts are directed at utilizing the biocatalytic electron transfer functions of enzymes to assemble biofuel cells that convert organic fuel substrates into electrical energy [7, 8]. Exciting opportunities exist in the electrical

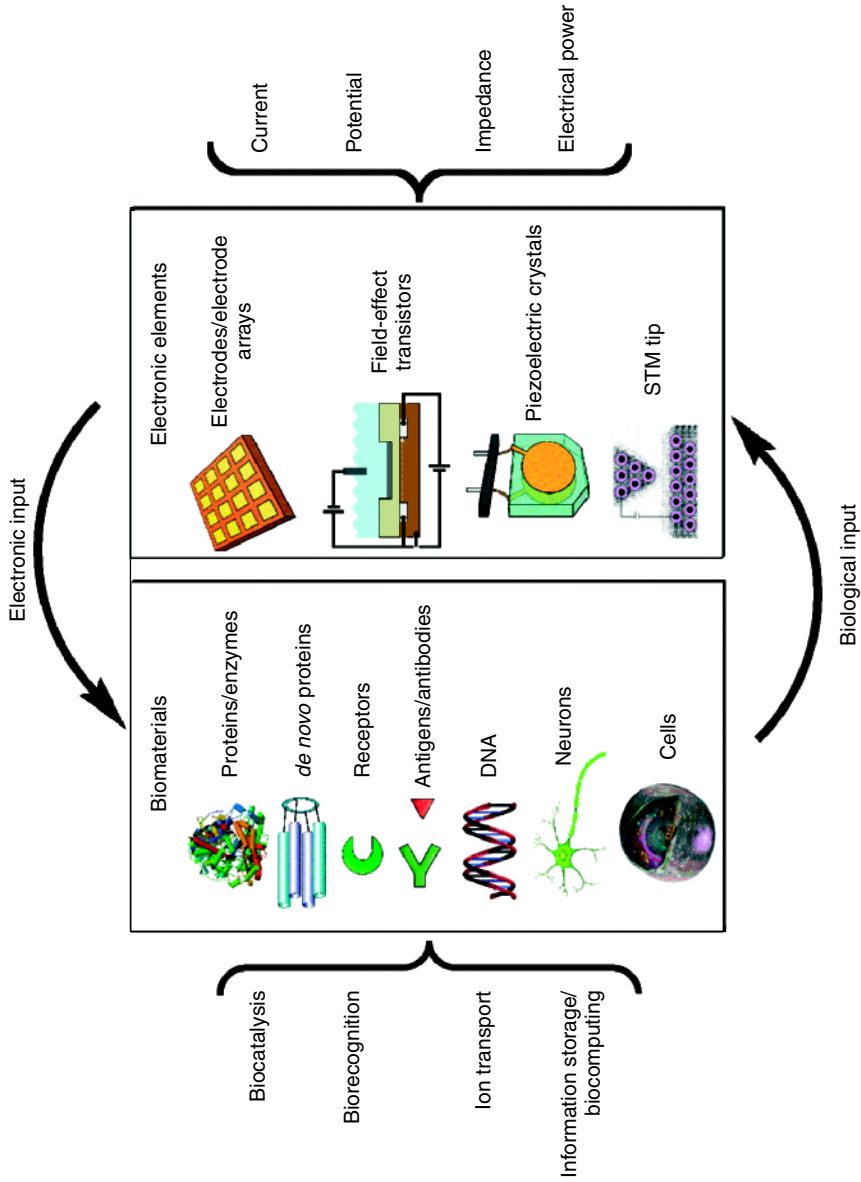


Fig. 1.1 Integrated systems of biomaterials and electronic elements for bioelectronic applications.

interfacing of neuronal networks with semiconductor microstructures. The excitation of ion conductance in neurons may be followed by electron conductance of semiconductor devices, thus opening the way to generating future neuron-semiconductor hybrid systems for dynamic memory and active learning [9]. The recent progress in nanotechnology and specifically in nanobiotechnology adds new dimensions to the area of bioelectronics. Metal and semiconductor nanoparticles, nanorods, nanowires, and carbon nanotubes represent nano-objects with novel electronic properties. Recent studies revealed that the integration of these objects with biomolecules yields new functional systems that may yield miniaturized biosensors, mechanical devices and electronic circuitry [10–12].

