

# Fabrication of Electrochemical-Based Bioelectronic Device and Biosensor Composed of Biomaterial-Nanomaterial Hybrid

# 17

Mohsen Mohammadniaei, Chulhwan Park, Junhong Min, Hiesang Sohn, and Taek Lee

## 17.1 Introduction

With the rapid advances in biotechnology (BT), nanotechnology (NT) and information & communication technology (ICT), a new technology field called bioelectronics emerged (Christof and Chad 2004; Itamar and Eugenio 2005; Noy 2011). Since 2000, the bioelectronics has led to the development of biochips (Farzadfard and Lu 2014; Michael 2007; Qiu et al. 2013), biosensors (Lee et al. 2007; Sarkar et al. 2014), biomedical devices (Deng et al. 2014) and bioelectronic devices for computation (Nikitin et al. 2014). Specifically, bioelectronic devices have been investigated in several fields, such as electrical engineering, nanobiotechnology, mechanical engineering and chemistry (Offenhäusser and Rinaldi 2009; Ren et al. 2017; Strukov and Kohlstedt 2012). The bioelectronic computation system can be at the center of these advances, as

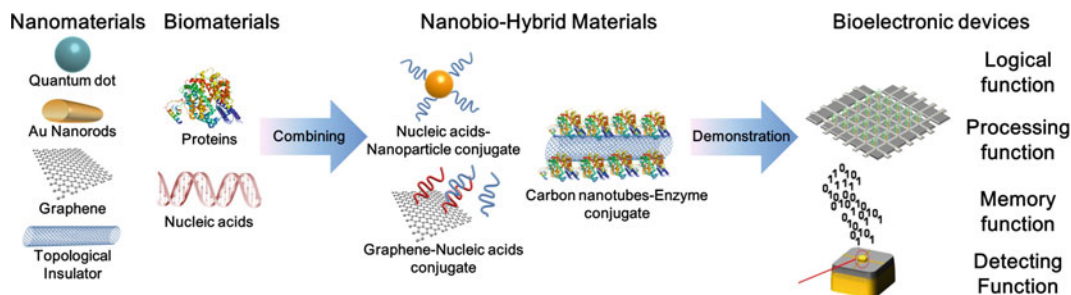
the elements that control information storage (Choi et al. 2007), determination, process (Benenson et al. 2004; de Silva and Uchiyama 2007), and logical behavior (Baron et al. 2006a; Fujibayashi et al. 2008; Win and Smolke 2008) provide appropriate functions to constitute the integrated molecular circuit (IMC). The advantages of bioelectronic devices include miniaturization, new functions, implantable devices that will be able to replace silicon-based (digitalized) systems in the future. Several groups have proposed the biocomputation concept (Adleman 1994; Ausländer et al. 2012; Rinaudo et al. 2007; Weber et al. 2008; Yin et al. 2008).

Bioelectronic devices for computation are usually composed of biomolecules such as metalloprotein (Chen et al. 2012; Lee et al. 2014b), enzyme (Katz and Privman 2010), DNA (Okamoto et al. 2004) and RNA (Win and Smolke 2008). These biomaterials have intriguing properties in nature, and their original properties have been mimicked in order to use computation on to the chip. For the immobilization of those biomolecules onto the inorganic substrate, the self-assembly technique was suitable for this application (Lu and Suo 2002; Schwartz 2001). Various groups have used biomolecules to demonstrate the logic gate (Baron et al. 2006b; Hild et al. 2010; Willner and Katz 2000; Zhang et al. 2013; Zhou et al. 2009), information storage device (Min et al. 2010; Yagati et al. 2009a, b, 2010; Yoon et al.

M. Mohammadniaei  
Department of Chemical and Biomolecular Engineering,  
Sogang University, Seoul, South Korea  
e-mail: [mniaei@sogang.ac.kr](mailto:mniaei@sogang.ac.kr)

C. Park · H. Sohn (✉) · T. Lee (✉)  
Department of Chemical Engineering, Kwangwoon  
University, Seoul, South Korea  
e-mail: [hsohn@kw.ac.kr](mailto:hsohn@kw.ac.kr); [tlee@kw.ac.kr](mailto:tlee@kw.ac.kr)

J. Min  
School of Integrative Engineering Chung-Ang University,  
Seoul, South Korea  
e-mail: [junmin@cau.ac.kr](mailto:junmin@cau.ac.kr)



**Fig. 17.1** Schematic diagram shows biomaterials and nanomaterials combination allow their integration in nanobio hybrid materials for bioelectronic devices

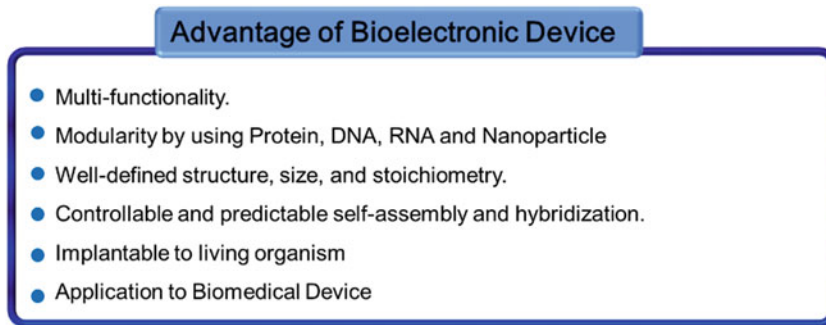
2014), transistor (Artés et al. 2012; Keren et al. 2003; Meng et al. 2011), computation device (Liu et al. 2000; Xie et al. 2011). Usually, those biomolecules were combined with nanomaterials such as nanoparticle, graphene and quantum dot that provide the multi-functionality (Luo et al. 2018) (Fig. 17.1).

Willner Group suggested several enzyme-based logic gates (Baron et al. 2006a; Itamar and Eugenio 2005; Katz and Privman 2010). They introduced a pair of enzymes, horseradish peroxidase (HRP) and glucose dehydrogenase (GDH), for gate performance. As an input material, hydrogen peroxide ( $H_2O_2$ ) and glucose were used to show the AND, XOR Gate functions. Recently, they proposed that the DNA computing circuit comprised libraries of DNAzymes. The system operated the parallel logic gate that depends on input markers. This operation process intends to regulate the anti-sense molecules and aptamer, which inhibit the enzymes. They provided a potential biochemical computer for the therapeutic control of biomedical applications. Furthermore, various enzyme-based logic gates were established (Baron et al. 2006b; Willner and Katz 2000; Zhang et al. 2013; Zhou et al. 2009). Also, Smolke group proposed that the information processing devices consisted of an RNA aptamer and RNA ribozyme (Win and Smolke 2008). The information processor received, processed and transmitted the input materials to express the green fluorescent protein as an output. In this study, the RNA aptamer and ribozyme combination can be used as the activating materials for a biomolecule-based

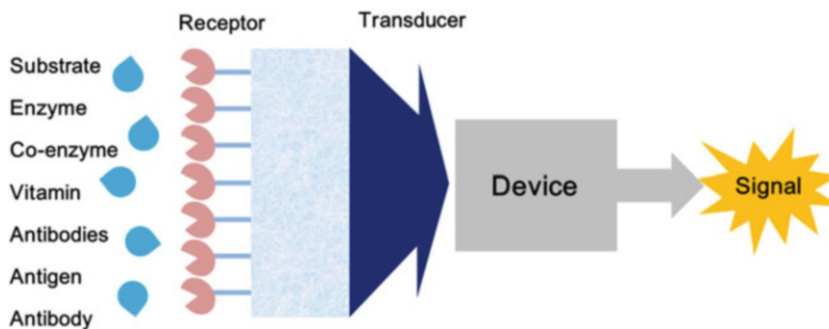
processor. Furthermore, some pioneering groups suggested the RNA or DNA molecule-based biocomputation systems for medical applications or cell system analysis (Liu et al. 2000; Weber et al. 2008; Xie et al. 2011). Huang's group demonstrated the protein-based transistor. For transistor fabrication, Huang developed the antibody-two gold nanoparticle complex immobilized onto the molecular gap that connected to the source and drain electrodes. This protein-based transistor provides a versatile platform for studying single molecule-based electronic devices. The fabricated bioelectronic device has the following advantage (Fig. 17.2).

Biosensors are powerful analytical devices, usually composed of biological sensing materials (bioreceptors) and physicochemical transducers (Fig. 17.3). These devices are employed to recognize and detect the desired target molecules with high specificity, selectivity and sensitivity either at the trace amounts or in the complex environments (Hunt and Armani 2010; Tamayo et al. 2013). Amongst all the sensing devices, electrochemical biosensors have received intensive attention for the detection of clinical biomarkers and analytes due to their fast response, low fabrication cost, high sensitivity and selectivity as well as simplicity and miniaturization capability, which have made them considerable candidates for the point-of-care diagnostics (Liu et al. 2017; Thévenot et al. 2001).

Electrochemical method is fundamentally based on the process of electron transfer between the electrode surface and the electroactive substances in solution (electrolyte). The electrode



**Fig. 17.2** Schematic diagram of main advantage of bioelectronics device



**Fig. 17.3** Schematic diagram of working mechanism of biosensor

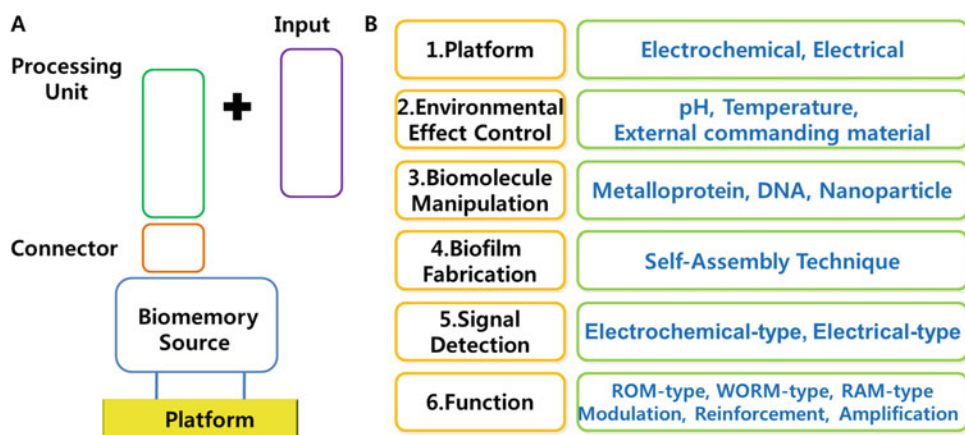
surface could be composed of metal (such as gold, platinum, etc.), conducting polymers, carbon or composite materials. The electroactive materials which are meant to have redox properties (oxidation/reduction) generally consist of ions, organic/inorganic materials and enzymes (Grieshaber et al. 2008; Pingarrón et al. 2008; Ronkainen et al. 2010; Wang 2002). Electrochemical biosensors are normally set up in a three-electrode electrochemical cell composed of working electrode (Target substrate, where the reaction of studied species occurs), counter electrode (To detect the electrochemical current/signal) and standard electrode, possessing a stable and fixed potential (Usually Ag/AgCl, due to its construction simplicity) (Pumera et al. 2007). The recorded electrochemical signal is directly proportional to the concentration of the electroactive species and is defined as the detection signal. There are mainly two analytical techniques applied for illustrating the experimental data; current vs. potential ( $i-v$ ) which is called

voltammetry, and current vs. time ( $i-t$ ) that is called chronoamperometry, in which, the working electrode is maintained at a constant potential (Kimmel et al. 2012).

As the major sensing element of biosensors, the biological sensing materials are substances with well immobilization properties to be attached strongly onto the electrode surface and high selectivity features to specifically detect the target analytes. They are mainly categorized in two groups: (1) Proteins and (2) Nucleic acids.

## 17.2 Protein-Based Electrochemical Bioelectronic Device

The protein-based biomolecular information storage device was proposed to alter the current silicon-based information storage device (Choi et al. 2007). Consequently, there are several types of biomemory devices proposed, for example, the WORM-type biomemory device (Yagati



**Fig. 17.4** (a) Schematic representation shows the expected structure of electrochemical bioelectronic device. (b) Classification of bioelectronics device constitution

et al. 2009b), multi-bit biomemory device (Yagati et al. 2009a), multi-level biomemory device (Lee et al. 2010), multi-functional biomemory chip (Lee et al. 2011a), signal-enhanced biomemory device (Yuan et al. 2013), and the bioprocessor (Ko et al. 2011). Such devices could control and modulate the electrochemical signal due to the redox property of metalloprotein in order to achieve the store and release the electron by external potential. Usually, the bioelectronic devices were classified with six categories (Fig. 17.4). This article briefly introduced device ranging from the basic concept of a biomolecular memory device to the various function validations of multi-functional biomemory devices and bioprocessors.

