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Bioelectrochemical Interface Engineering: Toward the Fabrication of Electrochemical Biosensors, Biofuel Cells, and Self-Powered Logic Biosensors

MING ZHOU AND SHAOJUN DONG*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P.R. China

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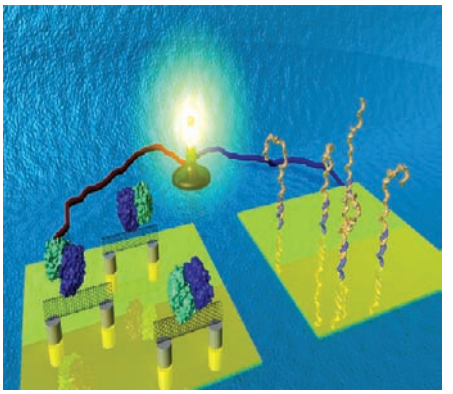

Over the past decade, researchers have devoted considerable attention to the integration of living organisms with electronic elements to yield bioelectronic devices. Not only is the integration of DNA, enzymes, or whole cells with electronics of scientific interest, but it has many versatile potential applications. Researchers are using these ideas to fabricate biosensors for analytical applications and to assemble biofuel cells (BFCs) and biomolecule-based devices. Other research efforts include the development of biocomputing systems for information processing.

In this Account, we focus on our recent progress in engineering at the bioelectrochemical interface (BECI) for the rational design and construction of important bioelectronic devices, ranging from electrochemical (EC-) biosensors to BFCs, and self-powered logic biosensors. Hydrogels and sol−gels provide attractive materials for the immobilization of enzymes because they make EC-enzyme biosensors stable and even functional in extreme environments. We use a layer-by-layer (LBL) self-assembly technique to fabricate multicomponent thin films on the BECI at the nanometer scale. Additionally, we demonstrate how carbon nanomaterials have paved the way for new and improved EC-enzyme biosensors. In addition to the widely reported BECI-based electrochemical impedance spectroscopy (EIS)-type aptasensors, we integrate the LBL technique with our previously developed "solid-state probe" technique for redox probes immobilization on electrode surfaces to design and fabricate BECI-based differential pulse voltammetry (DPV)-type aptasensors. BFCs can directly harvest energy from ambient biofuels as green energy sources, which could lead to their application as simple, flexible, and portable power sources. Porous materials provide favorable microenvironments for enzyme immobilization, which can enhance BFC power output. Furthermore, by introducing aptamer-based logic systems to BFCs, such systems could be applied as self-powered and intelligent aptasensors for the logic detection. We have developed biocomputing keypad lock security systems which can be also used for intelligent medical diagnostics.

BECI engineering provides a simple but effective approach toward the design and fabrication of EC-biosensors, BFCs, and self-powered logic biosensors, which will make essential contributions in the development of creative and practical bioelectronic devices. The exploration of novel interface engineering applications and the creation of new fabrication concepts or methods merit further attention.

1. Introduction

Bioelectronics corresponds to a field of biomolecular electronics that investigates the use of living organisms (e.g., DNA, enzymes, and whole biological cells) in electronic devices.[1−6] In the past decade, bioelectronics has shown considerable promise largely because evolution has often solved problems of a similar nature to those that must be solved in creating electronic devices from organic compounds.[3−7] These make the interfacing of man-made electronics with living organisms not only tell us a great deal about the

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levels of sophistication active in biology but also pave the way to exactly utilize this in derived bioelectronic devices.[7]

One major activity in the bioelectronics field is related to biosensors for a wide range of applications in clinical diagnostics, forensic chemistry, food quality control, and so forth.[4−9] Among these diverse analytical applications,[10,11] electrochemistry is always one of the most popular techniques due to the combined advantages of high sensitivity, small volume requirements, low cost, and the possibility of mass production via the microelectronic industry. Though lots of publications in such a field have been reported, the stability and reproducibility problems of the bioelectrochemical interface (BECI) still hamper electrochemical (EC-) biosensor applications in potential commercial medical diagnosis and real-time environmental monitoring.[4−9]

Another important aspect in bioelectronics is utilizing the biocatalytic electron transfer functions of enzymes or microbes to assemble biofuel cells (BFCs).[1,2] The uses of biomass, such as glucose, endogenously existing in biological systems, suggest the important potential applications of BFCs as one kind of implantable power source for biomedical devices.[1] In order to achieve such a goal, a lot of promising works have been reported.[1,2] However, two critical issues with regard to short lifetime and poor power density being dependent on enzyme stability, electron transfer rate, and enzyme loading still need to be addressed before BFCs become competitive in practical applications.[1]

Recently, the other promising field, the design of biocomputing, also begins to attract increasing attention of bioelectronic researchers. Two different branches of the biocomputing system are being developed in different directions. One is for competing with traditional electronic computation taking advantages of parallel computing performed by numerous biomolecules.[12] However, so far, most of these devices still cannot compete with the classical semiconductor-based processors.[12] The other direction is not aiming at any complex computation but rather at creating a "smart" information processing interface between biological and electronic systems,[13] which would be further used as logic biosensors for potential intelligent medical diagnostics[14−17] and may also lead to a better understanding of nature.[7,18]

In this Account, we will review our recent progress on BECI engineering for bioelectronic device construction, mainly highlighting our research advances on the rational design and fabrication of EC-biosensors, BFCs, and self-powered logic biosensors. Finally, future challenges and perspectives toward BECI engineering construction are described.

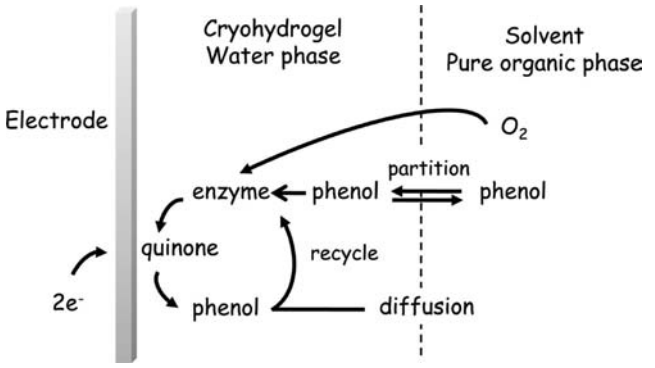

FIGURE 1. Schematic illustration of tyrosinase PHC-cryohydrogel biosensor and the amplification cycle. (Adapted with permission from ref 20. Copyright 1995 American Chemical Society.)

2. Electrochemical Biosensors

2.1. Electrochemical Enzyme Biosensors. Since the concept of an enzyme-based device was presented by Clark in 1962,[19] enzymes have been used in conjunction with various electrodes for constructing electrochemical (EC-) enzyme biosensors due to the inherent selectivity shown by the enzymes to promote selective detection of the enzyme substrates.[4−9] One of the most important steps for building EC-enzyme biosensors is to immobilize/integrate enzyme stably at a bioelectrochemical interface (BECI) and efficiently maintain the functionality of enzyme, while providing accessibility toward the target analyte and an intimate contact with the BECI.

