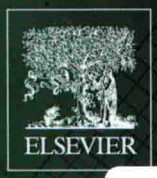

BIOMOLECULAR ELECTRONICS

Bioelectronics and the Electrical Control of Biological Systems and Reactions

Paolo Facci

Micro & Nano Technologies Series

Biomolecular Electronics

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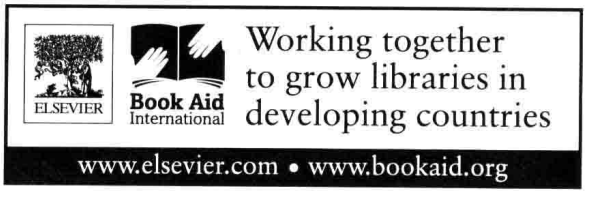

Biomolecular Electronics

To Bianca, Maria, and Benedetta

Una lux illuminat omnia
(G. Bruno, Sigillus Sigillorum)

Preface

At the beginning of the 1990s biomolecular electronics asserted itself as a powerful novel approach to combine the most advanced technology humankind has ever developed with the most sophisticated type of matter known in the Universe, biological matter. Expressions such as self-assembly, specific recognition, natural evolution, and so on, suddenly became very popular and widely used (and sometimes, abused), including by people lacking a rigorous biological education.

I think that words (and the concepts that these words signify) do not belong to anyone in particular and everybody has the right to use them at will. Sometimes, this can turn out to be a little annoying for educated people but more often, especially in science, it helps to provide an undeniably advantageous change of paradigm that can foster cultural advancement. Apparently something similar has happened also in the development of biomolecular electronics. Cross-contamination among basic and applied disciplines helped a lot to shape the brand new research field that aimed at selecting and exploiting molecules of biological origin for implementing novel hybrid devices. These last were characterized by a novel concept according to which the molecule itself constituted the device, at variance with the classical top-down approach borrowed from fabrication processes used in solid-state electronics.

In this regard, the concomitant advent of nanotechnology resulted in a powerful boost for biomolecular electronics, which naturally faced the issue of connecting electronic circuits and leads to single molecules.

After more than 20 years from its birth one must admit, however, that real practical biomolecular electronics applications, in terms of operating devices on the market, are still dramatically missing and the legitimate doubt arises that they will never appear!

This outcome is indeed quite common in applied science and often accompanies those cases that were characterized by a generalized over-excitement about their expectations. To date, not many experts trust in the future advent of transistors made from proteins or DNA.

At any rate the positive side-products, brought about by more than two decades of research in this field, are numerous and range from a deep understanding of electron transport through single biomolecules, to the elucidation of the key role of contacts with metal leads and that of redox electronic levels in assisting electron transport, to the correct appreciation of conformational variations in modulating molecular transport properties. The essential role of

water in enabling and preserving functional molecular conformations has once more been clarified.

I would list in the positive outcomes of the research activity in biomolecular electronics also the realization that its true added value is not likely to be in competing with much better-established technological paradigms to replace already existing and well performing devices (e.g., nanotransistors); rather, it is in having developed a series of approaches suitable for the effective interfacing of biological molecules and reactions with conventional electronic systems.

Indeed, this fact enables electronic control over the functional activity of biological molecules (e.g., metalloenzymes) and related reactions and paves the way to a future scenario where biological phenomena and systems will be controllable and driven technologically by external electric signals. The first steps towards this novel, charming scientific and technological adventure are what the present book is about.

During my activity in the field of biomolecular electronics I have had the opportunity of sharing with numerous young collaborators, students, and colleagues many ideas, efforts, lack of success, and, sometimes, also the happiness of exalting results. I am grateful to all of them. In particular, I thank Andrea Alessandrini, Victor Erokhin, Gerard Canters, Lorenzo Berti, Marco Salerno, Dario Alliata, Laura Andolfi, Carlo Augusto Bortolotti, Mimmo Gerunda, Paolo Petrangolini, Marialuisa Caiazzo, Elena Angeli, and Loredana Longo, who more than others have collaborated with me in this field and without whom I could not have reached the present view of this subject.