In previous time, they proposed the bioelectronic device composed of metalloprotein. The purpose of this electronic device was to accomplish electronic functions of the information storage. For this reason, Choi group introduced the redox protein for making biomemory device by self-assembly technique and validating the electrochemical biomemory functions (Choi et al. 2007). Recently, the protein/DNA-based bioprocessor was demonstrated to show the multi-functionality in one defined devices corresponding to input materials (Lee et al. 2014b). Here, we review from the protein-based biomemory device, protein/DNA-based bioprocessor, also briefly

survey DNA/RNA biologic gate and device which could be one of alternative standard device format in biocomputation system.

### 17.2.1 Protein-Based Information Storage Device

In the early stage, the metalloprotein-based biomemory device was proposed to overcome the limitation of inorganic molecule-based information storage device by Choi group (Choi et al. 2007). The metalloprotein contained the metal ion in the protein molecule that can be enabled to store the electron corresponding to input potentials. To fabricate the biomemory device, the immobilization and orientation of biomolecule technique should be required. To immobilize the biomolecule onto the inorganic substrate, a self-assembly (SA) method has been widely used to immobilize biomolecules on the substrate (Mitsumasa et al. 2010). The immobilization of biomolecules on the substrate needs an additional linker which anchors between the biomolecule and the substrate. Chemical linkers like (3-aminopropyl)triethoxysilane (APTES), 2-mercaptoacetic acid (2-MAA) 6-mercaptohexanoic acids (6-MHA) can be used to make connections between the gold substrate and the biomolecule (Chung et al. 2011; Robles-Águila et al. 2014; Yoo et al. 2011). However, direct

immobilization of biomolecules on the substrate without chemical linkers is more effective for the fabricating a well-ordered biomolecular monolayer than the use of chemical linkers. To achieve direct immobilization, a cysteine-modified azurin was introduced as an electron storage element to fabricate a biomolecular memory device. On the basis of this technique, *pseudomonas aeruginosa* azurin was modified to possess cysteine residue for direct immobilization on the gold surface by covalent bonding. This recombinant azurin was immobilized directly on the gold substrate, and its orientation was investigated by atomic force microscopy (AFM) and surface plasmon resonance (SPR). Then, the redox property of azurin was investigated by cyclic voltammetry (CV).

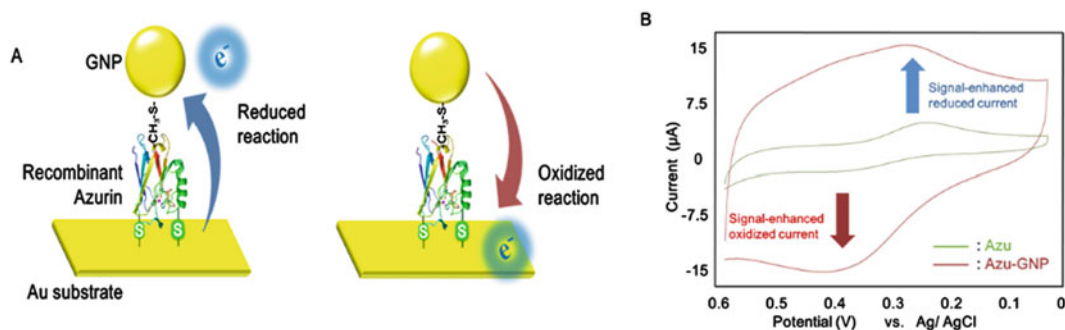
The basic mechanism of the proposed biomolecular memory device is that electrons flow into the recombinant azurin. A reduced copper ion in azurin gives the 'write' state and outflowing of electron from the recombinant azurin, and the oxidized metal ion in azurin gives the 'erase' state. Like this process, based on metalloprotein, electrons can flow in and out of the recombinant azurin by applied voltage. The quantity of the stored charge can be calculated as the memory performance of the fabricated biomolecular memory device using chronoamperometry (CA). Also, the recombinant azurin showed unique redox potential peaks and memory functions. From experimental results, this proposed biomemory device indicates new conceptual approach to bioelectronic application. Furthermore, the biomolecular memory devices have been developed by several groups (Ko et al. 2011; Mitsumasa et al. 2010; Yuan et al. 2013). Yu group suggested the electrochemical based biomemory device based on living bacteria *Shewanella oneidensis* (Yuan et al. 2013). Furthermore, Cho group investigated the electrical bistable property of ferritin to develop a resistive memory device application (Ko et al. 2011). Among those biomolecules, metalloprotein was widely investigated to use a biomemory source due to its bistable redox properties (Choi et al. 2007).

The main advantage of biomemory is functionality compared to inorganic molecule-based memory device. Usually, the biomolecule can be

easily tailored and conjugated with other biomolecule or nanoparticle that can provides the additional functionality such as signal-amplified current or dual-level information storage. It is hard to perform the inorganic molecule-based memory device and silicon-based memory device because of simple molecular structure. However, the biomemory can be extended to the various functionalities based on combination of biomolecule and nanoparticle. The natural property of a biomolecule can be modified and extended by introduction of nanoparticles. Yang's group conjugated the tobacco mosaic virus and quantum dot nanoparticles, and electrical investigation of that virus/nanoparticle conjugates for digital memory application (Tseng et al. 2006). Moreover, the functional benefits of a biomolecule are its original redox property and recombinant technique for feasible formation that can be applied to bioelectronics devices.

The gold nanoparticle on the recombinant azurin monolayer was developed an electrochemical signal enhanced biomemory device (Lee et al. 2011b) (Fig. 17.5). In this study, they introduced various gold nanoparticles to recombinant azurin monolayer (5 nm ~ 60 nm) to optimize the gold nanoparticle size which transfer the maximum electron transfer. For this reason, the recombinant azurin was immobilized directly on the gold substrate by cysteine residue, and 1-Octadecanethiol was used as a connector between the recombinant azurin and gold nanoparticle. From the electrochemical results acquired by CV, in a small particle range, the electrochemical signal of recombinant azurin/gold nanoparticle decreased, but in a large particle range, the electrochemical signal only originated from a gold nanoparticle without recombinant azurin. Therefore, 5 nm size of gold nanoparticle was determined as the optimal size. After that, biomemory device composed of recombinant azurin and gold nanoparticle was fabricated on the gold substrate. The confirmation of recombinant azurin and gold nanoparticle immobilization was verified by SPR and AFM. Then, the electrochemical investigation of the recombinant azurin/gold nanoparticle was carried out to evaluate the electrochemical signal enhancing effect compared to a recombinant azurin





**Fig. 17.5** (a) Scheme of the proposed signal-enhanced biomemory device composed of recombinant azurin and gold nanoparticle. (b) Cyclic voltammogram of recombinant azurin (green plot), recombinant azurin/gold nano particle

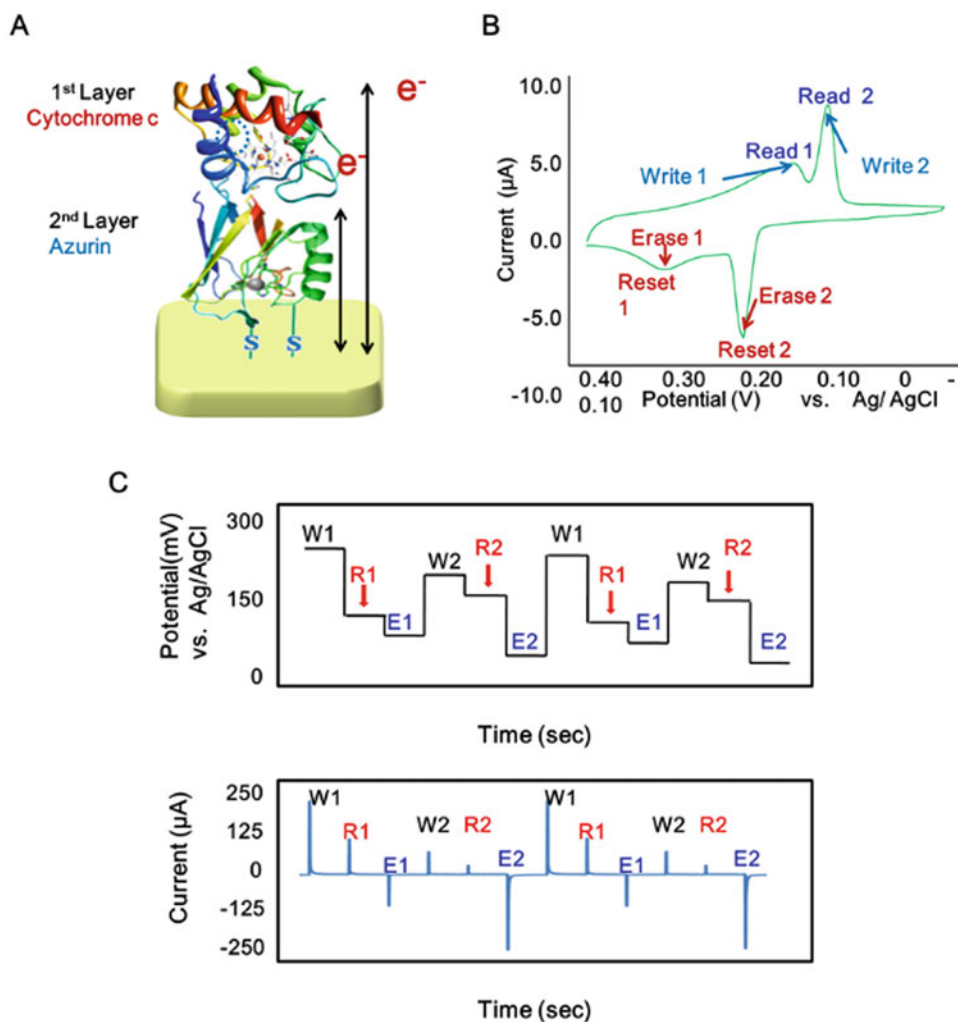
(red plot). (Figures adapted from Lee et al. (2011b), with permission from © WILEY-VCH Verlag GmbH & Co. KGaA 2011)

monolayer without a gold nanoparticle by CV and CA. The electrochemical signal of the recombinant azurin/gold nanoparticle was five times greater than the recombinant azurin monolayer. Also, the stored charge amount of the recombinant azurin/gold nanoparticle, measured as memory performance, was  $4.503 \mu\text{C}$ , and  $1.14 \mu\text{C}$  in the case of the recombinant azurin monolayer. This signal enhanced biomemory device suggested the possibility of bioelectronic development at a single molecular level with subjugation of a signal detecting limitation. As seen in this research, biomolecules have a huge potential with limitless functional expansion through the combination of various materials (Jensen et al. 2009).

In the conventional field of electronics, researchers have tried to improve memory density and circuit-integration efficiency to develop an advanced computing system. However, as the approach applies a scale of a less than 50 nm for fabricating semiconductor-based chip, economic and technical limitations have been identified. In terms of the field of bioelectronics, a Moreover, the biomemory can be developed to increase the memory density in the defined area. Biomolecular based electronic system might be an alternative option to overcoming this limitation. This combination can be used to realize a biomolecular memory device with improved memory density.

The multi-level biomolecular memory device composed of recombinant azurin and cytochrome

c to increase memory density with multiple redox states (Lee et al. 2013) (Fig. 17.6). The heterolayer composed of recombinant azurin and cytochrome c was fabricated through self-assembled layer-by-layer formation on the gold substrate. First, recombinant azurin was immobilized directly on the gold substrate, and then cytochrome c was immobilized on the recombinant azurin layer through electrostatic interaction. At pH 7.0, the isoelectric point of recombinant azurin was 6.03 and that of cytochrome c was 9.59, so recombinant azurin had a negatively-charged surface and cytochrome c had a positively-charged surface. Confirmation of the heterolayer formation was operated by SPR and AFM. After biochip fabrication, the electrochemical properties of the heterolayer were investigated. Using CV, the heterolayer showed that both redox peaks of recombinant azurin and cytochrome c had an obvious shape. The oxidation potential peak and reduction potential peak of recombinant azurin and cytochrome c were 0.062 V, 0.131 V and 0.131 V, 0.294 V, respectively. These redox values coincided with copper ion in recombinant azurin and iron ion in cytochrome c. Two different metal ions in recombinant azurin and cytochrome c played key roles as storage for controlling the various data in defined memory sector. Those acquired redox potential values were used as oxidation potential for the 'write' and reduction potential for 'erase', and open circuit potential was applied as the 'read'



**Fig. 17.6** A schematic diagram represents (a) The electron transfer mechanism of a cytochrome *c*/recombinant azurin heterolayer on Au surface. (b) Cyclic voltammogram of cytochrome *c*/recombinant azurin heterolayers. (c). Multi-

level biomemory performance by OCPA. (Figures adapted from Lee et al. (2010), with permission from © WILEY-VCH Verlag GmbH & Co. KGaA 2010)

step. Accordingly, the multi-level memory function was evaluated by open circuit potential amperometry (OCPA). From OCPA results, the fabricated heterolayer showed exceptional multi-level memory performance by applied potentials. This biomolecular memory device offered the potential of a biomolecular based memory device with high memory density. Recently, they proposed a new method to fabricate a multi-level biomolecular memory device (Lee et al. 2014c). As seen in this section, the combination of

metalloproteins and nanoparticles can be applied to develop the functional biomemory devices.