Since the 1990s, the hydrogel was chosen as an early but efficient material in our laboratory for the design of EC-enzyme biosensors operating in extreme environments. As shown in Figure 1, a tyrosinase biosensor based on highly hydrophilic polyhydroxyl cellulose-cryohydrogel (PHC-cryohydrogel) was fabricated to determine phenols in pure chloroform and chlorobenzene.[20] Such a biosensor can be stored in dry state for >3 months with no activity loss. The improved lifetime can be attributed to the essential water in the cryohydrogel layer, which is required for enzyme catalytic activity and stable operation in pure organic solvents. Based on enzyme inhibition, a tyrosinase PHC-cryohydrogel biosensor was proposed to detect phenols in chloroform, chlorobenzene, and 1,2-dichlorobenzene,[21] which would enlarge the practical applications of EC-enzyme biosensors. We extended such a system to an enzyme dimethylformamide-PHC (DMF-PHC) organohydrogel electrode, which can work in aqueous buffer, water/oil mixtures, and anhydrous

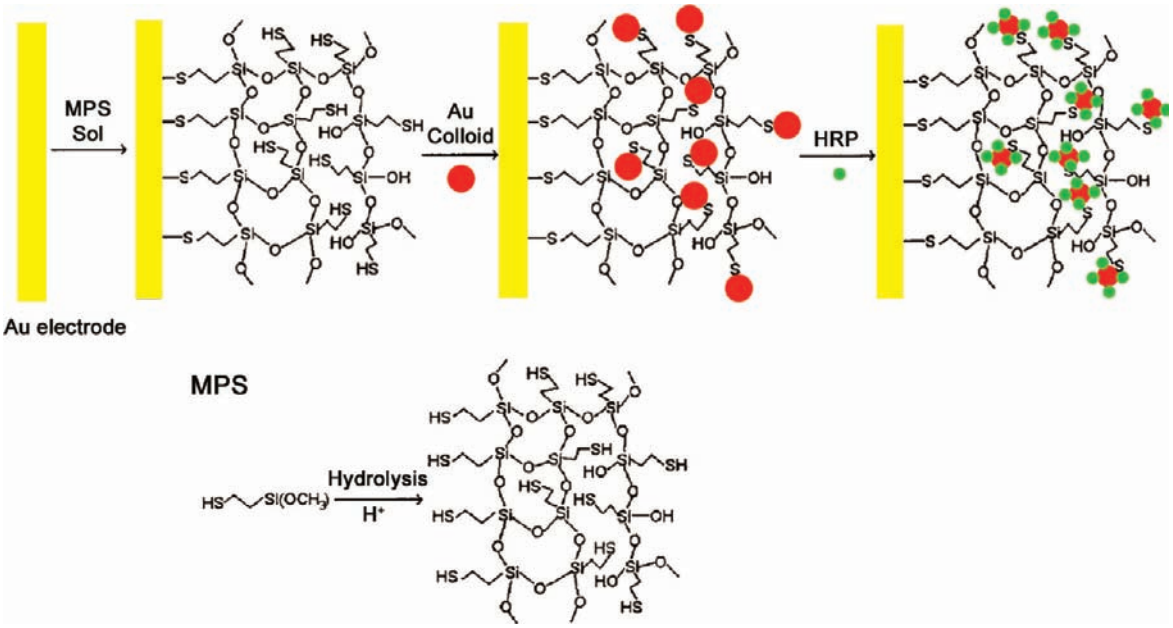

FIGURE 2. Hydrolysis of MPS and the stepwise biosensor fabrication process. (Adapted with permission from ref 25. Copyright 2002 American Chemical Society.)

organic solvents.[22] Though hydrogels exhibited many advantages for enzyme immobilization, hydrogel swelling is often a problem, which stimulated us to search for better materials or/and methods to prepare novel enzyme BECI for EC-enzyme biosensors with improved performance.

Later, sol–gel technology was in sight. It is particularly attractive for biosensor fabrication because sol–gel-derived materials can be prepared under ambient conditions and exhibit tunable porosity, physical rigidity, chemical inertness, and high thermal stability and experience negligible swelling in aqueous/organic solvents. By combining the merits of silica sol and poly(vinyl alcohol) grafting 4-vinylpyridine copolymer, we made use of such two kinds of carriers as matrix for enzyme immobilization,[23] which prevented the silica glass from cracking during the sol–gel transition and eliminated the swelling of hydrogel at the same time. Then, sol–gel matrix has been also successfully used to fabricate EC-soybean peroxidase and EC-soybean peroxidase biosensors for determining H_2O_2 in acid medium (linear range of 0.02–2.6 mM).[24] Furthermore, by self-assembling gold nanoparticles (AuNPs) to a thiol-containing sol–gel network (Figure 2), we developed a third-generation horseradish peroxidase (HRP) biosensor exhibiting the direct electrochemical behavior toward H_2O_2 detection (linear range of 0.005–10 mM.[25]

Due to its simplicity, controllability and versatility in combination with high quality and uniform coating, layer-by-layer (LBL) self-assembly technique is another powerful method to construct multicomponents at the nanometer scale on BECI.[26] By LBL self-assembly of ferrocene derivatized poly(allylamine) modified carbon nanotubes (CNTs) and glucose oxidase (GOD) on an indium tin oxide (ITO) surface, a multilayered GOD biosensing interface was fabricated.[27] The cyclic voltammograms (CVs) revealed the bioelectrocatalytic response is directly correlated to the number of deposited bilayers. By combining the concepts of "glue molecule" (proposed by our group in 2005[28]) and LBL, we further presented an EC-glucose dehydrogenase (EC-GDH) biosensor based on thionine cross-linked CNTs and AuNPs multilayer (Figure 3).[29] Interestingly, the analytical performance of the biosensors can be tuned by visible light, which may provide an operational access to develop new kinds of photocontrol enzyme-based bioelectronics. However, EC-dehydrogenase biosensors with NAD^+ in base solutions may need improvements before potential real applications, since the NAD^+ cofactor has to be well coupled to the electrode and to the active site of the dehydrogenase.

