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Keypad Lock Security System Based on Immune-Affinity Recognition Integrated with a Switchable Biofuel Cell

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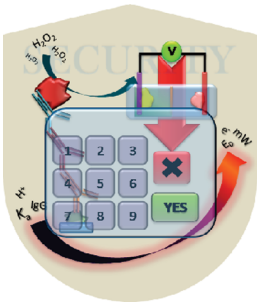
ABSTRACT An immune-based biorecognition system mimicking a keypad lock device was integrated with a switchable biofuel cell resulting in the power output change upon the correct input of the "password" encoded in the antibody-sequence.

SECTION Macromolecules, Soft Matter



Recently emerged chemical systems mimicking Boolean logic gates and their networks are considered as a novel platform for unconventional computing (information processing by chemical means).[1−4] They have been also applied to model various digital electronic devices[5] including molecular comparator,[6] multiplexer−demultiplexer,[7−9] encoder−decoder,[10,11] keypad lock,[12−17] as well as flip-flop and write/read/erase memory units.[18−20] Biomolecular systems based on proteins/enzymes,[21−24] DNA,[25−27] RNA,[28] and whole cells[29,30] offer advantages for assembling higher complexity logic systems due to natural specificity and compatibility of biomolecules. Association of the chemical logic systems with electronic interfaces allows effective transduction of the chemically processed signals to electronic signals.[31] Recently pioneered enzyme-based logic systems[32−36] were integrated with Si-chips,[37] conducting electrodes,[38] and complex bioelectronic devices (i.e., biofuel cells)[39] providing their control by complex combinations of external biochemical signals. The switchable systems, controlled by biocomputing logic networks, laid the foundation for the advanced "smart" systems integrating a biomolecular "decision"-making part with an operating bioelectronic device. The main avenue for future development of these "smart" bioelectronic devices is directed to biomedical applications providing interface between biological and electronic processes.[40] However, some other interesting applications are feasible.

We have recently designed an enzyme-based model of a keypad lock system performing the implication logic operation when the final result of biochemical reactions depends on their correct sequence.[41] This allowed the signal "YES" (mimicking opening of the "lock") when the correct "password" represented by a specific order of biochemical inputs was applied. Otherwise the system stayed mute (the access was denied). However, the number of possible chemical inputs was limited by cross-reactivity of the applied enzymes. Much more combinations could be achieved providing higher security of the biomolecular "lock" when biorecognition systems based on DNA or immune interactions are applied. Extensive data on the DNA computing[25−27] provides excellent background for this specific application, which is waiting for the challenging research. On the other hand, very few systems performing logic operations with the use of immune-recognition are known yet.[42] The present paper explores the possibility of designing a novel bioelectronic device where a switchable biofuel cell is controlled by different permutations of immune-signals operating as a self-powered keypad lock system.

Regular ELISA systems[43] for immune-specific analysis are based on the binding of a secondary enzyme-labeled antibody to a complex produced by recognition of an antigen and primary antibody−analyte. If the complex is not formed, the secondary antibody does not bind to the modified surface. To our best knowledge, this process has never been extended to a longer sequence of biorecognition events to yield a multicomponent sequence of antibodies. A multistep binding of complementary antibodies finished with a signal-generating enzyme-labeled antibody was used in the present study to engineer a biomolecular keypad lock system.

An antigen, 3-nitro-L-tyrosine (NT), bound to a carrier protein (bovine serum albumin, BSA) was physically adsorbed on polystyrene wells of an ELISA plate. The surface functionalized with the NT−BSA conjugate was stepwise reacted with different antibodies defined as input signals for the system, each time washing out the nonreacted species. An antibody bound to the surface in a previous step served as an antigen for binding another antibody in the next step. The antibodies were bound to the surface only if they found corresponding complementary antigens attached to the interface in the previous reaction steps; otherwise they were removed upon the washing steps. The correct sequence of the biorecognition steps included the binding of rabbit-origin IgG anti-nitrotyrosine (input signal **1**), goat-origin IgG specific against rabbit IgG (input signal **2**) and mouse-origin IgG specific against goat IgG

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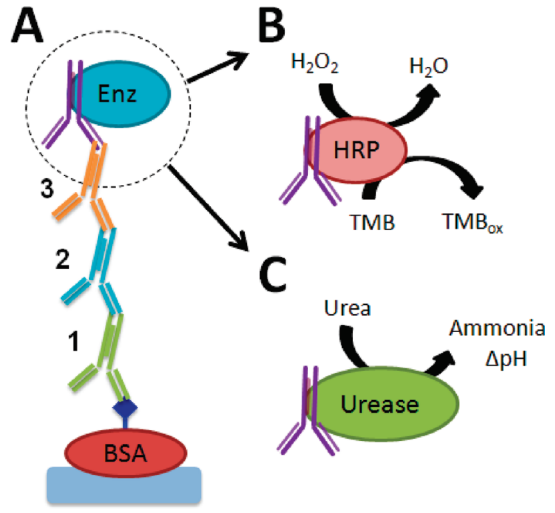
Scheme 1. (A) The Multicomponent Immune-Recognition System Assembled upon Application of the Antibody-Signals in the Correct **1**,**2**,**3** Sequence; (B) The Biocatalytic Reaction Producing the Optical Output Signal upon Oxidation of TMB in the Presence of HRP Bound to the Multicomponent Immune Complex; (C) The Biocatalytic Reaction Producing the pH Changes upon Formation of Ammonia in the Presence of Urease Bound to the Multicomponent Immune Complex