### 17.2.2 Enzyme-Based Logic Gate

As seen in the part of the front section, biomolecules have been widely used for bioelectronic devices including information storage device. Furthermore, those materials also have been introduced to develop the biologic gate. In conventional electronic field, logic gates

have been developed to act as performing component in electronic device for logical operation such as Boolean function using field-effect transistor (FET), metal–oxide–semiconductor field-effect transistor (MOSFET) (Lee et al. 2015; Tomohiro et al. 2004).

However, with the miniaturization of electronic devices, scale down of logic gate has been researched in electronic researching areas. Thus, molecular-based logic gate or nanomaterial-based logic gate was developed to demonstrate logic function at molecular level (de Ruiter and van der Boom 2011; de Silva and Uchiyama 2007; Huang et al. 2001). Biomolecules also had been researched as a candidate to apply logic gate with miniaturization (Bychkova et al. 2010). However, in recent years, biomolecules have been recognized as a logic component because of the new advantages compared to conventional logic devices. The first advantage is that biomolecules can offer the possibility of homogeneous system fabrication to develop the uniform three-dimensional logic system compared to two-dimensional solid-state device which is widely used (Gdor et al. 2013). Thus, biomolecules-based system can provide the integration of complex reacting process for the development of high-order logic gate (Katz 2015). The second is that biomolecules-based logic gate can be operated by various input signals and output signals including light energy instead of electronic input signal (Prokup et al. 2012). Thus, biomolecules-based logic gate may not be bound to the electronic system for logic operation.

Among various biomolecules, enzyme and DNA have been widely used for biologic gate development. The advantage of enzyme used for logic gate is that various input signals can be used according to the used enzymes and chain reaction by related enzymes can be used for logic function. Thus, the complex logic systems have been demonstrated by enzyme (Baron et al. 2006a). In case of DNA, DNA can be used to build the complicated geometric structures by specific binding and conformational changes of DNA which is able to develop logic gate only using DNA itself (Okamoto et al. 2004; Seelig et al. 2006). In addition to these biomolecules, bacteria

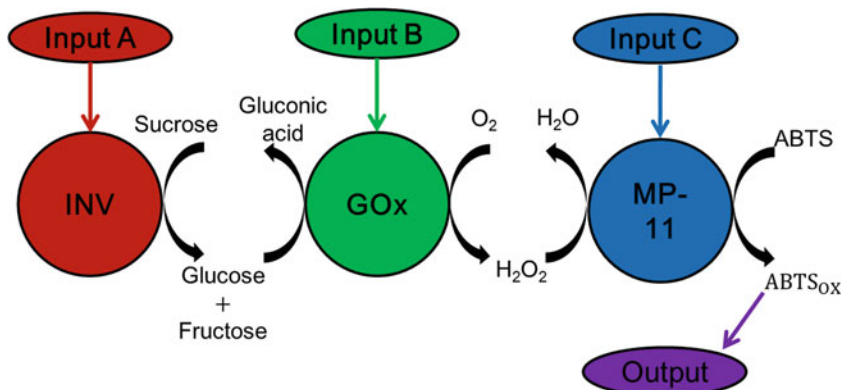
also have been investigated for logic gate fabrication (Arugula et al. 2012).

Enzyme has been used in bioelectronics field due to its unique properties like structure-folding and specific interaction with the substrate. Based on the property of enzyme, different output materials and signals can be induced by different input substrate injected into the enzyme. Thus, various combination of enzyme and the substrate have been used to develop logic gates like “AND” or “NAND” logic gate (Zhou et al. 2009). Katz group has been developed various functional enzyme-based logic gates. They developed the Boolean logic gate using enzymes including glucose oxidase, glucose dehydrogenase as input signals for logic operation (Strack et al. 2008b).

They developed the concatenated enzyme-based logic gate by highly specific recognition chain reactions using invertase, glucose oxidase and microperoxidase-11. Figure 17.7 shows the schematic mechanism of this logic gate. Sucrose, glucose and hydrogen peroxide were used as input materials. These input signals were considered “1” when they were existed and “0” when they were absent. To measure the output signal of this logic gate, the absorbance change of ABTS, biocatalytically oxidized dye, was used with defined threshold value ( $O.D = 0.3$ ). Thus, the output signal with overthreshold was defined as “1”, otherwise “0”. According to the associated chain reaction of this logic system, only when all three inputs were injected, the output signal overpassed the defined threshold value. So, only (h), defined as “1,1,1”, showed the “1” as the output signal (Strack et al. 2008a). This logic system, where individual reactions were interrelating and input materials were compatible, could be used as an alternative solution for assembling complex logic process which is difficult to demonstrate with synthesized chemical molecules due to limitation of synthetic complexity and scale up.

In addition to the logic gate based on interaction of enzyme and input substrate, there exists the other type of logic gate using induced folding and unfolding of polypeptide chain in protein (Deonaraine et al. 2003; Muramatsu et al. 2006).





**Fig. 17.7** Schematic mechanism of the developed concatenated enzyme-based logic gate by highly specific recognition chain reactions using invertase, glucose oxidase and microperoxidase-11

Looking in detail, structure of genetically and chemically engineered chaperonin azo-GroEL was changed by ATP and light. According to light as input signal, photomechanical gate of GroEL was induced to trans-to-cis isomerization and cis-to-trans isomerization by UV and visible lights, respectively. Also, its geometrical structure was changed by ATP. Using these two inputs, “AND” logic gate was developed.

Besides these logic gates, enzyme-based logic gates using interfacial pH change (Pita et al. 2009a), enzyme-functionalized nanoparticles (Pita et al. 2008) and supramolecular enzyme-hydrogel hybrids (Ikeda et al. 2014) were developed. These enzyme-based logic gates offer a huge potential for application in wide areas including clinical field for drug delivery and physiological conditioning assessment (Mailloux et al. 2014; Pita et al. 2009b; Radhakrishnan et al. 2013).

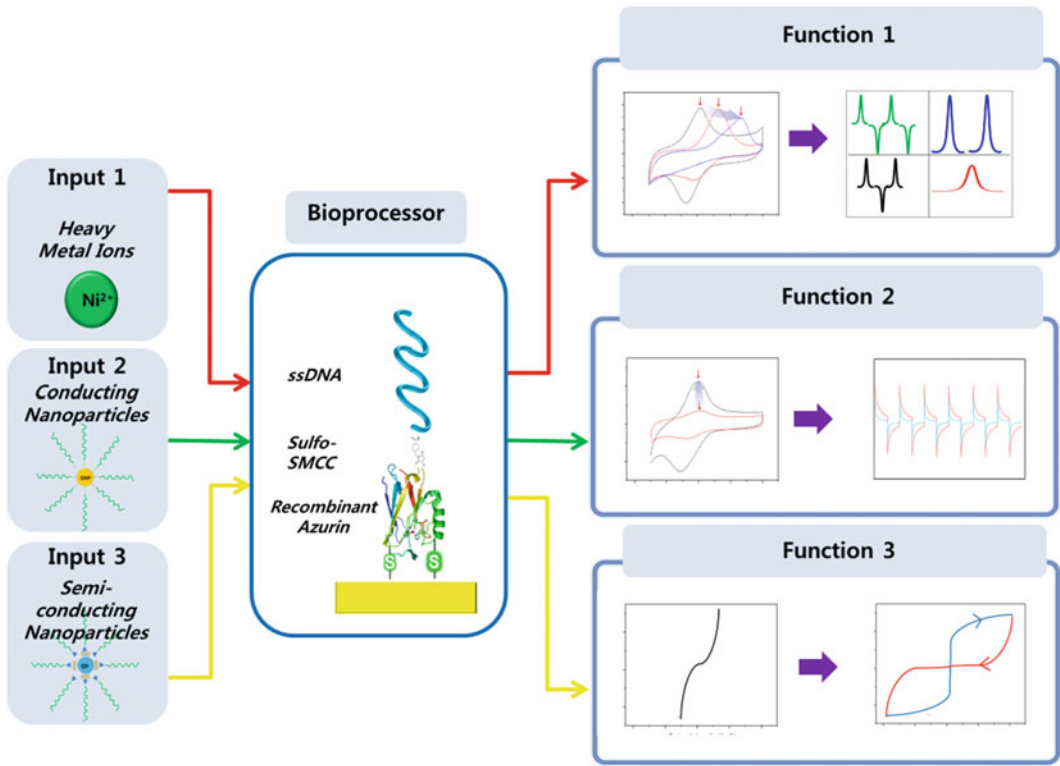
### 17.2.3 Protein-DNA-Based Bioprocessor

The conventional information processor is a programmable device that performs the various functions according to input data, then, the proper output produced-based on defined functions. The biomolecule can be applied to construct the bioprocessing device. The protein-DNA hybrid molecule-based information-processing device

was developed for mimicking the information process in cellular signal (Lee et al. 2014b). The proposed bioprocessing device performed three functions: ‘information regulation’, ‘information reinforcement’, and ‘information amplification’. The information process system is based on the biomemory platform consisting of metalloprotein/DNA hybrids, and could be regulated by surrounding commands (metal ions, conducting nanoparticles and semiconducting nanoparticles) (Fig. 17.8). The core material (redox material) in the biomemory platform was re-engineered from a simple metalloprotein into protein/DNA hybrids to receive the surrounding’s commands and to store the information based on input.

The azurin was rolled as the memory core and the ssDNA can be used as the processing receptor. When cDNA-nanoparticle or various metal ions are hybridized, the ssDNA can be hybridized or intercalated with metal ions for bioprocessing functions. The information reinforcement and information regulation functions were validated based on input materials by the chronoamperometry (CA) method. The ssDNA arm has a charged backbone that can bind to various heavy metal ions, such as Cu, Zn, Ni, Co, Fe, Mn.

To assess the information amplification function, a scanning tunneling spectroscopy experiment was carried out on the recombinant azurin/DNA hybrid when cDNA-quantum dot (QD: CdSe-ZnS) added to bioprocessor. In the case of



**Fig. 17.8** Schematic diagram of bioprocessing device comprised with recombinant protein/DNA hybrid corresponding to input materials. That shows the proper pre-defined functions

the recombinant azurin/DNA hybrid, the I-V result shows the semiconductor behavior, as after 0.2 V of the applied bias, the recombinant azurin/DNA hybrid shows a non-ohmic behavior. However, in the case of the recombinant azurin/DNA-cDNA/QD complex, the result shows that the bi-electrical stability ranges from  $-2.0$  to  $+2.0$  V. In this case, the recombinant azurin/DNA hybrid-biotin-tagged cDNA/streptavidin-coated QD conjugate is initially in a low conducting state until it reaches about 0.8 V. After 0.8 V, the I-V curve drastically changed which indicates a transition of the recombinant azurin/DNA hybrid /biotin-tagged cDNA/streptavidin-coated QD complex from a low conducting state to a high conducting state. This state change can be defined as ‘information amplification’. Thus, the protein/DNA-based bioprocessor has complex functionality with the suggested concept and this functionality can be extended with new input materials such as

graphene, nanoparticles, proteins and RNA. This concept provides the possibility for biomolecular-based computing systems; analog style memorizing, fuzzy type determining, and the environment are affected.