By combining the advantages of nano- and carbon-materials, carbon nanomaterials have paved a new way to improve EC-enzyme biosensors.[9] For example, CNTs film electrode showed a promotion toward the direct electron transfer (DET) of MP-11 compared to no obvious DET at MP-11 modified glassy carbon (GC) electrode.[30] By introducing ionic liquids (ILs) into CNTs, we proposed the first use of CNTs/ILs films as a sensing interface in the direct electrochemistry

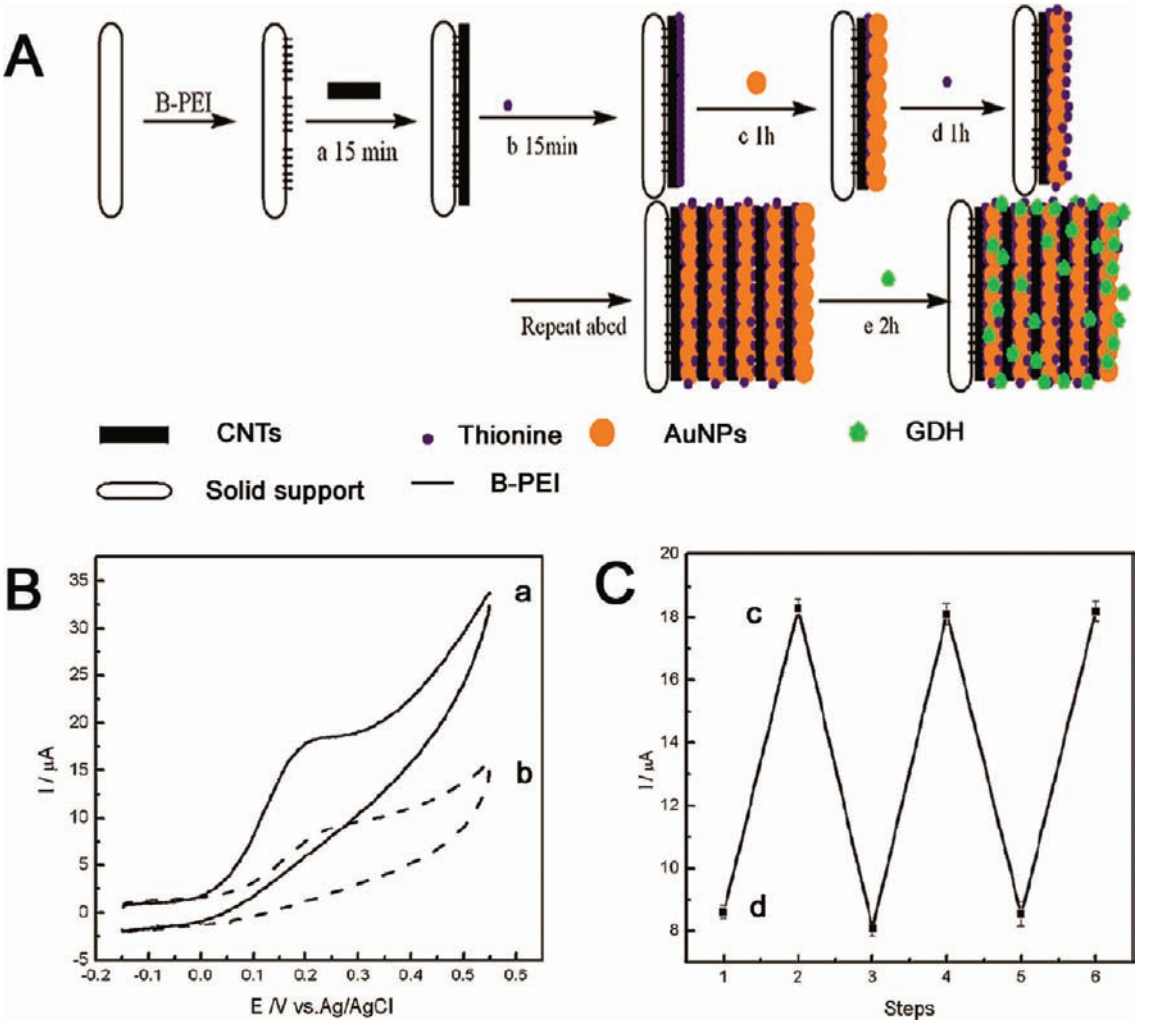

FIGURE 3. (A) Schematic structure of $(CNTs/thionine/AuNPs)_5$/GDH modified ITO.[29] (B) CVs of $(CNTs/thionine/AuNPs)_8$/GDH modified ITO for NADH electrooxidation with (a) and without light irradiation (b). (C) Reversible switch of the currents generated by $(CNTs/thionine/AuNPs)_8$/GDH modified ITO in NADH solution with (c) and without light irradiation (d). (Adapted with permission from ref 29. Copyright 2008 Elsevier.)

of protein for O_2 and H_2O_2 electroreduction.[31] By entrapping GOD into CNTs-chitosan (CNTs-CHIT) matrix, GOD/CNTs-CHIT films showed a higher electron transfer rate of GOD (7.73 s^{-1}) than that of flavin adenine dinucleotide adsorbed on CNTs (3.1 s^{-1}).[32] In addition to the DET between enzymes and conventional electrode substrates mediated by CNTs, the greatly reduced overpotentials for NADH and H_2O_2 electrocatalysis at CNTs-electrodes make CNTs attractive for EC-dehydrogenase and EC-oxidase biosensors design.[9] Banks et al. explained the observed improvement in electrochemistry at the CNTs-electrode to be the presence of high edge plane density on CNTs.[33] Inspired by this principle, we replaced CNTs with ordered mesoporous carbons (OMCs) containing higher density of edge-plane-like defective sites as a substrate for EC-enzyme biosensing applications.[34] On the basis of the enhanced electrochemical reactivity of NADH and H_2O_2, OMCs-based alcohol dehydrogenase (ADH) and GOD electrodes showed faster response time, wider linear range, lower detection limit, and higher sensitivity compared to CNTs-based enzyme electrodes for ethanol and glucose, respectively. In another work, we tried another novel carbon material, graphene (GN), with the nature of a single sheet as the matrix of GN-based EC-biosensors, which showed a better analytical performance for glucose and ethanol detection compared with graphite- or GC-based bioelectrodes.[35]

2.2. Electrochemical Aptamer Biosensors. Aptamers are oligonucleotides (DNA or RNA) that possess high recognition ability to the specific targets.[5] They are generated by an in vitro selection process called SELEX (systematic evolution of ligands by exponential enrichment) which was first reported in 1990.[36] Compared with protein binding-antibodies, such