A fundamental requirement of any bioelectronic system is the existence of electronic coupling and communication between the biomolecules and the electronic supports. Special methods to immobilize biomolecules on solid supports while preserving their bioactive structures were developed. Ingenious methods to structurally align and orient biomaterials on surfaces in order to optimize electronic communication were reported [13]. Although impressive advances in the functional tailoring of biomolecule electronic units–hybrid systems were accomplished, challenging issues await scientific solutions. The miniaturization of the bioelectronic systems is a requisite for future implantable devices, and these types of applications will certainly introduce the need for biocompatibility of the systems. The miniaturization of the systems will also require the patterned, dense organization of biomolecules on electronic supports. Such organized systems may lead to high throughput parallel biosensing and to devices of operational complexity. The development of methods to address and trigger specific biomolecules in the predesigned arrays is, however, essential. This book attempts to highlight different theoretical and experimental topics that place bioelectronics as a modern interdisciplinary research field in science.

The understanding of charge transport phenomena through biological matrices attracted in the past decades, and continues to evolve, intensive theoretical and experimental work. The seminal contributions of the Marcus theory [14], the superexchange charge transfer theory [15], and the definition of superior tunneling paths in proteins [16] had a tremendous impact on the understanding of biological processes such as the electron transfer in the photosynthetic reaction center, or the charge transport in redox-proteins that are the key reactions for numerous electrochemical and photoelectrochemical biosensing systems. A continuous feed back between elegant experimental work employing structurally engineered proteins and theoretical analysis of the results led to the formulation of a comprehensive paradigm for electron transport in proteins [17]. This topic is addressed in detail in Chapter 2. The charge transport through DNA has recently been a serious scientific debate [18, 19], and contradicting results claiming conductive [20], superconductive [21], semiconductive [22] or insulating [23] properties of DNA were reported. Theories describing charge transport through DNA (electrons or holes) that included hopping mechanisms, tunneling paths, or ion-assisted electron transfer were developed [24, 25]. Charge transport through DNA is anticipated to play a key role in the electrical detection of DNA and in the analysis of base mismatches in nucleic acids, in the use of DNA

nanowires as circuitry in devices, and as a means to readout sequence specific DNA structures (DNA computers).

The electrical contacting between biomolecules and electrodes is an essential feature for most bioelectronic systems. Numerous redox enzymes exchange electrons with other biological components such as other redox-proteins, cofactors or molecular substrates. The exchange of electrons between the redox-centers of proteins and electrodes could activate the biocatalytic functions of these proteins, and may provide an important mechanism for numerous amperometric biosensors. Nonetheless, most of the proteins lack direct electron transfer communication with electrodes, and the lack of electrical communication between the biomaterials and the electronic elements presents one of the fundamental difficulties of bioelectronic systems. Although the barriers for charge transport between redox-proteins are easily explained by the Marcus theory and the spatial insulation of the redox-centers of enzymes by the protein matrices, they hinder the construction of electrically communicated biomolecular-electronic hybrid systems. Ingenious methods for the electrical contacting of biomolecular assemblies associated with electronic units were developed in recent years [13]. The structural engineering of proteins with electron relays [26], the immobilization of redox enzymes in conductive polymers or redox-active polymers [5], the steric alignment of proteins on electron relays associated with electrodes [27], or the incorporation of redox-active intercalators in DNA [28] represent a few means to electronically communicate the biomolecules with the electronic elements. These aspects are addressed in several sections of the book (Chapters 3 and 4) and are exemplified here with the electrical communication of redox enzymes with electrodes for the generation of amperometric biosensors and biofuel cells, and with the intercalation of a redox-label into double-stranded DNA for the electrical probing of DNA. The integration of glucose oxidase, which lacks direct electrical communication with electrodes, into a redox-active hydrogel film consisting of tethered Os(II)-polypyridine complex (1) units, and linked to the electrode, facilitates the electrical contact between the enzyme and the conductive support, Figure 1.2(A). The flexible redox-units linked to the polymer electrically wire the redox-center of the enzyme with the electrode by mediated electron transfer. Glucose sensing electrodes based on this charge transport concept are already on the market, and the design of micro-sized electrically wired enzyme electrodes for invasive continuous monitoring of glucose are close to commercial realization [29]. A different application of electrically contacted enzyme electrodes rests in the design of biofuel cells [7, 8], Figure 1.2(B). Fuel cell systems represent a well-established technology, where electrical power is generated by two complementary oxidation and reduction processes occurring at a catalytic anode and cathode, respectively. While the generation of electrical power by electrically contacted redox enzymes, in a biofuel cell configuration has probably little value in global energy production, the systems might have important merit as implantable devices that generate electrical power from body fluids. For example, a glucose-based biofuel cell utilizing electrically contacted enzyme electrodes could use blood as a fuel for the electrical powering of pace makers, insulin pumps or prosthetic elements.