### 17.3 Nucleic Acid-Based Bioelectronic Device

Nucleic acid has been received attention due to its functionality and programmability. By specific recognition and hybridization with complementary nucleic acid sequence, Especially, DNA has become an attractive biomolecule for bioelectronics application including biosensor and biologic gate (Campolongo et al. 2011). Widely studied DNA logic gate is based on the specific-sequence recognizing and binding property of DNA itself. For example, in DNA hybridizing mechanism, longer-specific

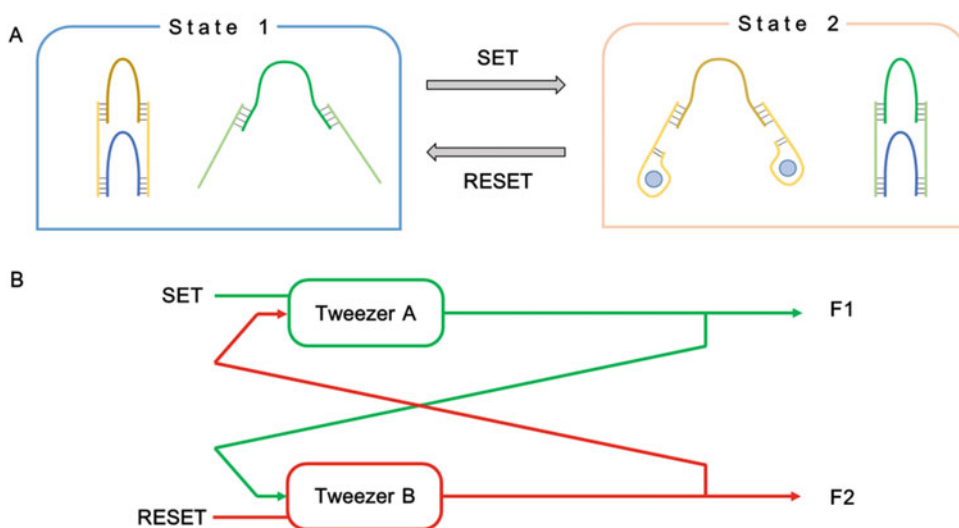
complementary DNA can disrupt the hybridized DNAs formed with shorter-specific complementary DNA. Then, longer-specific complementary DNA replaces the shorter-specific sequence and shorter-specific complementary DNA is de-hybridized from interaction. This mechanism is fit to develop logic gate like DNA displacement-based logic gate (Frezza et al. 2007). In the field of DNA logic gate, Willner group and Winfree group have developed various logic gates based on functionality of DNA (Liu et al. 2012b; Seelig et al. 2006).

### 17.3.1 DNA-Based Logic Gate

Willner group designed and developed the aptamer-based DNA tweezer structure for “SET-RESET” logic demonstration (Elbaz et al. 2009a) (Fig. 17.9). This DNA tweezer, composed of four different nucleic acids, could be trans-shaped its structure, opened shape or closed shape, by insertion of specific materials such as adenosine monophosphate (AMP), adenosine deaminase (AD) and inosine monophosphate. This DNA tweezer possessed fluorescence dye (Cy5) and quencher (Iowa black RQ) located on tweezer frame nucleic acid. Thus, in the case of closed

shape, fluorescence intensity was quenched, but in the case of opened shape, high fluorescence intensity was detected. By this mechanism, shape-change of DNA tweezer was verified through fluorescence intensity. Two different but related DNA tweezers which were called “Tweezer A” and “Tweezer B” respectively, possessing two different dyes and quenchers each other, were used to develop “SET-RESET” logic gate in this paper, and these tweezers were trans-shaped in contrast to each other. By addition of AD, the system composed of two different DNA tweezers could become “state1” as defined “RESET”, and the system could become “state2” by addition of AMP as “SET”. Each state was confirmed by fluorescence intensity. Figure 17.9a, b show the schematic image and process of this logic system and “SET-RESET” logic data with exist of two different states.

In addition to this research, G-quadruplexes, one type of DNA sequence composed of stacks of guanine tetrads by Hoogsteen hydrogen bonding, have been wide used for DNA logic gate due to the easy modulation of DNA structure under benign conditions (He et al. 2013; Wang et al. 2012). For example, G-quadruplex-hemin complex was formed by the insertion of potassium ion and hemin into G-quadruplex, then specific



**Fig. 17.9** (a) Scheme of coherent activating logic gate of two tweezers using adenosine monophosphate and adenosine deaminase as input materials. (b) Schematic diagram of the SET-RESET system

structure was formed and hemin used as electrochemical probe was inserted inside that structure. That change could be detected by electrochemical technique. Using this mechanism, “AND” logic was demonstrated. Both potassium and hemin were added to G-quadruplex, then the output signal was passed over the defined threshold value, 1, however, addition of only one of them to G-quadruplex showed the under-threshold value, 0. Also, the output signal was under-threshold value without potassium ion and hemin (Wang et al. 2012).

In addition to these achievements, pH, nanoparticles have also used to fabricate DNA logic gates similar to enzyme-based logic gates (Elbaz et al. 2009b, 2012; Freeman et al. 2009). Also, there exists logic gate using mismatching of hybridized DNA, insertion of mercury ion in thymine–thymine (T–T) mismatch in hybridized DNA and insertion of iron ion in cytosine–cytosine (C–C) mismatch in DNA duplexes, for electrochemical logic outputs (Li et al. 2011).

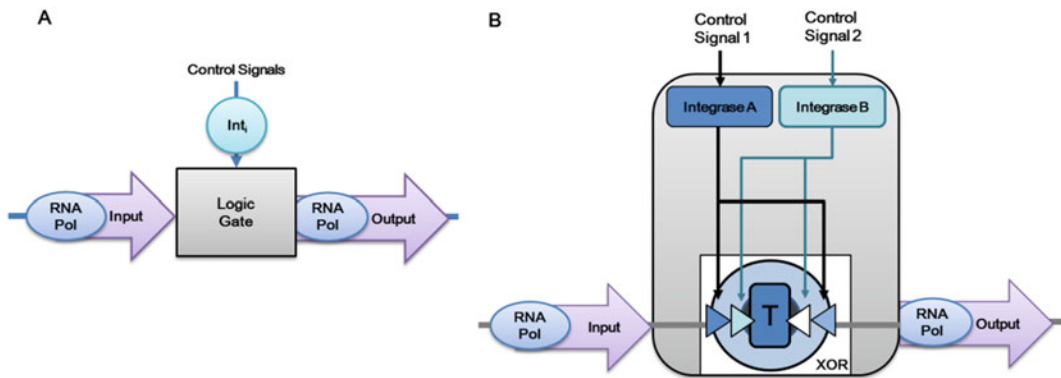
These developed DNA logic gates can be applied for DNA computing development. Winfree group developed the digital circuit computation with multilayer circuits by DNA displacement cascades within all logical operation (Qian and Winfree 2011). Furthermore, using DNA displacement cascades, they developed the DNA computing system mimicking neural network computation which even showed the property of Hopfield networks associative memory (Qian et al. 2011). As seen in this chapter, biomolecules, especially enzyme and DNA, have been widely investigated for the development of logic gate. There is also the case of programmable DNA-enzyme conjugates fabrication for logic gate (Gianneschi and Ghadiri 2007). These biomolecular logic gates give a chance to develop the effective complex computing functions demonstration for biocomputer system development (Ogihara and Ray 2000), also enrich the life of mankind due to the expanded application of biomolecules in clinical field (Mailloux et al. 2014; Pita et al. 2009b; Radhakrishnan et al. 2013).

### 17.3.2 RNA-Based Biologic Gate

The RNA molecule is a powerful source for constructing molecular logic gate owing to its intriguing characteristics. Compared to DNA molecule, RNA has various functionality and applications (Haque et al. 2012; Jaeger and Chworos 2006). Those functionalities of RNA molecules were originated from the proper folding and assembly of RNA molecule tertiary structures using the formation of hairpin loops, dove-tail, bulges, and internal loops. Those tertiary structures of RNA give a unique functionality such as aptamers, ribozymes, and riboswitches (Grabow and Jaeger 2014). These functional RNA molecules can be easily designed to constitute the logic gate core for performing specific function such as gene expression, diagnosis, or cell signaling (Benenson et al. 2004; Rinaudo et al. 2007). The RNA-based logic gate is usually activated through the RNA hybridization or displacement that gives conformational change or ligand binding according to input molecule (Benenson 2009; Xie et al. 2010).

Benenson group reported the RNAi-based logic evaluator to perform Boolean logic behavior-based on to input molecules (Rinaudo et al. 2007). The constituted biological circuit was composed of a couple of mRNA species to produce the fluorescent protein in human kidney cell. Those mRNA species were constituted with the different non-coding region to perform the logic behavior. Then, the specific designed DNA contained plasmid was applied to control the gate function in the cell through transfection. The siRNA was used to regulate the mRNA degradation as the gate input. Then, the mRNA produced the fluorescence protein corresponding to input signal and this expression of fluorescence protein level was used to the output signal.

Recently, the field-effect transistor structure-based genetic RNA logic gate was suggested (Bonnet et al. 2012, 2013). The bacteriophage serine integrase was used to regulate the state of double stranded DNA. Interestingly, this study defined the input and output signals are the



**Fig. 17.10** (a) Three-terminal transistor based gates use integrase (Int) control signals to modulate RNAPolymerase (RNA Pol) flow between a separate

gate input and output. (b) The logic element within a three-terminal Boolean integrase XOR gate such that gate output is high only if control signals are different

transcription rates of the flow of RNA polymerase according to DNA at the logic element boundaries (Fig. 17.10). The integrase-serine control invert or delete the DNA encoding transcription, thus terminating or promoting the transcription rates. With this mechanism, the AND, OR, XOR, NOR and XNOR gates have been fabricated with one/two asymmetric transistor. Those results demonstrated RNA molecule can be extended to construct new concept of logic gate which is hard to achieve in molecular logic gate.

### 17.3.3 RNA-Based Bioprocessor

The RNA has a unique functionality such as catalytic property, recognition, self-folding, self-splicing and etc. (Grabow and Jaeger 2014; Haque et al. 2012; Jaeger and Chworos 2006). Those properties of RNA molecule can be extended to use of bioprocessor unit (Benenson 2009; Win and Smolke 2008). Usually, the bioprocessor composed of RNA molecule can be received the chemicals or RNA sequences and it process the information. The RNA molecule can be used to the molecular information processor operating in living systems with a biological environment (Rinaudo et al. 2007; Win and Smolke 2008), RNA-based

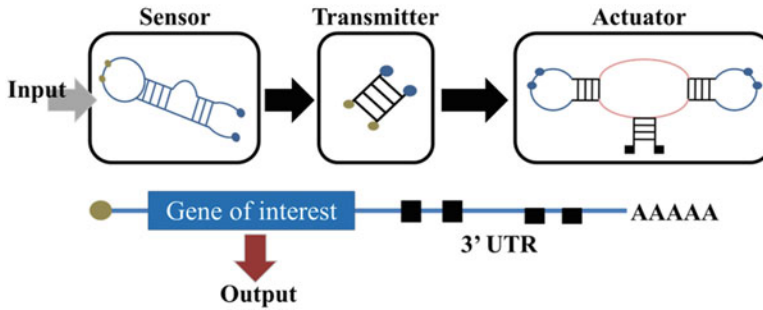
biocomputation system would be powerful alternative to solve the current limitation of silicon-based computation, for example, (1) RNA molecule-based computation can be directly used to diagnostic system such as cancer or hereditary disease. (2) The combination of RNA-molecule can be used to detect RNA-related virus sensing system (3) it may provide new type of computation that gives the analogue-based result corresponding to RNA input material. (4) the silicon-based computation system is hard to operate in a living organism (Xie et al. 2010).

The synthetic RNA-based information processing devices was fabricated to perform the logic gates, signal filtering, and cooperativity functions (Win and Smolke 2008). The RNA-based bioprocessor that constituted with ribozymes and RNA aptamers was received the molecular input. Then, the processed input was transmitted to control the expression of the green fluorescent protein as output. The RNA aptamer that rolled as the sensor part was composed of a hammerhead ribozyme for cleaving of the aptamer. Also, the information transmitter part has the complementary RNA sequences for binding to RNA aptamer and ribozymes parts. Like this, RNA molecule can be used to bioprocessor module to perform the multi-functional information processor development (Fig. 17.11).



## Functional composition of an RNA

### *Modular components and information flow*



**Fig. 17.11** Functional RNA device composition framework. The color scheme for all figures is as follows: brown, aptamer or sensor component; purple, catalytic core of the ribozyme or actuator component; blue, loop regions of the actuator component; green and red, strands within the transmitter component that participate in the competitive hybridization event. A functional composition

framework for assembling RNA devices from modular components. Information in the form of a molecular input is received by the sensor and transmitted by the transmitter to a regulated activity of the actuator, which in turn controls the translation of a target transcript as an output

The interesting concept of RNA-based autonomous bioinformation processor was reported to program a biomolecular computing device to work inside a living cell (Ausländer et al. 2012). In this study, the autonomous biomolecular information processor was fabricated to control the disease-related gene expression for small-cell lung cancer and prostate cancer detection system. To operate the autonomous bioinformation processor, the regulation of specific mRNA level and chemicals level should be required to control the point mutation as the input material. Then, the bioprocessor gives a short ssDNA which control the gene expression level for anticancer effect as the output. The automation bioprocessor regulates 'positive state' and 'negative state' corresponding to specific gene expression level. They demonstrated RNA-based computation system can be directly applied to the gene expression control system for future diagnostic detection. Like this, the advances in RNA-based information processing system demonstrate the promise for biocomputation with new functionality.