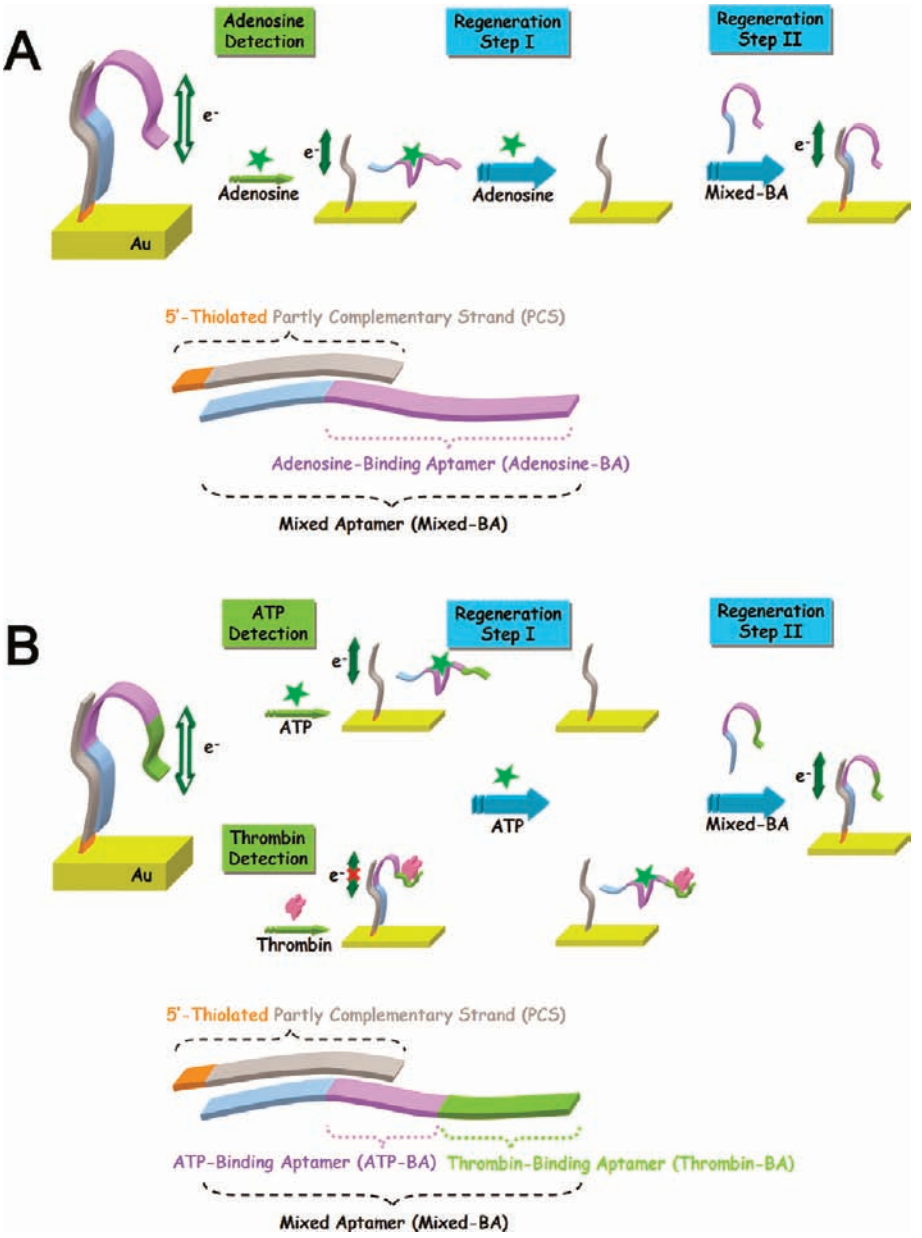

FIGURE 4. Schematic illustrations of EIS-type reusable and label-free EC-aptasensors for (A) single target detection and (B) parallel detection. (Part A adapted with permission from ref 37. Copyright 2007 Royal Society of Chemistry. Part B adapted with permission from ref 38. Copyright 2008 American Chemical Society.)

nucleic acid aptamers have multiadvantages.[8] Aptamers possess a wide range of targets ranging from proteins to amino acids, drugs, metal ions, and even whole cells.[8] It has been generally recognized that aptamer affinity is comparable to or even higher than that of antibodies.[5] Aptamers are isolated in test tubes and can be chemically synthesized in large quantities, whereas antibody production often requires animals or cell cultures, aptamers are relatively more cost-effective for most applications.[5] Additionaly, the immobilization of aptamers is easier than that of antibodies, because chemical modification of nucleic acids is simple and straightforward compared with that of antibodies.[8] By chemical synthesis, modifications in aptamers can be introduced, enhancing the stability, affinity, and specificity of the molecules.[5] Furthermore, aptamers can be thermally denatured and renatured for many cycles without losing binding ability, while denatured antibodies usually cannot be renatured.[8] Thus, aptamers are considered as novel recognition elements in molecular recognition and detection with various techniques.[5,8] Until now, a great amount of EC-aptamer biosensors (EC-aptasensors) have been developed,[5,8,11] among which our lab contributed