Paolo Facci, Genova

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Biomolecular Electronics 1

CHAPTER OUTLINE

1.0 What is biomolecular electronics?

With the expression "biomolecular electronics" we refer nowadays to that branch of technology that exploits molecules or molecular systems of biological origin for interaction with modern electronics.

The idea of using biomolecules to assemble hybrid electronic devices stems from molecular electronics (Aviram & Ratner, 1974) and, as such, is intimately connected with the advent of nanosciences and nanotechnologies, dating back to the beginning of the last decade of the past century. The interest in biomolecules stems not only from their size, which is typically in the nanometer range (at least in two spatial dimensions). Rather, it is often connected to a particular standpoint from which one can regard them.

Indeed, an interesting point of view is that of regarding biomolecules in general and proteins in particular as self-contained, nanometer-sized functional units that are highly specialized and efficient in performing a certain functional task. Their efficiency in performing a particular activity is traced back to the fact that biomolecules, being parts of living beings, have naturally evolved over billions of years, reaching the current "optimal" degree of specialization for accomplishing a given task.

One can often come across this opinion reading the specialized literature or attending topical conferences; however, it appears quite questionable in light of a slightly less naïve understanding of the Theory of Evolution (Fodor & Piattelli-Palmarini, 2010); nevertheless, it is perhaps a good enough starting point to understand the historical motivations which led to the

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remarkable interest shown by a sizable part of the interdisciplinary scientific community in the use of biomolecules for assembling electronic devices.

Another element motivating the interest in proteins as components of electronic circuits is their sizes. Indeed, these molecules often configure self-consistent, functional units with typical sizes in the nanometer range. Therefore, it is conceivable to imagine functional devices as small as a single molecule, especially in view of the fantastic progresses in miniaturization and parallelization made with nano-lithographic techniques.

Furthermore, the rich chemistry that particularly characterizes protein-forming monomers, i.e., the 20 natural amino acids, makes these molecules amenable to various kinds of chemical functionalization, thus improving their reactivity towards desired functional groups and enabling, as a consequence, their ready chemisorption on pre-functionalized structures and surfaces. Moreover, the exceptionally fine-tuned recognition properties of biological structures (e.g., complementary nucleic acid strands, DNA-binding proteins, antibody-antigen or receptor-ligand pairs, etc.) could allow for self-assembling of more complex structures in the nanometer-sized gap between meso/nanoscale electrodes.

Among the various classes of molecules of biological origin, two of them have been especially focused upon by researchers in the field: DNA and proteins. The reasons for this choice are different; in the case of DNA, its robustness and ease of handling are relevant factors. Nevertheless, a further important aspect fostering DNA as an interesting molecule in biomolecular electronics stemmed from the idea that, if we could understand the mechanism by which electrons are driven along the double helix during DNA repair of damage caused by oxidative stress, we would obtain, as a byproduct, a way to produce molecular wires, that is, one-molecule-thick conducting wires, to be used in assembling (bio)molecular circuitry. If DNA can be regarded in terms of molecular wire, what else does one need to assemble molecular circuits? Some functionally relevant electronic elements, of course. Those were identified as the proteins, which are indeed deputed to perform the main functional tasks in the organisms that express them.

At this point a relevant question arose that has not been completely answered yet, about the electrical conductivity of biomolecules. It is indeed obvious that at the basis of any electronic circuit there is a need to deal with wires and elements that can conduct or, more generally, interact functionally with electron flows, i.e., electron currents.

Whereas this issue is still open in the case of DNA, which displays good conductivity over short ranges (of the order of nanometers) and appears to be

an insulator over larger distances (Guo et al., 2008), the situation regarding proteins is more complex and requires detailed analysis because of the large variability of their properties (mirroring the differences in protein structures).

In the case of DNA, its poor conductivity, at least in the mesoscopic range, has prompted various approaches for enhancing it, based on doping (with metal ions or intercalating agents (Ban et al., 2009)) or on the use of DNA as a molecular template for metal nanowire growth (Berti et al., 2005). In the case of proteins, which possess an intrinsically higher chemical and structural variability compared with DNA, it is not surprising that specific transport proteins, carrying either electrons or ions, soon attracted the interest of researchers.