### 17.3.4 RNA-Based Biomemory

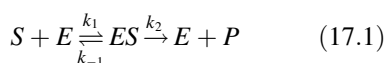
In recent years, Choi's group reported the RNA and semiconductor nanoparticle hybrid can be used to the resistive memory device application (Lee et al. 2015). To construct the resistive memory device, the thermodynamically stable pRNA 3WJ from the phi29 DNA packaging motor was used and conjugated with quantum dot nanoparticle (CdSe-ZnS). The pRNA 3WJ was easily conjugated with the quantum dot using Sephadex G100 resin-recognized RNA aptamer-based site-specific conjugation method. The prepared pRNA 3WJ/QD hybrid was immobilized onto Au substrate by self-assembly technique. The pRNA 3WJ was rolled as the connector between QD nanoparticles and Au substrate for the resistive memory performance. Furthermore, the pRNA 3WJ rolled as the insulator between the QD and Au substrate. As a semiconductor, the QD was rolled to storing the electron for memory function. And, the metal Au substrate was rolled to

m. The electrical bi-stability property (I-V curve) of pRNA 3WJ/QD hybrid was confirmed by scanning tunneling spectroscopy (STS). As a result, the pRNA 3WJ/QD hybrid exhibited the resistive memory property. The proposed resistive memory device using a combination of RNA and nanoparticles can be applied to bioinformation storage device.

## 17.4 Protein-Based Electrochemical Biosensor

Protein-based electrochemical biosensors (ECBs) can be divided into two classes of enzyme-based and antibody-based biosensors (Vestergaard et al. 2007). However, in comparison with immunosensors, enzymatic biosensors have been most regularly used in disease diagnosis and point-of-care applications. They are biological catalysts and can be exploited in the purified forms and be engineered for the particular reactions. Whereas, antibodies (used for immunosensors) are non-catalytic biological elements which are well capable to specifically bind with their corresponding antigens. Although, they have a very high specificity, their applications are limited and their handling needs a considerable experimental proficiency (Li et al. 2009; Ramanavičius et al. 2006; Rocchitta et al. 2016).

Enzymes are large macromolecules, mostly proteins, which usually harbor prosthetic groups (one or more metal ions). These metal ions enable the enzymes to undergo oxidation and reduction upon the reaction with their corresponding analytes. This redox action which is corresponding to the presence of the analyte, can be detected electrochemically as the function of enzymatic ECBs. The basic mechanism for enzyme catalysis is as follows:



Where, S is substrate, E is enzyme, ES is enzyme/substrate complex, and P is product.

The enzymatic ECBs are highly selective and fast with high sensitivity due to their catalytic activities and they can be effectively immobilized onto the substrate (transducer) due to their 3D structure. However, they are relatively expensive and still suffering from the loss of activity after a prolonged usage, due to the deactivation and/or substrate detachment (Rocchitta et al. 2016).

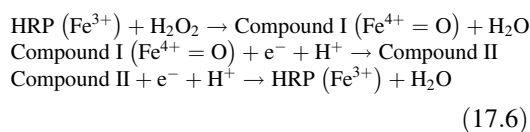
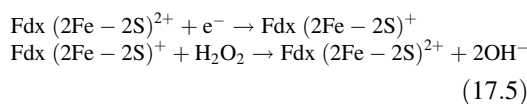
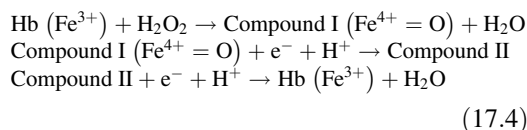
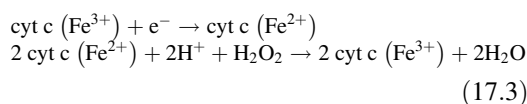
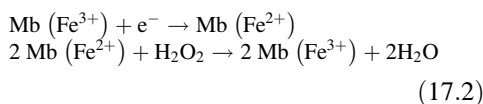
Basically, analytes can be directly oxidized/reduced at ordinary solid electrodes. However, employment of conventional electrodes have been restricted because of their slow electron transfer kinetics and high overpotentials, which reduce the sensing performance of the biosensors (Pumera et al. 2007). It has been reported that, incorporation of enzymes with nanostructured solid electrodes can enhance the electron-transfer rate between the modified electrode and the solution interface (Wang 2005). Moreover, the size and structure of enzymes can be engineered for the further amplification of sensing performances (Dolatabadi et al. 2011; Malekzad et al. 2017). On the other hand, enzyme immobilization onto the electrode surface is a very important issue to be studied. The successful immobilization normally requires; (i) enzyme stability and specific affinity towards the surface, (ii) surfaces uniformity, (iii) maintenance of the natural enzymes' biological functions, and (iv) controlling the enzyme orientation for the achievement of maximum surface density (Zhang et al. 2009).

There are usually two methods of immobilization: (1) Indirect immobilization; (2) Direct immobilization. The indirect immobilization technique is based on the modification of the electrode surface with suitable linkers for the establishment of functional groups to be further used for the enzyme binding reaction. Whereas, the direct immobilization method does not require extra chemical linkers and involves either physical adsorption or specific interaction between the enzyme and the electrode, such as immobilization by means of Au-thiol (sulfhydryl) interaction (Rao et al. 1798; Singh et al. 2016; Wong et al. 2009). Although, the enzyme-based ECBs have been utilized for the detection of various toxins and analytes, such as glucose (Wang 2008), urea (Chen et al. 2011; Cho and Huang 1798), nitric

oxide (Nagase et al. 1797; Yoon et al. 2017b) and hydrogen peroxide, here we demonstrate some examples of enzyme-based ECBs for the detection of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ).

#### 17.4.1 Protein-Based Electrochemical Biosensor for $\text{H}_2\text{O}_2$ Detection

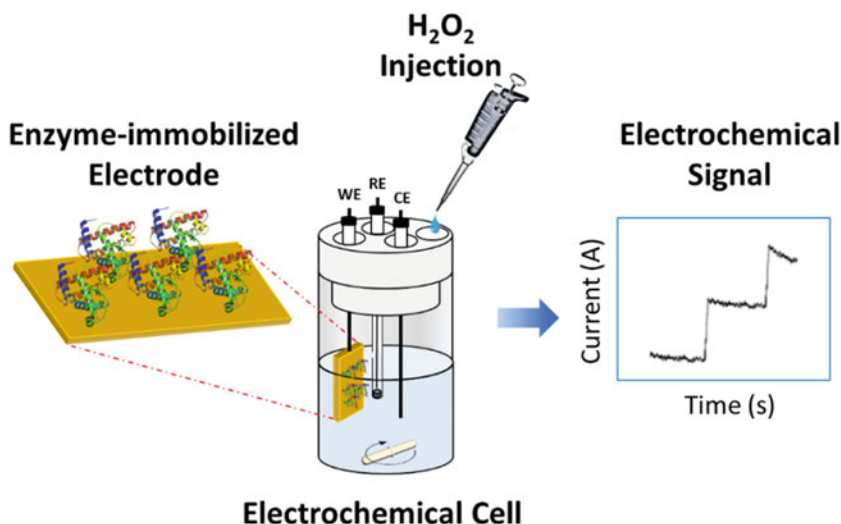
Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is an important biomarker of the major reactive oxygen species (ROS) whose mediated pathways have been related to various bodily disorders such as neurodegenerative diseases, Alzheimer, asthma, cancer and inflammatory arthritis (Andre et al. 2013; Giorgio et al. 2007; Rojkind et al. 2002; Schalkwijk et al. 1786). Therefore, detection of low concentration of  $\text{H}_2\text{O}_2$  in a rapid and selective fashion is highly demanding. Up until now, many enzymes (redox-active proteins) have been exploited to develop various  $\text{H}_2\text{O}_2$  ECBs, such as cytochrome c (cyt c), Myoglobin (Mb), Hemoglobin (Hb), Ferredoxin (Fdx) and Horseradish Peroxidase (HRP). Their corresponding electrocatalytic reactions towards  $\text{H}_2\text{O}_2$  is shown as follows:



A typical experimental setup for the electrochemical detection of  $\text{H}_2\text{O}_2$  is shown in Fig. 17.12. Basically, amperometric technique (i-t) is employed, from which, the applied voltage is kept constant at the value where all the species are in the reduced states. Then, an identical aliquot of  $\text{H}_2\text{O}_2$  with various concentrations is injected inside the  $\text{N}_2$ -saturated buffer solution with the constant time intervals, while the solution is continuously stirring. The current versus time is recorded for further analysis.

The Mb, cyt c, Hb, Fdx and HRP as the class of metalloproteins are heme proteins containing iron cation(s). Due to the redox capability of heme proteins, these metalloproteins have been widely incorporated for enzyme-based biosensors, particularly  $\text{H}_2\text{O}_2$  biosensors. However, as we mentioned earlier, to increase the electron transfer rate of the solid electrodes, the modification of electrode prior to the enzyme immobilization is necessary.

Gold nanoparticle-modified iridium tin oxide (Au NP/ITO) has been reported to enhance the electrochemical properties of the cyt c (Yagati et al. 2012). It was observed that, the Au NP provided not only conduction enhancement but also a very high surface to volume ratio for the effective and dense immobilization of cyt c. This led to a clear quasi-reversible redox current signals resulting from the  $\text{Fe}^{3+/2+}$  redox center, which showed a good electron exchange between the protein and solid electrode. Later, Cho's group developed new electrode by coupling gold nanoparticles with graphene oxide as the precursor for the immobilization of HRP to be further used for the sensitive detection of  $\text{H}_2\text{O}_2$  (Yagati et al. 2014). The electrode was fabricated using chronoamperometry method based on electrochemical co-reduction of graphene oxide/nanoparticle (ERGO-NP) composite films onto ITO electrode. The ERGO-NP/ITO electrodes demonstrated a very high conductivity (ca. 5 times higher than the unmodified electrodes) which was attributed to well-distribution of immobilized enzyme onto the surface as well as the high surface area and highly conductivity of the substrate. The sensor showed excellent sensitivity of  $1808.9 \mu\text{A mM}^{-1} \text{cm}^{-2}$



**Fig. 17.12** Schematic diagram of the enzyme-based ECB experimental setup. WE, RE and CE stand for working, reference and counter electrode, respectively

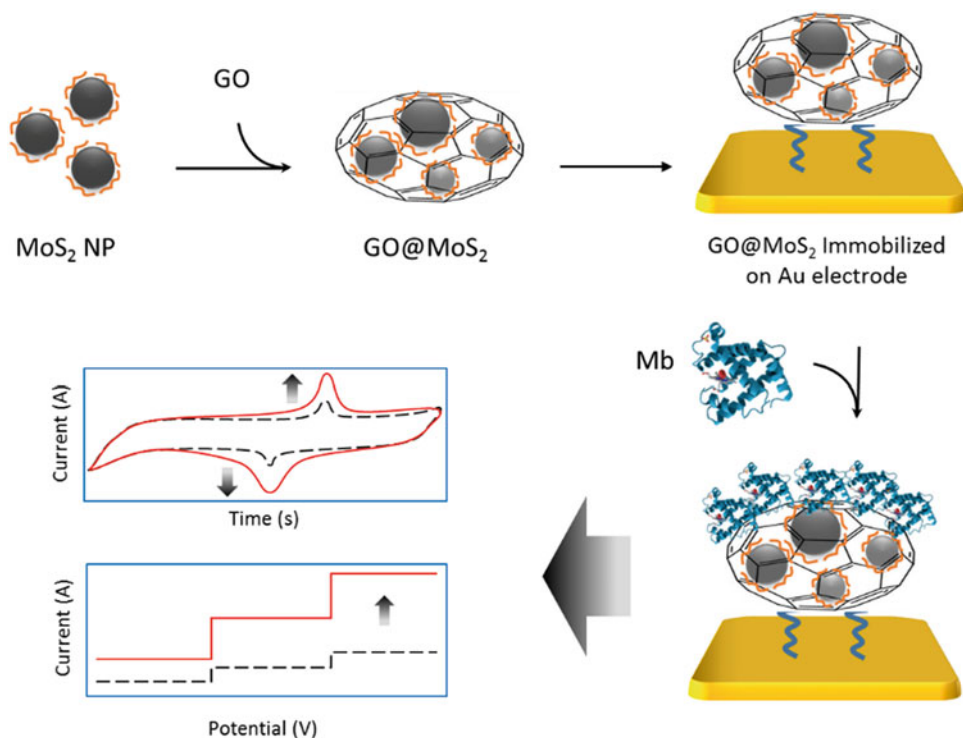
and selectivity with a linear dynamic detection range and the detection limit of  $0.6 \mu\text{M}$ .