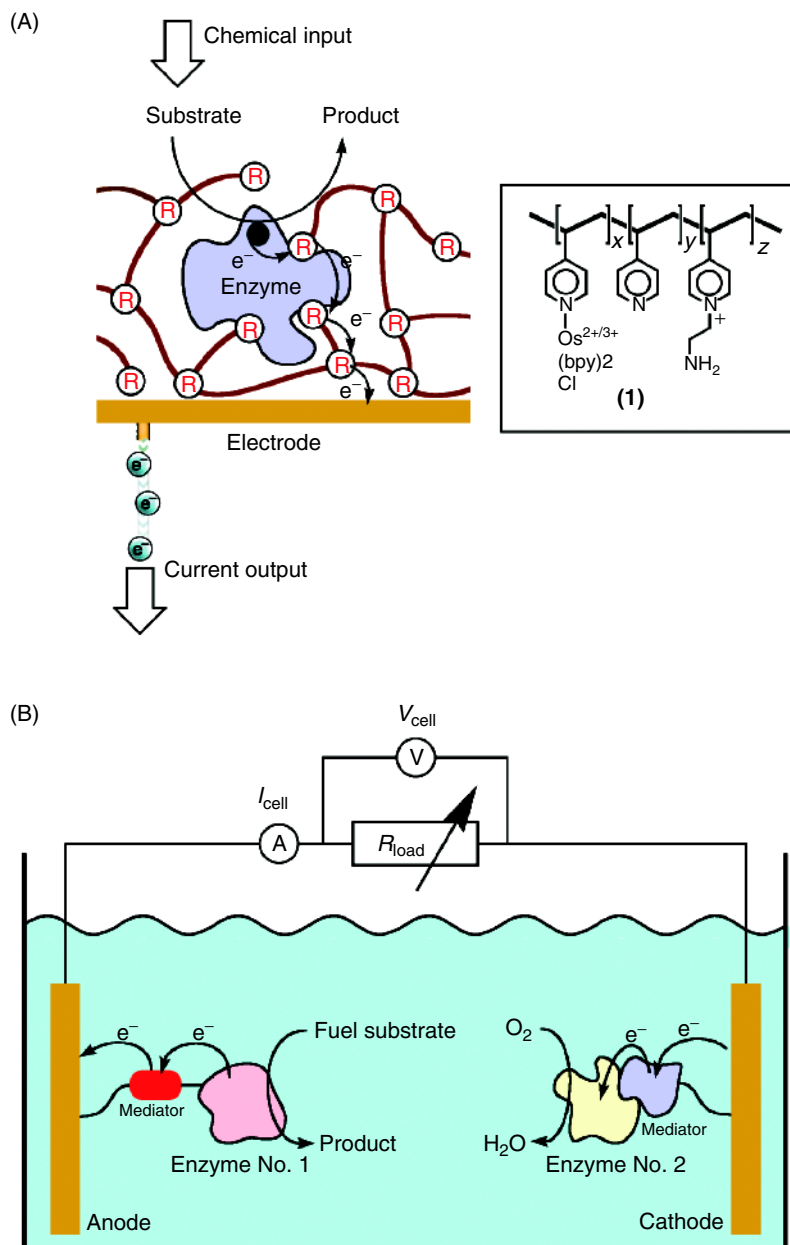


Fig. 1.2 (A) Electrical contacting of a redox-enzyme with an electrode by an electroactive polymer and the application of the system as an amperometric biosensor. (B) A biofuel cell configuration based on electrically contacted enzyme electrodes.