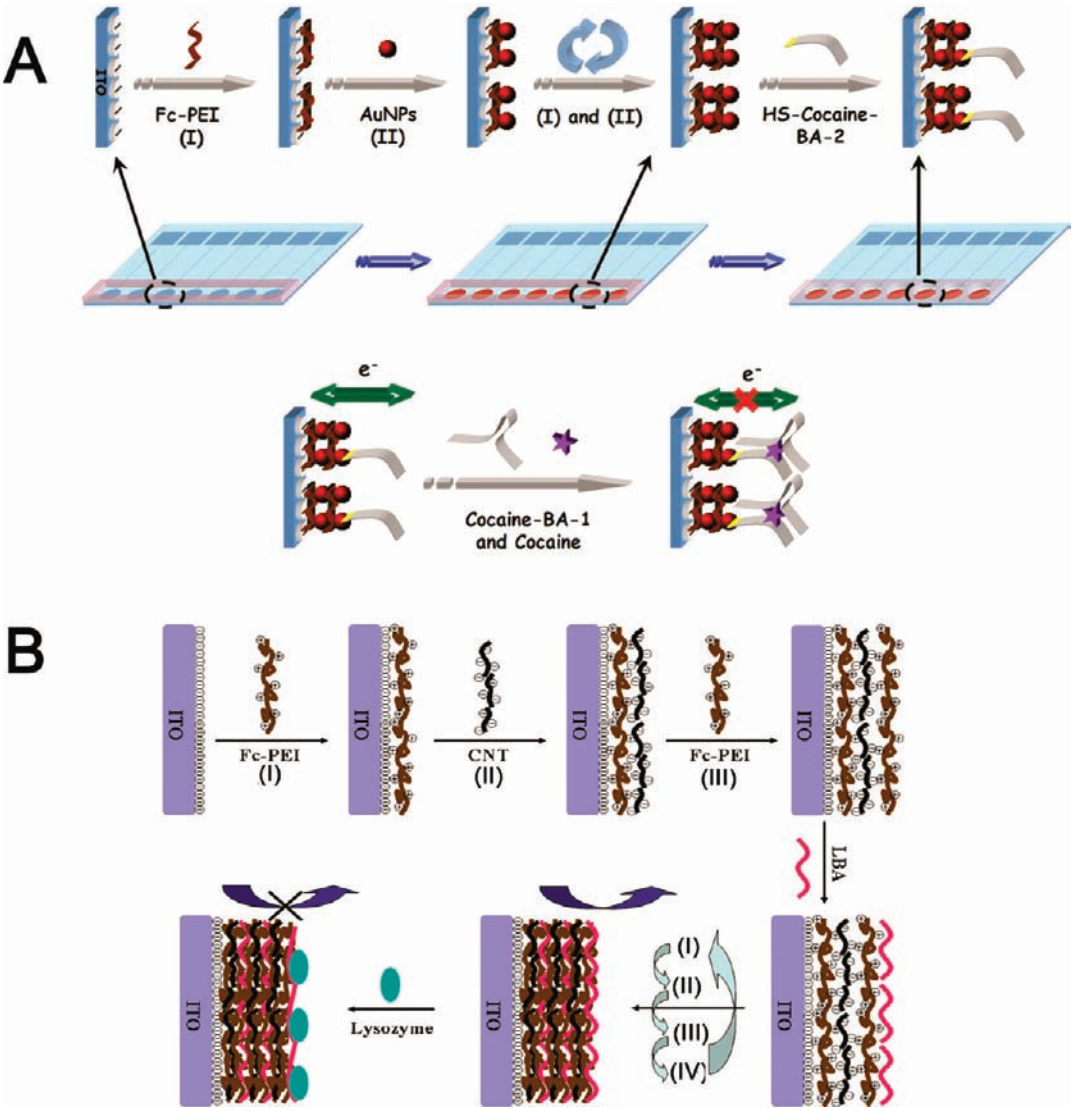

FIGURE 5. Schematic illustrations of DPV-type EC-aptasensors for target detection based on target-binding aptamer (A) on the outermost layer of multilayer and (B) within each layer of multilayer. (Part A adapted with permission from ref 39. Copyright 2010 American Chemical Society. Part B adapted with permission from ref 40. Copyright 2010 Elsevier.)

much effort on how to use aptamers to simplify the sensing process and decrease the cost of a biosensor. A series of strategies without the modification of "special electrochemical probes", also called "label-free strategies",[5,11] were thus developed.[37−40]

In 2007, we proposed a reusable and label-free EC-aptasensor for small molecule detection based on electrochemical impedance spectroscopy (EIS), a technique for detecting impedance variations on aptasensing BECI systems.[37] As shown in Figure 4A, the sensing interface was fabricated by treating a gold surface with a partly negative DNA duplex that comprises a partly complementary strand (PCS) and an adenosine-binding aptamer strand (adenosine-BA). If small molecule (adenosine as the model) is present in the system, the duplex will lose its relatively longer adenosine-BA to the target molecule, but keep the shorter PCS on gold surface. This process directly leads to great loss of the negative charge density of the electrode, which in turn can be monitored by EIS as the impedance decrease. The response range between 1 and 100 μM with the detection limit of $\sim$0.1 μM and satisfied selectivity for adenosine detection were obtained. This approach not only skips the probe-labeling process but also provides a regeneration ability of the sensing interface by retreating it with adenosine-BA. Moreover, it does not depend on the molecule size or the structural change of the aptamer and, therefore, may be available to a wider range of targets. To demonstrate this point, we further extended such a strategy

to protein detection.[38] As illustrated in Figure 4B, the improvement is that the part DNA duplex was redesigned as a PCS and a mixed aptamer strand (mixed-BA) containing both adenosine triphosphate-binding aptamer (ATP-BA) and thrombin-binding aptamer (thrombin-BA). If thrombin is present, the thrombin-BA part in the mixed-BA on gold surface would catch thrombin, which will then increase system impedance. After treating mixed-BA with ATP, the sensing interface can be recovered. The detection range for thrombin is from 0.01 to 100 nM with the detection limit of 0.01 nM. Due to the inherent structure, such aptasensing BECI is not suitable for detecting ATP and thrombin simultaneously, but it is a good platform to construct the unique self-powered and "smart" aptasensors for logic detection[15] as demonstrated in section 4.

The "solid-state" technology concept originates from our efforts on $Ru(bpy)_3^{2+}$ immobilization at an electrode surface for electrochemiluminescence (ECL),[41] which can decrease the consumption of expensive reactant and enrich the ECL probe to enlarge the signal. Elicited by such concept, we imported the integration of the "solid-state probe" concept and LBL technique into the label-free EC-aptasensors to realize drug detection for the first time.[39] As shown in Figure 5A, the aptasensing BECI was formed by the LBL assembly of ferrocene-appended poly(ethyleneimine) (Fc-PEI) and AuNPs on an ITO array electrode, followed by the covalent label of hydrosulfonyl modified cocaine-binding aptamer strand-2 (HS-cocaine-BA-2) onto the outermost AuNPs layer. When the target cocaine and cocaine-binding aptamer strand-1 (cocaine-BA-1) were present simultaneously, the HS-cocaine-BA-2 layer hybridized partly with cocaine-BA-1 to bind cocaine, which led to a decreased differential pulse voltammetry (DPV) signal of Fc-PEI and in turn can be detected by DPV. Interestingly, when we introduced more aptamer strands into the BECI (Figure 5B), a wider detection range was obtained.[40] Besides the aptasensing applications, the integration of "solid-state probe" and LBL techniques was further employed in the intelligent aptasensing system construction on microfluidic biofuel cells (BFCs)[14] as proposed in section 4.

Compared with labeled aptasensors, label-free stragegies do not need complicated steps (e.g., label, separation, and immobilization) for aptasensing. However, this may also lead to the difficulities in selectively distinguishing a real binding event from a false signal that originates from nonspecific contaminants or aptasensor degradation. So, until now, most label-free aptasensing systems are still far from practical applications in complex samples. It is hoped that, with more efforts and the development of advanced techniques, the advantages of labeled and unlabeled strategies can be effectively integrated and amplified, which would give more considerable and ideal development prospects in analytical fields.

3. Biofuel Cells

Biofuel cells (BFCs) based on enzymes and microbes have been recently paid considerable attention because they are recognized as a new kind of energy conversion technology that possesses striking properties, such as operation in mild conditions and potential to be used as in vivo power sources for bioelectronics including micropumps, pacemakers, and so forth.[1,2] Several strategies have been applied to improve the performance of BFCs.[1,2] Willner's group used ferrocene, pyrroloquinoline quinone, AuNPs, or CNTs as electron relays in the linkers, and subsequent chemical cross-linking of the attached enzyme molecules with glutaraldehyde has been used to stabilize the layer for BFC contruction.[1] A major development in Heller's group has been that the enzymes are embedded into a conducting hydrogel polymer to which Os-complexes are attached as electron mediators of BFCs.[2] Recently, based on the mechanical confinement of enzymes and mediators, Cosnier and colleague reported the first successful operation of a BFC inside an animal, which demonstrated implanted enzymes remained operational during 3 months.[42] On the basis of large amount of works in biosensors, we started to explore different approaches for BFCs design with enhanced power output/improved stability.

3.1. Biofuel Cells with Porous Materials as the Substrates. The enzyme immobilization procedure is critical for BFCs fabrication. It should keep enzyme activity while favoring its electrical connection with the underlying electrode, directly or via a redox mediator. In such a context, three-dimensional (3-D) electrode architectures are promising since they would greatly increase the reactive surface area and therefore the enzyme loading, allowing for current and stability enhancement.