Another report dealt with the Mb immobilization onto porous cerium dioxide ( $\text{CeO}_2$ ) which was priority electrodeposited onto the ITO glass (Yagati et al. 2013). The developed  $\text{CeO}_2$ /ITO film offered a nanoporous structure with a large surface area for the direct immobilization of Mb without any chemical linker to hamper the electron transfer between the interfaces. The biosensor represented a good selectivity and a sound current response of 10 s. Recently, a very sensitive enzyme-based  $\text{H}_2\text{O}_2$  ECB was reported which was based on the  $\text{MoS}_2$  nanoparticle encapsulated with graphene oxide (Fig. 17.13) (Yoon et al. 2017a). Owing to the unique electrochemical properties of topological insulator ( $\text{MoS}_2$ ) nanoparticle as well as the graphene oxide, the proposed biosensor exhibited high electrochemical signal which gave rise to the sensitive detection of  $\text{H}_2\text{O}_2$  at 20 nm. A comprehensive report of different enzyme-based  $\text{H}_2\text{O}_2$  ECBs is provided in Table 17.1.

#### 17.4.2 Protein/DNA-Based Electrochemical Biosensor for $\text{H}_2\text{O}_2$ Detection

The study of interaction between protein and DNA has become a very interesting topic in variety fields of biology, chemistry and biotechnology (Gromiha and Nagarajan 2013). The study on charge transfer between the redox proteins or enzymes and nucleic acids has attracted much attention, since it can provide deeper understandings of the electron transfer mechanism in real biological systems and establish a stepping stone for the fabricating of novel biosensors and biodevices (Gorton et al. 1799; Nowak et al. 2011). Here we review some examples of Protein/DNA-based ECBs for the detection of  $\text{H}_2\text{O}_2$  and oligonucleotides.

There are mainly two methods for the DNA/protein conjugation: electrostatic bonding and covalent bonding. Taking the advantage of electrostatic bonding, ECBs composed of DNA and hemoglobin (Hb) dropletting onto the gold electrode to detect  $\text{H}_2\text{O}_2$ . The DNA helped the Hb to keep its native structure and to less aggregate giving rise to its reducibility enhancement (Kafi et al. 2006). Another approach was based on HRP/DNA–silver nano hybrids and poly



**Fig. 17.13** Schematic depicting the synthesis route of the biosensor (Upper panel) and constitution of biosensor towards EC signal enhancement and H<sub>2</sub>O<sub>2</sub> detection improvement. (Figure reproduced with permission from Yoon et al. 2017a)

**Table 17.1** List of protein-based electrochemical biosensor for H<sub>2</sub>O<sub>2</sub> detection

Protein	Modified Electrode	LOD ( $\mu\text{M}$ )	Dynamic Range ( $\mu\text{M}$ )	References
<b>Cyt c</b>	Au NP/ITO	0.5	–	Yagati et al. (2012)
	MPCE	0.146	0.02–24	Zhang (2008)
	GNPs/RTIL/MWNTs/GCE	3.0	0.05–11.5	Xiang et al. (2008)
	RTIL-PDDA-AuNPs/MUA-MCH/au	5.0	0.04–3.45	Song et al. (2013)
	MPA/au	1.0	0–0.25	Suárez et al. (2013)
<b>Mb</b>	GO@MoS <sub>2</sub>	0.02	–	Yoon et al. (2017a)
	CeO <sub>2</sub> /ITO	0.6	3.0–3000	Yagati et al. (2013)
	Nafion/IL/GCE	0.14	1.0–180	Safavi and Farjami (2010)
	GNRs@SiO <sub>2</sub> /RTIL-sol-gel/GCE	0.12	0.2–180	Zhu et al. (2009)
	Clay-IL/GCE	0.73	3.9–259	Dai et al. (2009)
<b>Hb</b>	GNPs/MWNT/GC	0.08	0.21–3000	Jia et al. (2009)
	Graphene/Fe <sub>3</sub> O <sub>4</sub> /GCE	6.0	0.25–1.7	Wang et al. (2013)
	ZnO/MWCNT/GCE	0.02	–	Palanisamy et al. (2012)
	SDS/TiO <sub>2</sub> /GCE	0.087	0.5–70	Wang et al. (2011)
<b>HRP</b>	ERGO-NP	0.6	–	Yagati et al. (2014)
	Composite-3	0.009	0.01–0.22	Umasankar et al. (2012)
	PTMSPA@GNR	0.06	10–1000	Komathi et al. (2013)
	Au NAE	0.42	0.74–15,000	Xu et al. (2010)
	Au NP/MPA/au	0.16	0.48–1200	Wan et al. (2013)



(diallyldimethylammonium chloride) (PDDA)-protected gold nanoparticles (Ma et al. 2009). As shown in Fig. 17.14, at first, DNA-Ag + complex was electrochemically reduced onto the bare gold electrode to obtain negatively charged immobilization matrix (DNA-Ag) for the further immobilization of PDDA-Au particles. Then the process of A and B were repeated to achieve a more conductive bilayer structure. Next, the positively charged HRP ( $H_2O_2$  reducing enzyme) was bonded to the negative surface for the subsequent detection of  $H_2O_2$ . The reported biosensor represented a linear dynamic range over  $H_2O_2$  concentrations from 7.0  $\mu M$  to 7.8 mM and the detection limit of 2.0  $\mu M$  ( $S/N = 3$ ) with good selectivity and acceptable stability.

Using two different types of proteins (HRP and Cyt c) in conjugation with DNA, Yonghai et al. fabricated an  $H_2O_2$  biosensor to mimic the charge transfer and electrocatalytic mechanism of two proteins in living organisms (Song et al. 2012). According to their results, a faster charge transfer rate was observed for the bi-protein bio-interphase than the single protein biointerphase, demonstrating a synergetic effect to better the electron transfer. The DNA role was to provide a network film as a biocompatible microenvironment for the proteins adsorption and an essential pathway for the charge transfer between the electrode and proteins (Fig. 17.15).

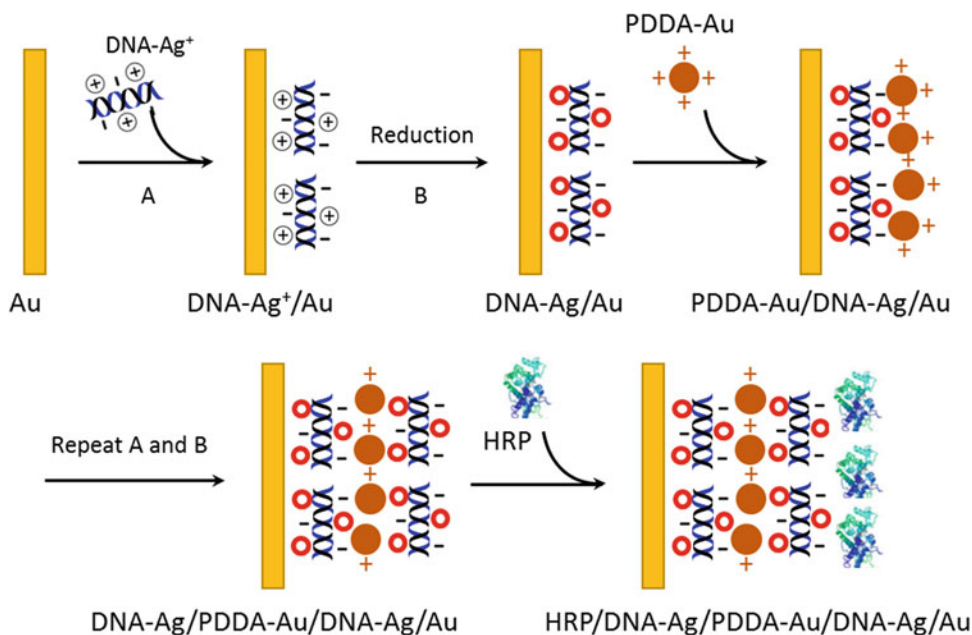
### 17.4.3 Protein/DNA-Based Electrochemical Biosensor for Oligonucleotide Detection

Interaction between DNA and protein has been also studied to develop various ECBs for the detection of different genomic DNA/RNA strands. One of the well-known protein-ligand covalent conjugation techniques is the streptavidin (STV)-biotin. (Dundas et al. 2013; González et al. 1799) Making use of STV-biotin interaction, Shanlin et al. fabricated a chemical

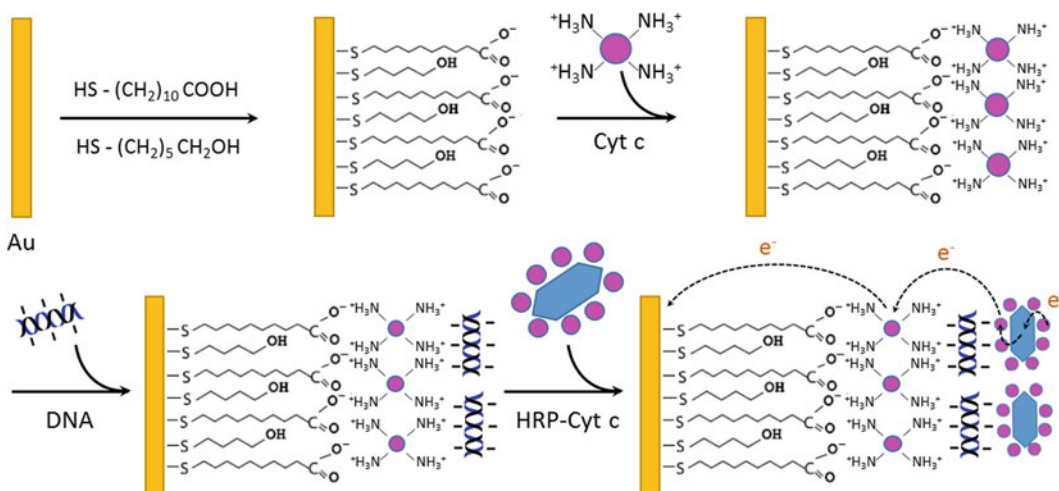
controllable electrode for EC detection of DNA using EIS method (Pan and Rothberg 2005). As depicted in Fig. 17.16, bare gold electrode was firstly modified with the mixed monolayer of 2-mercaptoethanol (ME) and 11-mercaptopundecanoic acid (11-MUA) to provide enough space for the conjugation of STV onto the free carboxyl group of the 11-MUA through amide bonding. A biotin-modified ssDNA was then bound to the STV through the robust STV-biotin chemistry. Using  $[Fe(CN)_6]^{4-/3-}$  redox reporter, the EIS measurement was performed. Before the target invasion, the ssDNAs hampered the redox probes to reach the gold electrode surface leading, whereas, after the target hybridization, the formation of the upright dsDNA facilitated approach of the redox probe to the surface. The resistance difference was monitored by EIS method to offer a very sensitive and selective ECB with the detection limit of 10 pM.

The redox active proteins or enzymes have been also implemented for the nucleic acid detection. For instance, using HRP as the redox reporter by reducing the  $H_2O_2$ . Gang et al. reported a very sensitive enzyme-based E-DNA sensor consists of a stemloop DNA probe which was labeled with biotin and digoxigenin (DIG) at its each end (Liu et al. 2008). The probe was immobilized onto an avidin-modified electrode through the biotin-avidin conjugation (another strong conjugation technique). In the absence of the target DNA, the DIG was shielded from being approached by bulky AntiDIG-modified HRP because of the steric effect. After the target induction and hybridization, the dsDNA forced the DIG to be detached from the surface and be accessible by the AntiDIG-modified HRP for the enzymatic transduction via  $H_2O_2$  reduction. The proposed biosensor exhibited a high sensitivity down to femtomolar with the ability of mismatch detection.