The electrical contacting between molecular species and electrodes may be stimulated by specific biorecognition events. For example, the intercalation of doxorubicin (**2**) into the double-stranded DNA formed between a primer nucleic acid associated with an electrode and the complementary analyte DNA enables the electrochemical reduction of the intercalator and the subsequent catalytic reduction of O_2 to H_2O_2 , Figure 1.3. The latter product induces in the presence of luminol and horseradish peroxidase (HRP) the formation of chemiluminescence as a readout signal for the DNA duplex formation on the electrode [28]. The analysis of DNA by different electrochemical methods is discussed in Chapter 5.

Scanning probe microscopy techniques have introduced exciting opportunities in surface science and specifically in the characterization of biomolecules on surfaces. Scanning tunneling microscopy allows one to probe tunneling currents through proteins, thereby imaging the structure of individual protein molecules. Atomic force microscopy (AFM) not only permits the imaging of single biomolecules on surfaces but also permits the specific affinity interactions between complementary

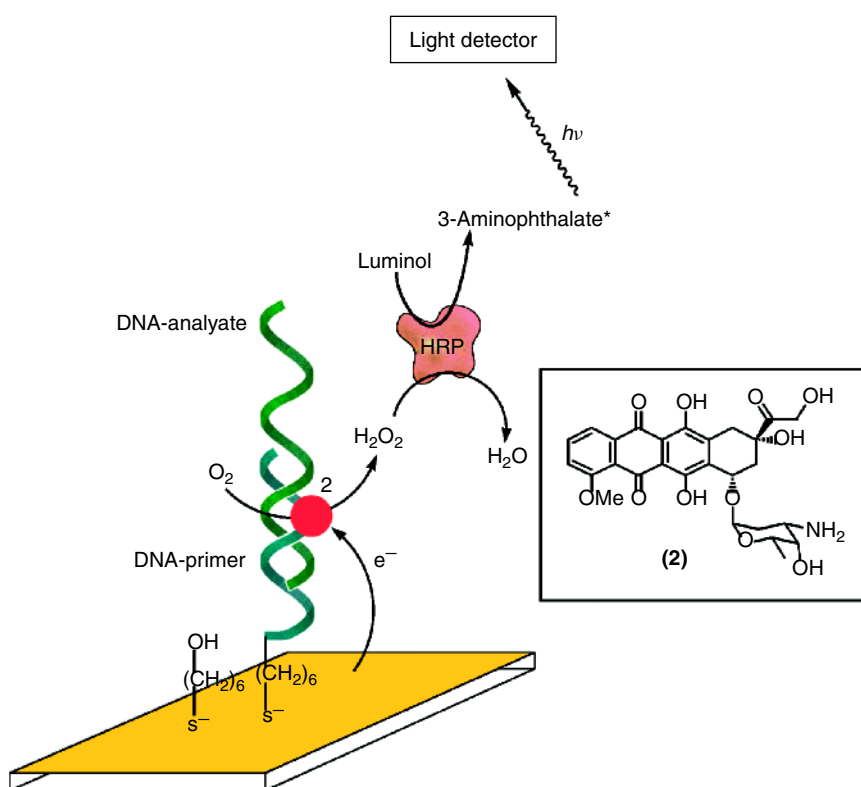


Fig. 1.3 The biochemiluminescent detection of DNA by the intercalation of a redox-active substrate into the double-stranded DNA assembly and its electrochemical activation.