We first tried to use porous carbons (PCs) as the matrix of BFCs.[43] GOD (or laccase (LAC)) was entrapped in CNTs-CHIT suspension, which was then cast onto PCs matrix to form the catalyst of bioanode (or biocathode). Ferrocene monocarboxylic acid (FMCA) (or 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS)) was used as the mediator of bioanode (or biocathode) for glucose oxidation (or O_2 reduction). The power of this BFC was higher than that of the cell prepared with GC matrix. After the continuous

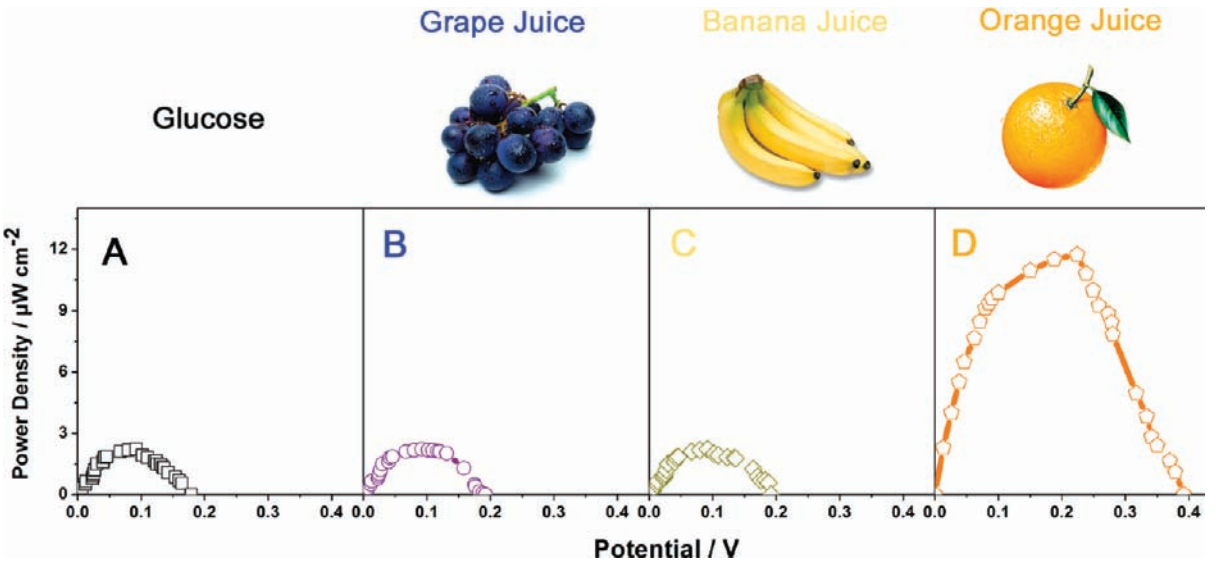

FIGURE 6. Power outputs of the glucose/O_2 BFC harvesting energy from (A) glucose, (B) grape juice, (C) banana juice, and (D) orange juice. The juices are pressed from related natural fruits. (Adapted with permission from ref 46. Copyright 2008 Elsevier.)

BFC operation for 3 h, there are no changes in the magnitude of cell voltage and current. Interestingly, the maximum power densities (P_{max}) were different at different pH values (99.8 μW cm^{-2} (pH 4.0), 14.75 μW cm^{-2} (pH 5.0), 7.94 μW cm^{-2} (pH 6.0), and 2.0 μW cm^{-2} (pH 7.0)), which may provide an alternative way to adjust the output as required.

Later, we demonstrated an OMCs-based glucose/O_2 BFC.[44] OMCs with 3-D interconnected pore topology were used as supports for both stably confining GDH on bioanode and LAC on biocathode. The unique physicochemical properties of OMCs (e.g., well-ordered pore structure, high specific pore volume, and high specific surface area) make OMCs-BFC exhibit higher open circuit voltage (V_{OC}) (0.82 V), P_{max} value (8.7 μW cm^{-2}), and stablility compared to CNTs-BFC (0.75 V and 2.1 μW cm^{-2}). Thus, OMCs are believed to be another "popular" carbon material besides CNTs for constructing BFCs with improved power output and stability. LAC bioactivity for O_2 electroreduction is sensitive to the pH value. Based on this principle, a biocomputing keypad lock system was fabricated based on OMCs-BFC[18] in section 4.

By the integration of porous materials and LBL technique, the BFC based on self-assembly multilayer modified 3-D ordered macroporous (3-DOM) gold electrodes was fabricated.[45] The P_{max} value of the cell with 3-DOM as matrix (178 μW cm^{-2}) was ~16 times larger than that with the flat electrode (12.6 μW cm^{-2}) due to the 3-DOM electrode and LBL technique.

3.2. Biofuel Cells Powered by Ambient Biofuels. We further constructed a fruit juice powered glucose/O_2 BFC by using CNTs-ILs as the electrode matrix.[46] Interestingly, in addition to glucose as the fuel, the BFC can harvest energy from natural fruit juices pressed from fruits. Especially, the V_{OC} and the P_{max} values of the BFC with orange juice as the fuel are higher than those with glucose, grape juice, or banana juice (Figure 6). This suggests that orange juice can greatly enhance the power output and could be attributed to some components in fruit juices, which can be oxidized by LAC and thus helpful to improve the power output.[46] The system based on fruit juices and air revealed the "green", renewable, and readily available characteristics of BFCs.

Many kinds of commercial soft drinks are rich in glucose, so in another work we employed commercial soft drinks as the fuels of the single-walled-carbon-nanohorn-based miniature glucose/O_2 BFC.[47] Compared with the performance with glucose as the fuel (V_{OC} value of 0.72 V and P_{max} value of 140 μW cm^{-2}), the BFC exhibited ehanced power output with carrot juice drink (0.71 V and 245 μW cm^{-2}) and aerated water (0.71 V and 33.6 μW cm^{-2}) as the fuel but decreased power output with iced red tea (0.60 V and 139 μW cm^{-2}) and peach juice drink (0.22 V and 26.2 μW cm^{-2}) as the fuel. Nevertheless, the BFC can still directly generate energy from soft drinks. When the cell operates continuously in glucose solutions for 12 h, it maintains ~80% of its power, indicating a comparatively stable power output process. Being different from the BFC above needing purification steps to harvest energy from fruit juices,[46] no complicated process is needed in this system; supermarket soft drinks can be used directly. Thus, the universality of the fuels and the simple cell construction could ensure a portable microenergy device.