An alternative approach for the oligonucleotide detection using protein/DNA-based ECB was to make use of metalloproteins in conjugation



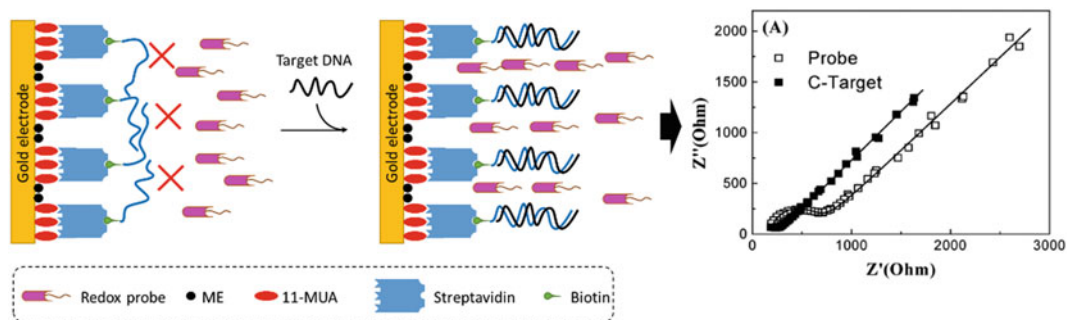
**Fig. 17.14** Layer-by-layer formation of the DNA-Ag/PDDA-Au/DNA-Ag/Au



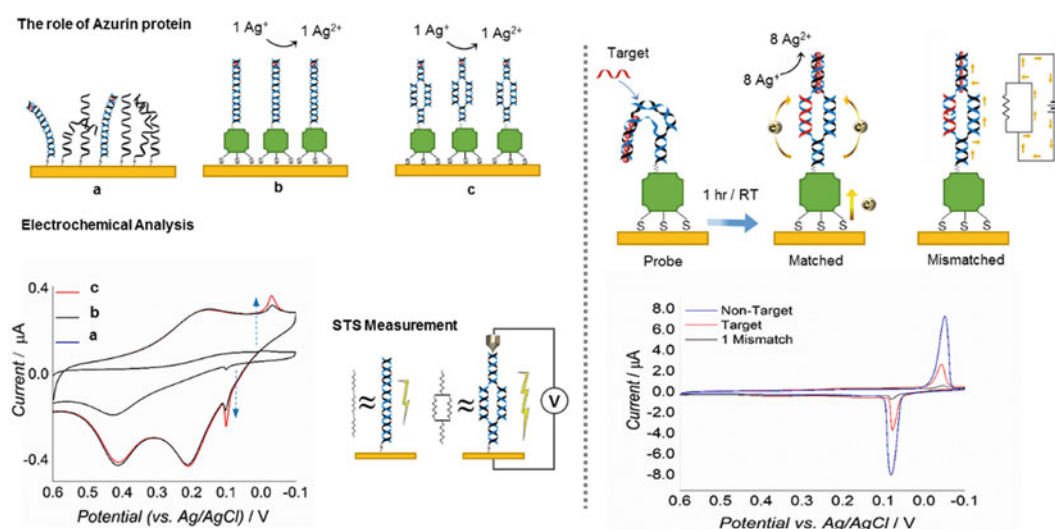
**Fig. 17.15** Surface functionalization of the biosensor composed of DNA and HRP-Cyt c

with nucleic acids. Recently, a novel parallel dsDNA and recombinant azurin hybrid was developed to have higher conductance than that of the canonical DNA and they conjugated it with recombinant Azurin protein (denoted as PSD/rAzur) for the general detection of various viral DNAs and miRNAs (Mohammadniaei et al. 2017). As depicted in Fig. 17.17, the immobilized

rAzur onto the gold electrode provided Cu<sup>+</sup>/Cu<sup>2+</sup> redox reaction and a stable anchoring site to remove the requirements of additional chemical linkers and rolled as a selective-arrayed molecule due to its appropriate cross-sectional diameter (~5 nm) and capability to receive only one DNA strand at its N-terminus. The electrochemical (EC) and scanning tunneling spectroscopy (STS) measurement



**Fig. 17.16** Surface functionalization of the biosensor and further EIS measurement. (Figure reproduced with permission from Pan and Rothberg 2005)



**Fig. 17.17** Schematic illustration of PSD/rAzu biosensor

confirmed higher electron conductivity of the PSD (resembling a parallel electrical circuit,) compared to the dsDNA. Silver ion bond between C-C mismatched base pairs on the top of each helix, functioned as the redox signal reporter for EC conductance measurement and sensing application. The single mismatch detection strategy was inspired by the short-circuit law in classical physics which illustrates that, in a parallel electrical circuit possessing two current flow paths, current migrates through the path with no electrical impedance. Therefore, the single mismatched duplex could be considered as the path with higher impedance, resulting a lower electrochemical signal. The developed biosensor could detect

miR-155, miR-21, miR-141, miR-143 as well as genomic MERS-CoV and HIV-1.

## 17.5 DNA-Based Electrochemical Biosensor

DNA-based electrochemical biosensors have been mostly used as the hybridization assays for the genetic analysis, due to the ability of the single stranded DNA (ssDNA), as the sensor probe, to seek out and hybridize with the target gene (Paleček and Jelen 2002; Wang 2002; Zhai et al. 1797). However, DNA has been also incorporated with different organic/inorganic

platforms to form various biosensors (Chowdhury et al. 2014; Gao et al. 2016; Liu et al. 2015a). In this section, we are going to review some examples of DNA-based ECBs towards  $H_2O_2$  detection and oligonucleotide detection.

Conventionally, the nucleic acids possess weak enzymatic properties due to the lack of prosthetic groups, in order to exploit them for the  $H_2O_2$  detection (catalytic reaction) the necessity for the incorporation of this biomolecule with metal ions is demanding.

### 17.5.1 DNA-Based Electrochemical Biosensor for $H_2O_2$ Detection

In 2006, researchers developed a dimension-controlled silver–DNA hybrid nanoparticles which was electrodeposited on a glassy carbon electrode based on the reduction of silver with the help of DNA (Wu et al. 2006). The DNA rolled to avoid aggregation of silver nanoparticles and enhanced the catalytic capability of the nanocomplex to further detect  $H_2O_2$  at the low concentration of 0.6  $\mu M$  and linear detection range of 2.0  $\mu M$ –2.5 mM.

Another amperometric  $H_2O_2$  biosensor was reported by Yasushi group, composed of DNA–Cu(II) and chitosan polyion (Gu et al. 2009). DNA/chitosan polyion complex membrane was employed as a precursor for entrapment of electrocatalytic copper ions, which could specifically bound to double stranded DNA (dsDNA) and further reduce the  $H_2O_2$  on the glassy carbon electrode (GCE). The sensor exhibited good sensitivity and selectivity towards ascorbic acid with the linear range from 10  $\mu M$  to 10 mM and the detection limit of 3  $\mu M$ .

DNA in the form of G-quadruplex DNAzyme has been also integrated with hemin to electrochemically detect  $H_2O_2$  in a very low concentration of 0.16  $\mu M$  (Wu et al. 2015). Deoxyribozymes (DNAzymes), are ssDNAs with particular catalytic features. G-quadruplex DNA is a self-assembled G-rich DNA sequence, whereas the hemin/G-quadruplex is formed by coordination of hemin inside the G-quadruplex

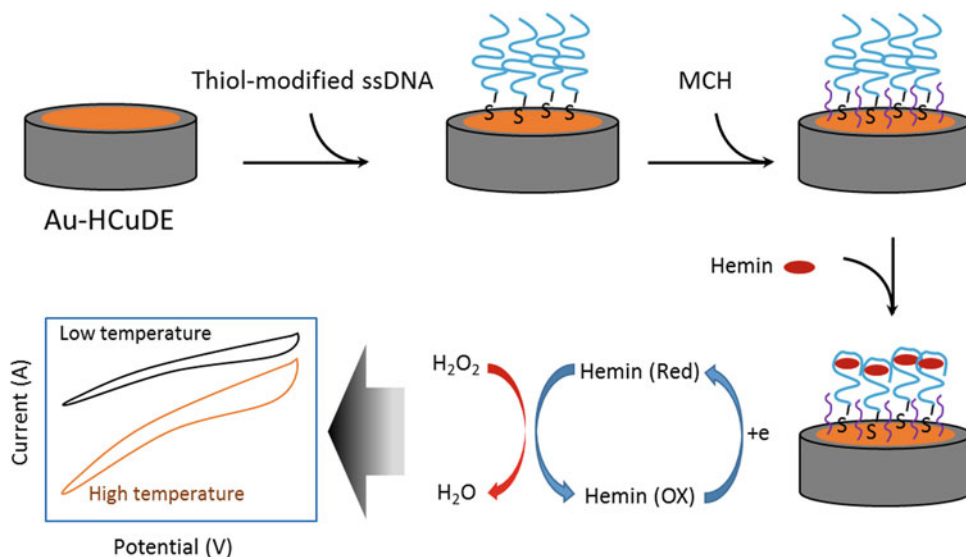
DNA (Li et al. 2016). Owing to the Fe ion of the hemin group, the hemin/G-quadruplex can effectively catalyze  $H_2O_2$ . As depicted in Fig. 17.18, the G-quadruplex was firstly self-assembled onto the gold particles modified heated copper disk electrode (Au–HCuDE), then the electrode was back-field with 6-mercapto-1-hexanol (MCH) in order to remove physical bindings of thiol-modified ssDNA to the surface. After that, the hemin was introduced to the structure to form the hemin/G-quadruplex. Enhancement of the electrode temperature to 50 °C, resulted in the amplification of the electrocatalytic activity in the developed biosensor.

Recently, a highly sensitive  $H_2O_2$  ECB with the ability of  $H_2O_2$  detection in the sterilize milk has been reported based on a novel “on-off-on” switch system. The electrode consisted of methylene blue (MB) as the charge mediator, gold nanoparticle as the electrochemical signal enhancer and iridium (III)/G-quadruplex to provide a hydrophobic layer (switch off). After the introduction of  $H_2O_2$ , iridium (III)/G-quadruplex was cleaved into DNA fragments. Releasing the DNA fragments from the electrode surface led to the signal recovering (switch on), which enabled  $H_2O_2$  detection.

### 17.5.2 DNA-Based Electrochemical Biosensor for Oligonucleotide Detection

It has been proved that, nucleic acid (DNA/RNA) detection and analysis is highly essential not only for obtaining genetic information but also for the sake of diagnosis, identification and classification of various diseases and genetic disorders (Abi et al. 2018; Zhai et al. 1797). Nucleic acid ECBs are usually based on the hybridization method. It involves monitoring the electrochemical signal response, resulting from the Watson–Crick base-pairing of the genomic DNA/RNA target with the sensor probe (Jolly et al. 2016).

In 1994, Millan et al. reported an electrochemical DNA sequence-selective biosensor which was a stepping stone for the development of various nucleic acid ECBs (Millan et al. 1794).

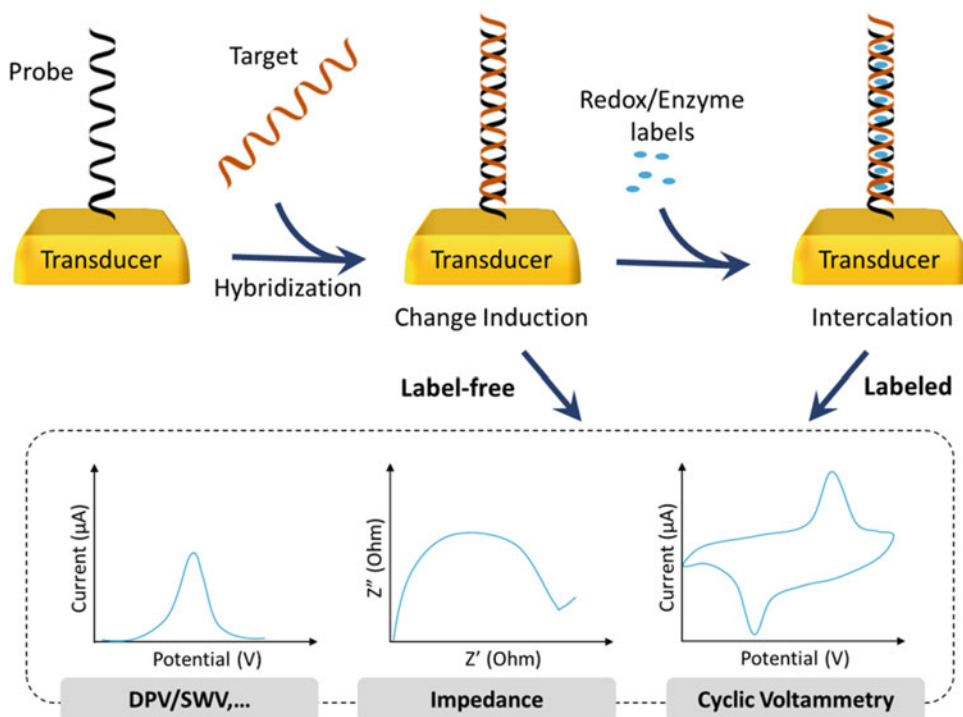


**Fig. 17.18** Schematic construction and performance of the biosensing platform for electrocatalytic reduction of H<sub>2</sub>O<sub>2</sub> at the elevated electrode temperature

Generally, in the nucleic acid ECBs, sensor probe (recognition platform) consists of ssDNA or ssRNA covalently self-assembled onto the electrode surface, which should be conductive, biocompatible and have a low over potential (Odenthal and Gooding 2007). The probe sensor is then immersed into the solution containing target ssDNA/ssRNA, which is complementary to the probe strand, to form a double stranded nucleic acid helix. Depending on the experimental design and whether the sensor is “signal-off” or “signal-on”, the hybridization process results in a notable change in the transduced electrochemical signal and further detection by the signal processor (Liu et al. 2012a). There have been usually two main techniques for the development of electrochemical nucleic acid biosensors: labeled and label-free approaches. In the labeled method, enzyme labels or redox labels are employed to bind to the nucleic acids. This binding can be specific, covalent or electrostatic. Whereas, the label-free approach is commonly based on the difference in the electrical properties of the single stranded and double stranded structures. Schematic diagram in Fig. 17.19 demonstrates the two mentioned approaches.