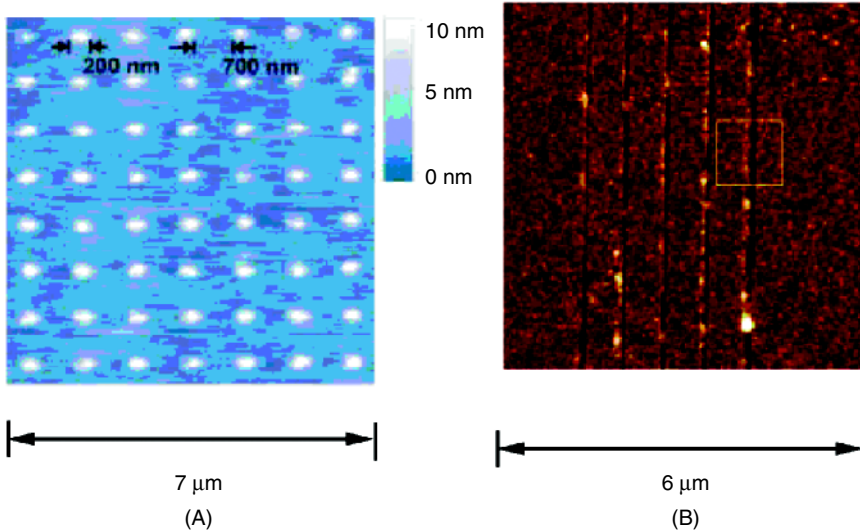


Fig. 1.4 (A) AFM image of a retronectin protein array generated by dip-pen nanolithography. (B) AFM image of a patterned surface consisting of a DNA monolayer treated with a DNase-modified AFM tip that cleaves off the DNA units upon contact with the surface. (Part A is adapted from [33] and Part B is adapted from [32], with permission).

antigen–antibody pairs, or double-stranded DNA complexes to be followed [30, 31]. Scanning probe microscopes also add new dimensions as tools for patterning surfaces with biomolecules. The use of dip pen–lithography for the generation of biomolecular patterns [32], Figure 1.4(A) or the application of enzyme-functionalized AFM tips as a biocatalytic patterning tool [33], Figure 1.4(B), are just two examples demonstrating the potential of these nano-tools to fabricate dense biomolecular arrays. Realizing that bioelectronics involves the intimate coupling of biomolecules to electronic supports, the use of scanning probe microscopy to characterize the structure–function relationships of single biomolecules, and to actuate single biomolecules are inevitable for the future development of the field. Some aspects of scanning probe microscopy for bioelectronic applications and the manipulation of single biomolecules are addressed in Chapters 6 and 10.

Self-organization of biomolecules leads to unique 2D- and 3D-nanostructures that include structurally defined pores or channels. These materials may act as templates for the assembly of other materials, and the generation of systems of hierarchical structural complexity. Figure 1.5 shows a scanning force microscopy image of S-layer protein from *Bacillus sphaericus* on a silicon surface exhibiting square lattice symmetry with a lattice constant of 13.1 nm. Alternatively, the pore or channel structures may be utilized as “microreactors” of predefined dimensions for the synthesis of metallic or semiconductor nano-objects. This topic is addressed in Chapter 13, where the applications of S-layer proteins in bioelectronic systems are discussed.