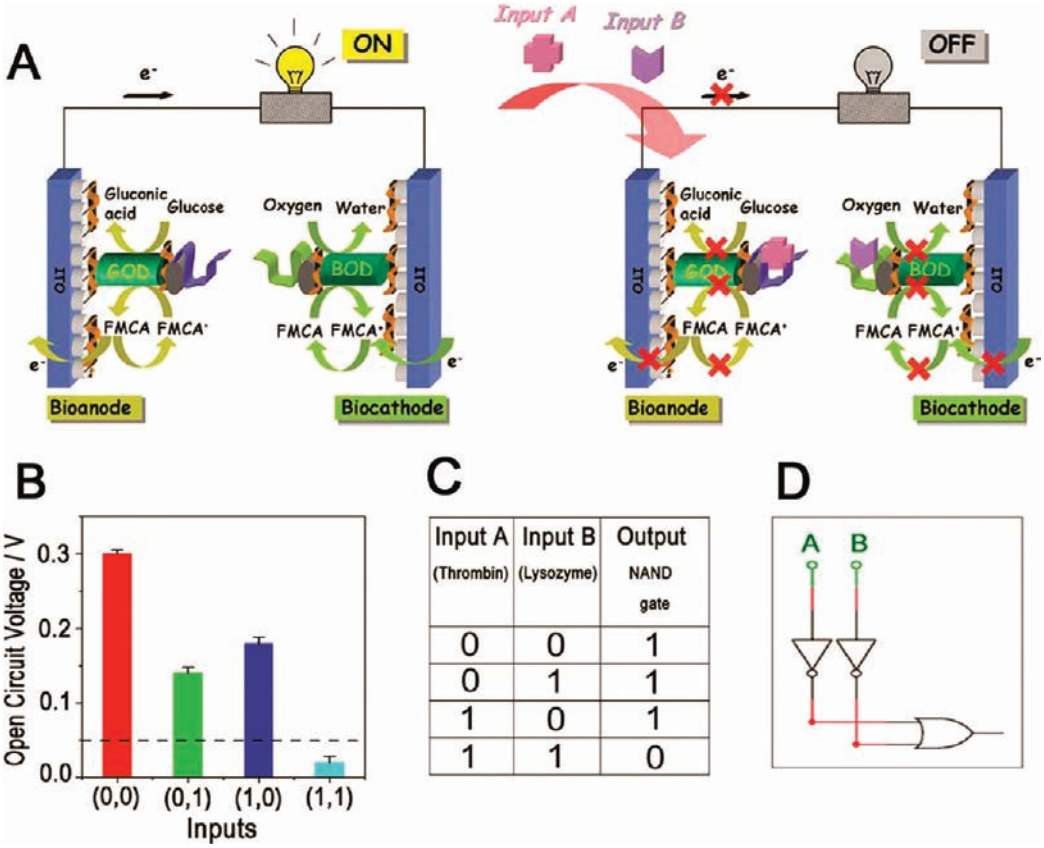

FIGURE 7. (A) Schematic illustration of the assembled aptamer-based BFC logically controlled by biochemical signals. (B) Bar diagram showing the V_{OC} value of BFC as the logic functions of different combinations of input signals. The dashed line shows the threshold. (C) Truth table for NAND logic gate. (D) Circuit for NAND logic gate. (Adapted with permission from ref 14. Copyright 2010 American Chemical Society.)

In addition to fruit juices and soft drinks, commercial ethanol and alcoholic beverages can also power BFC.[48] By adopting wine as the biofuel, ethanol/O_2 BFC based on ion-exchange capacity sol−gel and biopolymer CHIT composite exhibited better performance (V_{OC} value of 0.82 V and P_{max} value of 1780 μW cm^{-2}) compared with that operating in ethanol (0.86 V and 1560 μW cm^{-2}) and liquor (0.78 V and 680 μW cm^{-2}). The lifetime of the cell was approximately 36 days before they decreased to less than 20% of the maximum power, suggesting the great potential for the development and practical application of bioethanol BFC.

In 2010, we fabricated an on-chip BFC with GDH-bioanode and LAC-biocathode operating in cassava-based solutions.[49] The cyanide existing in solutions can affect the T2 Cu of the LAC active center and inhibit O_2 consumption on the biocathode, which accordingly makes the P_{max} value of BFC decrease and leads to the first use of inhibitors for self-powered sensing endogenous biological cyanide. Similaly, on the basis of the inhibiting effect of Hg^{+} on alcohol dehydrogenase (ADH) and bilirubin oxidase (BOD), we further developed a miniature BFC-type self-powered sensor based on ADH-bioanode and BOD-biocathode for the trace detection of Hg^{+} (10 nM) in tap, ground, and lake water.[50] The connection of several such BFCs in series may result in a "turn-off" sensor where the absence of analyte will result in the signal (e.g, LED lit, normally needing the V_{OC} value of ~2.0 V at least from the power source) and the presence of analyte will turn off the signal (e.g., LED goes dark) in the future.

4. Self-Powered Logic Biosensors

Biocomputing, belonging to a subarea of unconventional chemical computing and performed by living organisms, aims at the information processing using biochemical means without the involvement of electronic computers.[3,13] However, most biocomputing systems reported until now represent only the proof of the concept demonstrating the possibility of performing logic operations and are not ready yet for practical applications.[12] On the other hand, the application of biocomputing systems for analytical purposes could yield a novel class of logic biosensors to identify biomedical problems.[13] Being different from the traditional biosensors, logic biosensors are smart and are able