Several challenges have remained to develop nucleic acid ECBs such as surface immobilization control, single mismatch detection and fast response. Fabrication of a highly reproducible and credible nucleic acid ECB to discover genetic disorders caused by base pair mutation is highly demanding for early-stage diagnosis of different types of cancers and diseases (Baker 2006; Drummond et al. 2003). An acceptable nucleic acid ECB should compete against the standard sensing methods such as quantitative real-time polymerase chain reaction (qRT-PCR) (Maddocks and Jenkins 2017), northern blotting (NB) (Schwarzkopf and Pierce 2016) and microarray (Dastjerdi et al. 2014). However, microarray technique is prone to high cost which makes it less approachable to every user. Also the NB method is restricted due to the requirements for radiolabeling leading to cross contamination and low efficiency. The major limitation of qRT-PCR, regardless to its high cost, would be the detection of short strand nucleic acids such as microRNAs, due to their low melting temperature following by the complicated primer design to causes contamination experimental errors (Lee et al. 2014a; Wu and Qu 2015).



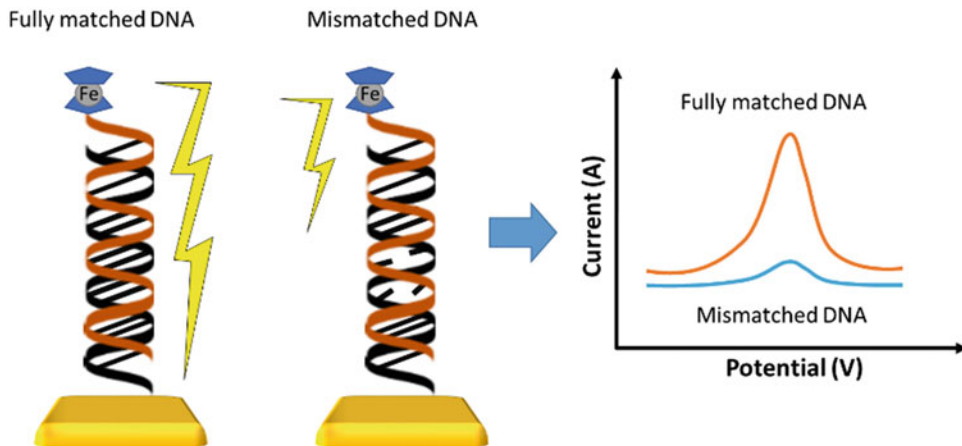


**Fig. 17.19** General diagram for the labeled and label-free electrochemical nucleic acid biosensor; DPV and SWV stand for differential phase voltammetry and square wave voltammetry, respectively

In 2005, Masahiko group reported a great capability of DNA ECBs for the detection of single mutation inside the dsDNA (Inouye et al. 2005). The idea was simply based on the defining the dsDNA as an electrical wire whose one end was attached onto the gold electrode and the other end was modified with Ferrocene as the EC redox reporter (Fig. 17.20). The duplex with mismatch base pair resulted in the rupture in  $\pi$ - $\pi$  orbital loss in the dsDNA to interrupt the charge transfer from the electrode surface to Ferrocene through the double strand helix leading to a significant electrochemical signal drop (SWV technique).

One of the famous methods for sensitive electrochemical detection of nucleic acids is hybridization chain reaction (HCR) (Trifonov et al. 2016). This biosensor was consisted of a gold electrode, fictionalized by thiol-modified ssDNA (1) which is partially complementary to the target oligonucleotide (2). Hybridization of the analyte (2), with the probe leads to the formation of a dsDNA containing a toehold sequence.

In the presence of the two hairpins of HA (3) and HB (4), the HCR triggers. The HCR mechanism is as follows: The toehold sequence of stand (2) opens the hairpin (3), exposing a new single-stranded toehold (W) which opens hairpin (4). The results in another free toehold (X) to open the hairpin (3) and the process keeps going until the hairpins supply is exhausted. The electrochemical detection method was EIS that is based on the semicircular diameter of the Nyquist plot from which the higher frequencies explains the higher charge transfer resistance ( $R_{et}$ ) corresponding to the more negative charge of the electrodes resulting from the layer-by-layer assembly of the electrode surface with oligonucleotides. The HEPES buffer (10 mM, pH = 7.2) was used which contained  $Fe(CN)_6^{3-/4-}$  as the negatively charged redox probe to indicate the electrode surface modification based on its repulsion from the surface, associated with the sequential addition of oligonucleotides (negative charge) to the surface. For a fixed incubation



**Fig. 17.20** Mechanism of the electrochemical detection of mismatched DNA

time of 45 min, different concentration of the analyte (2) was added to the biosensor to achieve the dynamic detection range. This method offered a detection limit of 1.2 nM of the analyte.

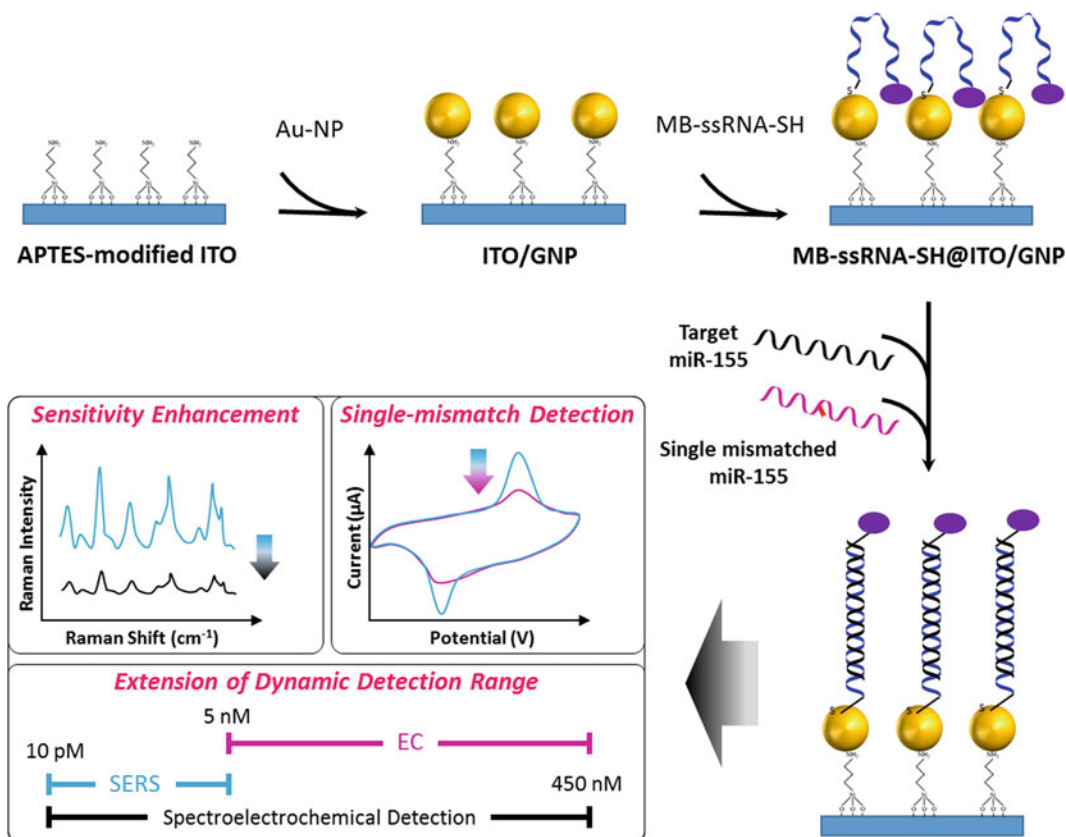
More recently, a simply-designed single-step miRNA biosensor was fabricated using the combination of EC and surface enhanced Raman spectroscopy techniques (SERS) (Fig. 17.21). With the strength of EC method, they removed the weakness of SERS technique for the single-mismatch detection. Also, in a back-to-back supporting situation, the combining method gave rise to the extension dynamic detection range of the biosensor from 10 pM to 450 nM (SERS: 10 pM ~ 5 nM and EC: 5 nM ~ 450 nM) (Mohammadniaei et al. 2018). In this report, a single stranded 3' methylene blue (MB) and 5' thiol-modified RNA (MB-ssRNA-SH) was immobilized onto the spectroelectrochemical-active gold nanoparticle-modified ITO (ITO/GNP) to detect the target miR-155. As a signal-off biosensor, upon the addition of target strand, the dsRNA transformed to an upright position resulting in a considerable decrease in SERS and EC signals of the MB.

In this section we tried to give the reader some insights into the different techniques for the detection of oligonucleotides based on nucleic acid-ECBs, although, there have been reported many outstanding DNA-based ECBs such as dichalcogenides based electrochemical

biosensors (Wang et al. 2017), rolling cycle amplification-based methods (RCA) (Cheng et al. 2009), isothermal amplification, (Zhang and Zhang 2012) aloe-like gold micro/nanostructures (Shi et al. 2013), three-mode system (Labib et al. 2013), (details of previous reports are provided in Table 17.2).

## 17.6 Future Prospective

In spite of initial achievement, the investigation of bioelectronic devices and biosensors are still required. The discussed results are intriguing for the electrochemical bioelectronics devices including biomemory device, biologic gate, bioinformation, biosensors and processor for future biocomputer systems. The biomolecule can easily be tailored and modified with other biomolecule or nanoparticles to embody the specific functionality. Not only the biomolecule can combine the original property with various nanoparticles and other biomolecules to the development of various bioelectronics computation platforms, but also the proto-type biocomputer can operate in living organism with hybrid molecule-neural cell connection. Moreover, unlike that of the silicon-based device, the future biomolecular-based computer could easily be integrated with the input module and energy module that give the new concept of output



**Fig. 17.21** Schematic diagram of the surface modification and microRNA detection of the MB-dsDNA-SH@ITO/GNP biosensor

**Table 17.2** Oligonucleotide detection using nucleic acid-based ECBs

Detection method	Detection steps	LOD	Labeling	Detection time	References
Cleavage-based signal amplification	10	69.2 aM	G-quadruplex hemin	> 2 h	Zhao et al. (2013)
Amperometric magnetobiosensor	4	0.4 fM	Biotin–strep-HRP	~ 3 h	Campuzano et al. (2014)
Three-mode electrochemical sensor	2	5 aM	None	~ 2 h	Labib et al. (2013)
Carbon nanotube-bridged field-effect transistor assisted by p19	2	1 aM	None	~ 2 h	Ramnani et al. (2013)
Tandem polymerization and cleavage-mediated cascade system	7	5 fM	None	~ 4 h	Liu et al. (2016)
DNA tetrahedral scaffold	4	10 aM	Biotinylated probe-avidin-HRP, poly-HRP80	~ 8 h	wen et al. (2012)
Oxidized carbon nano tubes and nanodiamonds	2	1.95 fM	DNAzyme based hybrid structure	~ 2 h	Liu et al. (2015b)

combination for the disease diagnostics and cancer cell identifications. That kind of characteristic is quite intriguing and potentially useful as a new concept of computing for bioelectronic medicine devices. On the edge of bioelectronics, biomolecule-based electronic devices can be envisaged as a powerful alternative, once appropriate fabrication technique and integrating circuit are achieved, then, the nano-scale system can be achieved with a biomolecule-nanoparticle hybrid.

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