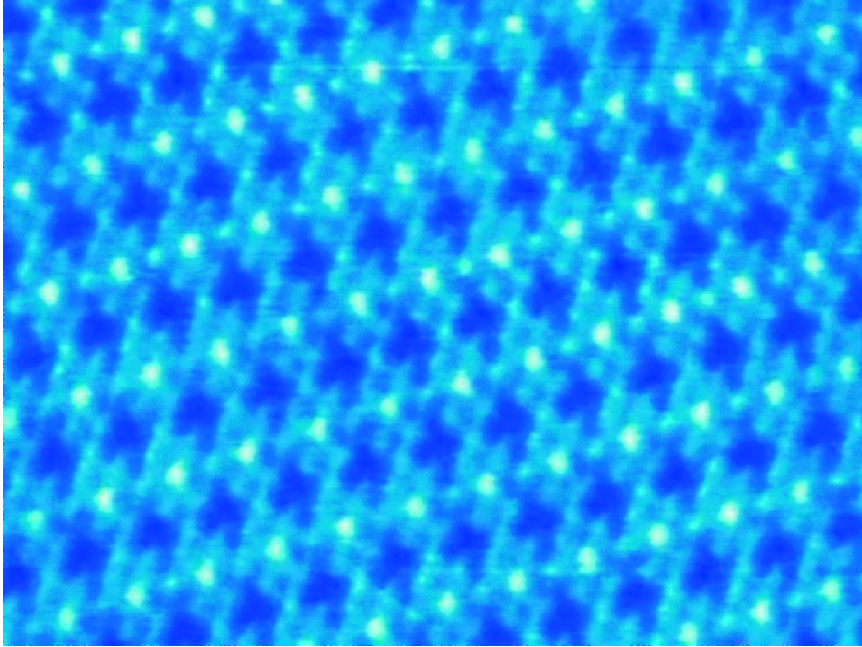


Fig. 1.5 AFM image of an S-layer protein from *Bacillus sphaericus* on a silicon surface. The image size corresponds to 150×113 nm. (Adapted from <http://nanotechweb.org/articles/news/2/3/15/1>, with permission).

Nanoparticles exhibit unique electronic, optical, catalytic and photoelectrochemical properties [34–36]. The dimensions of nanoparticles are comparable to those of biomolecules such as enzymes, antigens/antibodies or DNA. Not surprisingly, the conjugation of biomolecules with metal and semiconductor nanoparticles yields hybrid systems of new electronic and optoelectronic properties. Indeed, tremendous progress was accomplished in the realization of biomolecule–nanoparticle hybrid systems for various bioelectronic applications [37]. The electrical contacting of redox enzymes with electrodes by means of Au nanoparticles [38], the use of metal nanoparticle–nucleic acid conjugates for the catalytic deposition of metals and inducing electrical conductivity between electrodes [39], the electrochemical analysis of metal ions originating from the chemical dissolution of metallic [40] or semiconductor [41] nanoparticle labels associated with DNA, or the photoelectrochemical assay of enzyme reactions by means of semiconductor nanoparticles [42] represent a few examples that highlight the potential of biomolecule–nanoparticle hybrid systems in biosensor design. Recent advances in the integration of biomolecules with semiconductors and the application of biomolecule–nanoparticle hybrids in bioelectronics are highlighted in Chapters 7 and 8, respectively. Several other applications of biomolecule–nanoparticle or biomolecule–carbon nanotube systems are also discussed in other sections of the book.

Exciting opportunities exist in the applications of biomolecules as templates for the synthesis of metallic or semiconductor nanowires [43]. Such nanowires provide great promise for future nanocircuitry and for the assembly of nanodevices. The possibility of preparing DNA of desired shapes and base sequence, the availability of enzymes acting as biocatalytic tools for manipulating DNA, the binding of metal ions to the phosphate units of DNA chains, the specific intercalation of molecular components into the DNA biopolymers, and the specific DNA–protein interactions, turn DNA into an ideal matrix for its use as a template in the synthesis of nanowires consisting of metals or semiconductors. Indeed, tremendous progress has been accomplished by using DNA as a template for the generation of nanowires and patterned nanowires [44]. This subject is highlighted in Chapter 9, which demonstrates the use of patterned Au