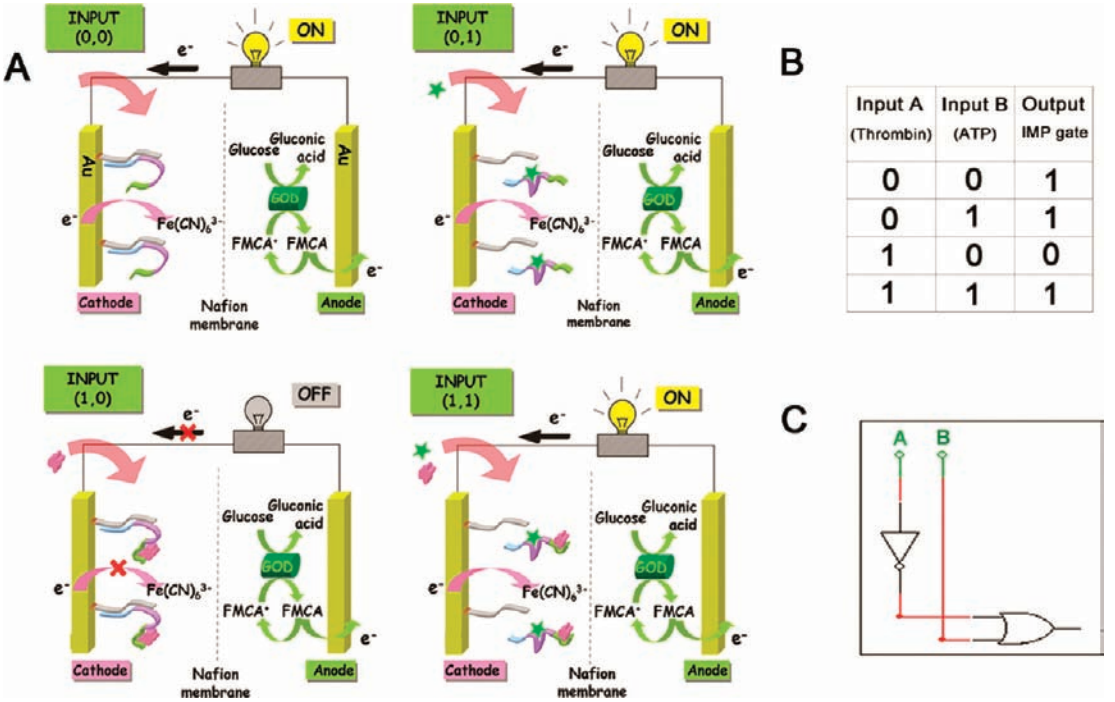

FIGURE 8. (A) Schematic illustration of the switchable BFC for logically controlling the power release by different combinations of aptamer-target recognition-based input signals. (B) Truth table for IMP logic gate. (C) Circuit for IMP logic gate. (Adapted with permission from ref 15. Copyright 2010 Royal Society of Chemistry.)

to intelligently analyze the relationship between different targets in complex samples according to the Boolean logic operations "programmed" into biocomputing systems.[14−17]

In 2010, we described the first model of controlled power release of BFCs by aptamer-based biochemical signals processed according to the Boolean logic operations "programmed" into biocomputing systems.[14] The BFC system was composed of two on-chip patterned ITO electrodes modified with an enzyme/aptamer-based self-assembled multilayer (Figure 7), which combines the similar structures and interesting properties of our reported LBL enzymatic electrode[27] mentioned in section 2.1 and EIS-type label-free aptasensor[27] demonstrated in section 2.2. According to the built-in NAND logic gate, the fabricated BFC controlled by aptamer logic systems enabled us to construct a self-powered and intelligent logic aptasensor, which can determine whether the two specific targets are both present in a sample. Such integration between aptamer logic systems and BFCs may not only give us an avenue to control BFCs power release by aptamer-based biocomputing systems, but also indicate an interesting "mutual benefits" concept between aptamer and BFCs, that is, utilizing aptamer-based biochemical signals as logic operation for controlling BFCs power release and applying BFCs as self-powered and intelligent biosensors for logic aptasensing. However, such a system was unreusable and can only realize the NAND logic gate, which may limit its potential application. So based on our recent work on BECI engineering of the DPV-type label-free aptasensor[38] mentioned in section 2.2, we further demonstrated an IMP-Reset logic based reusable, self-powered, intelligent, and microfluidic aptasensor (Figure 8).[15] Due to the unique function of IMP logic, the aptamer IMP-Reset logic system proposed can be used to "smartly" determine the presence of one specific target in the absence of another target in human serum in a single test.

In addition to the intelligent aptasensors, biocomputing security systems mimicking keypad lock functions are also attractive. Depending on enzyme-based parameters as "readin" and the V_{OC} value of BFC as "readout", we fabricated a self-powered and reusable biocomputing security system[18] on the basis of OMCs-BFC[44] mentioned in section 3.1. Similarly, another "nondestructive" biocomputing keypad lock based on gas-controlled BFC was proposed.[16] In addition to the prominent feature of a keypad lock, such a device could be used as a potential self-powered and "smart" implantable medical system with the diagnosis aim. Research on the topic of self-powered intelligent medical diagnostics is still underway in our group,[17] and some interesting and promising works will be reported in the near future.

5. Conclusions and Outlook

BECI is one of the key components of bioelectronic devices, which would make BECI configuration greatly determine the performance of bioelectronic devices. In this Account, we have summarized our recent achievements in developing BECI engineering, which accordingly provide rational and flexible ways to construct EC-biosensors, BFCs, and self-powered logic biosensors.

EC-techniques promise rapid, simple, and low-cost detection and also allow device miniaturization for samples with a very small volume. Focusing on EC-biosensors based on enzymes and aptamers, we described flexible modification approaches to BECI design for fabricating EC-enzyme and EC-aptamer biosensors. BFCs have been recently paid considerable attention because they are recognized as a new kind of energy conversion technology that possesses striking properties and potential to be used as in vivo power sources for bioelectronics. By rationally designing BECI of BFCs, we discussed flexible approaches to improve BFCs power output and stability. Biocomputing is aiming at information processing using biochemical means without the involvement of electronic computers. By coupling biocomputing with BFCs, we not only developed biocomputing systems mimicking Boolean logic but also created "smart" information processing interfaces of logic biosensors for potential self-powered intelligent medical diagnostics.

Although a great deal of progress has already been made in BECI engineering, several challenges and obstacles on bioelectronic devices still make them far away from real-world applications. For example, most EC-biosensors developed are tested in buffer systems in the laboratory. It should be deliberated on the sample matrix effects as well as the BECI stabilities for the potential commercial medical diagnosis and real-time environmental monitoring. Despite that BFCs have many advantages over traditional fuel cells, their practical applications still require solutions of many difficult engineering problems, particularly related to their short lifetime and poor power densities. Better understanding and further developments of BECI will expedite BFCs improvement. Until now, self-powered biocomputing systems are only realized in a few kinds of living organisms (e.g., enzymes, immune substrates, aptamers, and bacteria systems). Thus, diverse species are encouraged to be introduced into such self-powered logic systems, which will exhibit their own inherent and unique characteristics for the novel logic applications. With scaling up the complexity and diversity of biocomputing systems, data analysis for optimization and noise reduction is beneficial to their real applications. In the future, we may further continue our research on the above aspects, with particular focus on the creation of new concepts and methods of BECI engineering for bioelectronic device design and fabrication.

Finally, it should be noteworthy that the research from EC-biosensors to BFCs and self-powered logic biosensors can be considered as successive and systemic works. This means BFCs construction should be based on EC-biosensors design; accordingly, EC-biosensors and BFCs fabrications would both provide flexible and essential support for self-powered logic biosensors realization.

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BIOGRAPHICAL INFORMATION

Ming Zhou received his B.S. (2004) and M.S. degrees (2007, Professor Li-ping Guo's Group) from Northeast Normal University, China. In 2007, he joined Professor Shaojun Dong's group at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, and received his Ph.D. degree in 2010. He is currently a Postdoctoral Research Associate with Professor Joseph Wang in the Department of NanoEngineering at University of California, San Diego. His major research focuses on biosensors, biofuel cells, biocomputing systems, and nanomotors.

Shaojun Dong is a Professor of Chemistry at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. She obtained her B.S. degree from Furen University in Beijing, China (1952). She is the Member of the Academy of Sciences for the Developing World (TWAS) in Italy (1999). Professor Dong's research interests concentrate in electrochemistry with interdisciplinary fields, such as chemically modified electrodes, nanomaterials/nanotechnology, bioelectrochemistry, spectroelectrochemistry, biosensors, biocomputing, and biofuel cells.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: dongsj@ciac.jl.cn.

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