1.1 Proteins and biomolecular electronics

Typically, electron transport proteins are water-soluble molecules (rare exceptions apart; see, e.g., cytochrome b_{561}, a transmembrane three-heme cytochrome), whereas ion transport proteins are membrane molecules requiring specific environmental conditions (incorporation in a lipid bilayer) to be functionally active (see section 3.6.1). According to this difference, whereas the former are more easily implemented in electronic circuits, the latter are more demanding to exploit in a nano-circuit configuration. Ion channels have been used to gate transistors in hybrid devices thanks to their selectivity for specific ions (Bernards et al., 2006) and the inherent similarity of the gating mechanism of voltage-gated ion channels to the basic features of a field-effect transistor (Bezanilla, 2005). A specific application of ion channels in electronic circuits for sensing applications is the coupling of the channels reconstituted in lipid bilayers with other electronic nanostructures such as semiconductor nanowires (Misra et al., 2009). Examples of these applications are the possibility, by changing the pH of the solution, of controlling the conductance of a silicon nanowire covered with a lipid bilayer integrating peptide pores and the creation of nanobioelectronic transistors exploiting ion pumps such as Na^+/K^+-ATPase (Huang et al., 2010).

However, the most popular and charming class of proteins that seemed to be particularly suitable for implementing electronic devices is the redox metalloproteins. These very important molecules are indeed able to shuttle electrons between molecular partners by reversibly changing the oxidation state of the metal ions they contain (see sections 4.1, 4.1.1, and 4.1.2).

Following the route defined by conventional solid-state electronics, the research efforts in biomolecular electronics have generally focused on

demonstrating transistor-like behavior that proteins might display when positioned between two electrodes. However, considering the classic definition of a transistor, we should stress here a different meaning for the same word. A transistor represents a device composed of at least three electrodes (source, drain, and gate) which is able to amplify or switch the current between a pair of electrodes (e.g., source-drain) by controlling the voltage or current between two of these three electrodes (e.g., source-gate or drain-gate). In conventional electronics the controlling signal is an electric signal. In the context of proteins, among the possible signals that can function for gating a hybrid device we include also other signals of a chemical or physical nature. Among them one has to consider all the parameters that are able to modify, at constant bias voltage, the transport properties of a protein or of a protein layer sandwiched between two electrodes. In a similar way, protein conformational variations can induce changes in its transport properties or in those of another nanostructure to which the protein is coupled. Among these signals one can list variation in environmental pH, temperature, substrate recognition by enzymes and so on. The sensitivity to any of these signals confers sensing properties to the related devices.

We will consider the main results obtained along the pathway of understanding the behavior of redox metalloproteins when used as active parts of hybrid electronic devices in Chapter 4. Here, we continue by noting that other features of proteins have also attracted researchers' attention and have been used in various attempts to exploit the properties of natural biomolecules to implement electronic devices. There is, for instance, the case of antibodies. These molecules are part of an organism's immune system and are known for their specific recognition and binding capabilities against the corresponding antigens. Their use has been recently demonstrated in assembling single-protein devices (Chen et al., 2012). Particularly, antibodies of the IgG type (immunogammaglobulin) have been raised against Au nanoparticles, 5 nm in diameter. An IgG molecule can bind up to two of these gold nanoparticles, one per F_{ab} fragment, where the specific recognition sites for antigens are located. This specific binding provides a stable bond. Gold nanoparticles are attached to e-beam lithography-fabricated 10-nm gap electrodes that act as source and drain electrodes. Once the electrodes have been decorated with nanoparticles, they are exposed to IgGs that specifically recognize the nanoparticles, binding them firmly. This results in a configuration that can be gated electrically, by means of a proximal electrode, and optically, by the action of a CdSe Qdot attached to the IgG molecule at its F_c fragment. While irradiated, the Qdot absorbs light, providing an induced dipole moment