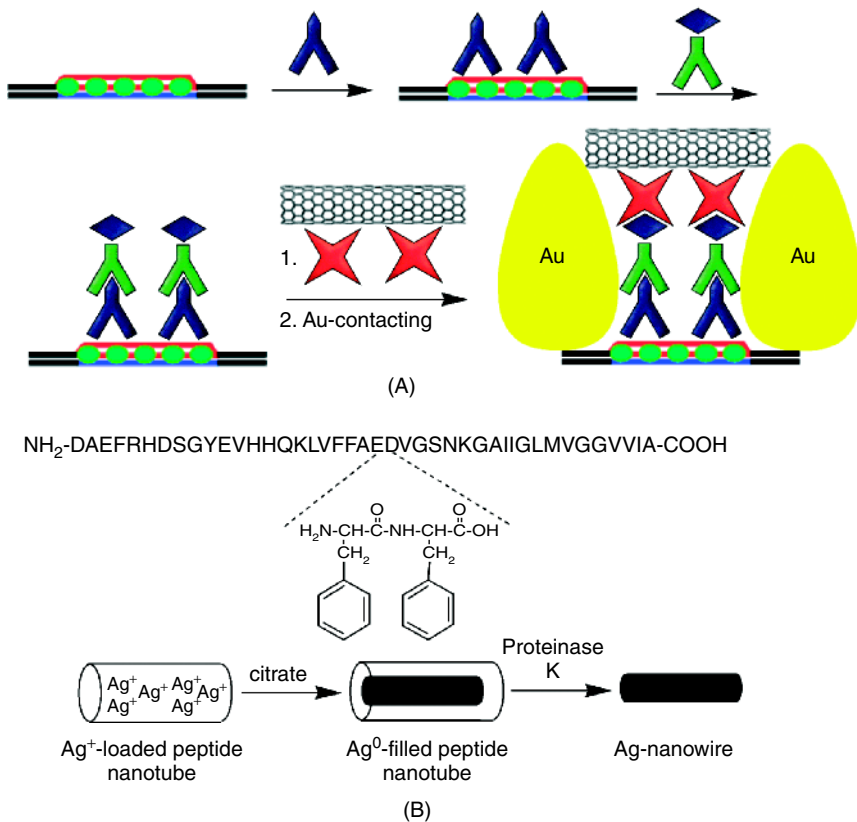
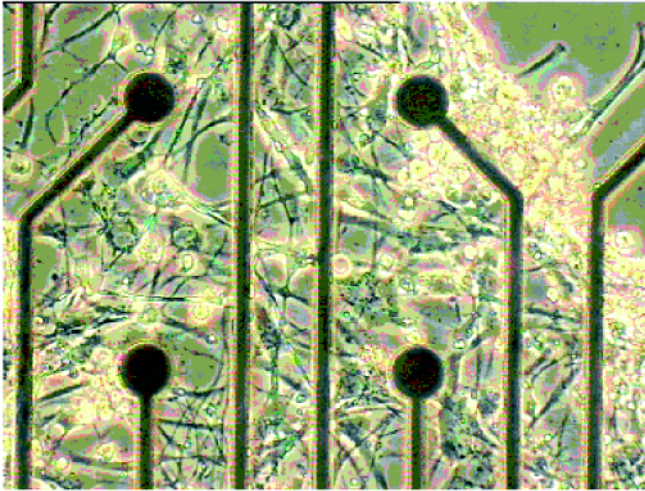
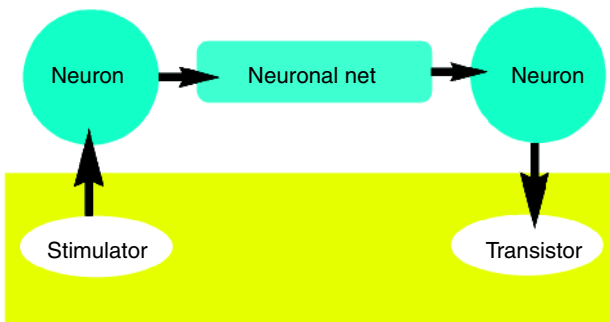


Fig. 1.6 (A) Assembly of a nanotransistor based on a carbon nanotube bridging two Au nanocontacts on a DNA template. The carbon nanotube is positioned on the DNA by the initial binding of RecA protein to the DNA, followed by the association of RecA-antibody and a biotinylated anti-antibody, and the fixation of avidin-coated tube to the assembly. (B) Formation of a Ag wire in the channel of a diphenylamine peptide tube, followed by the enzymatic dissolution of the peptide template.



(A)



(B)

Fig. 1.7 (A) Neurons on top of a multi-electrode array (adapted from <http://physicsweb.org/article/news/7/4/17#neuronsonelectrode> with permission). (B) A neuroelectronic hybrid system consisting of two neurons; the first neuron is activated by a capacitive stimuli, the signal transmission occurs through a neuronal network to a second neuron, where the information is recorded by a transistor.

nanowires on DNA as electrical contacts for the assembly of a nanotransistor. The construction of the biomolecule-base nanotransistor [45], Figure 1.6(A), is based on the assembly of a carbon nanotube between gold contacts formed on a DNA template using biorecognition events as driving motives for the construction of the nanodevice. Recent advances in this area suggest that self-assembled protein tubules or filaments may similarly be employed as templates for the synthesis of nanowire system [46].