(input signal **3**). The very final step was the attachment of the enzyme-labeled goat-origin IgG specific against mouse IgG to terminate the assembling process with the catalytic species (Scheme 1A). In order to read out the signal from the fully assembled antibody sequence by optical means, horseradish peroxidase (HRP, type VI, E.C. 1.1.1.7) was used as the catalytic label on the terminal antibody (Scheme 1B). The biocatalytic oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB, 41.6 μM) in the presence of HRP and H_2O_2 (1.5 mM) resulted in the absorbance increase (λ_{max} = 655 nm) signaling the full assembling of the antibody sequence (Figure 1). If any of the antibodies were applied in a wrong sequence and did not find the corresponding complementary species on the surface, the final antibody labeled with HRP was not attached to the surface, and the optical change was not observed. All possible six permutations of the antibody signals (**1**,**2**,**3**; **1**,**3**,**2**; **2**,**1**,**3**; **3**,**1**,**2**; **2**,**3**,**1**; **3**,**2**,**1**) were applied to the modified surface, and only the correct sequence of the signals (**1**,**2**,**3**) resulted in the formation of the optical signal as expected. This signal was considered as the "YES" output corresponding to the correct application of the **1**,**2**,**3** password. All other wrong sequences did not result in the attachment of the last HRP-labeled antibody, thus resulting in no signal produced, Figure 1. This was considered as the "NO" output, mimicking denying access in the keypad lock. Minor optical changes observed for the wrong permutations originate from nonspecifically adsorbed species. It should be noted that the difference between the optically read "YES" and "NO" outputs was much bigger when the reaction time allowed for the biocatalytic generation of the optical changes was longer (Figure SI-1 in the Supporting Information).

In order to couple the biorecognition events with a switchable biofuel cell[39] yielding a self-powered bioelectronic device,

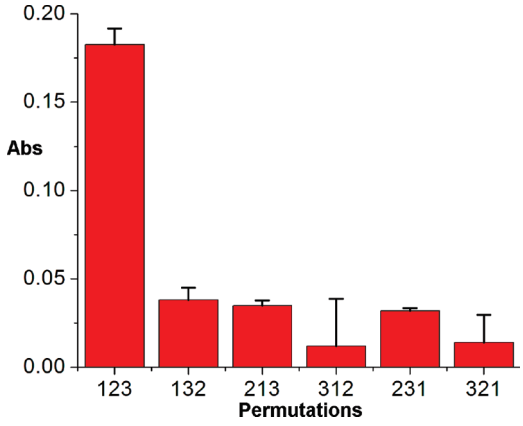


Figure 1. Absorbance increase at λ_{max} = 655 nm obtained upon application of the antibody-signals in different permutations finished with the HRP-labeled terminal antibody, followed by reacting the functional surface with TMB (41.6 μM) and H_2O_2 (1.5 mM) for 60 s.

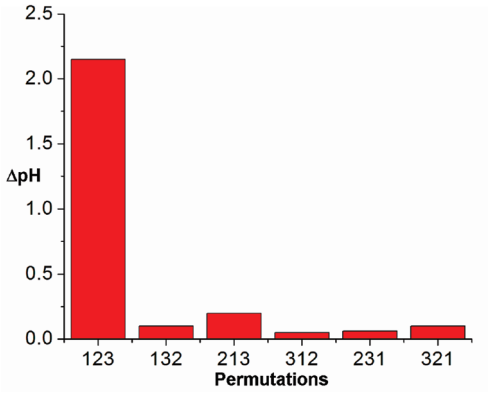


Figure 2. pH changes generated upon application of the antibody signals in different permutations finished with the urease-labeled terminal antibody, followed by reacting the functional surface with urea (10 mM) for 60 min.

the terminal goat-origin IgG specific against mouse IgG was labeled with urease (from jack bean, E.C. 3.5.1.5). This enzyme, when it is bound to the surface through the correct sequence of antibodies, resulted in a pH increase upon the biocatalytic formation of ammonia in the presence of urea (Scheme 1C). The pH changes generated in the system were measured after application of the antibody signals in different permutations (Figure 2). Only the correct sequence of the antibodies added according to the "password" **1**,**2**,**3** resulted in the attachment of the urease-labeled antibody, thus producing ΔpH > 2 (starting from pH = 4.2), while all incorrect permutations of the antibody signals yielded ΔpH < 0.3.

The solutions generated by the immune-recognition system were added to the cathodic compartment of a switchable biofuel cell (Scheme 2). A simple model biofuel cell was composed of two indium tin oxide (ITO) electrodes. The cathode was modified with a pH-switchable poly(4-vinyl pyridine) (P4VP; MW 160 kDa) brush[38] operating in the presence of 10 mM $K_3[Fe(CN)_6]$ used as a model oxidizer in

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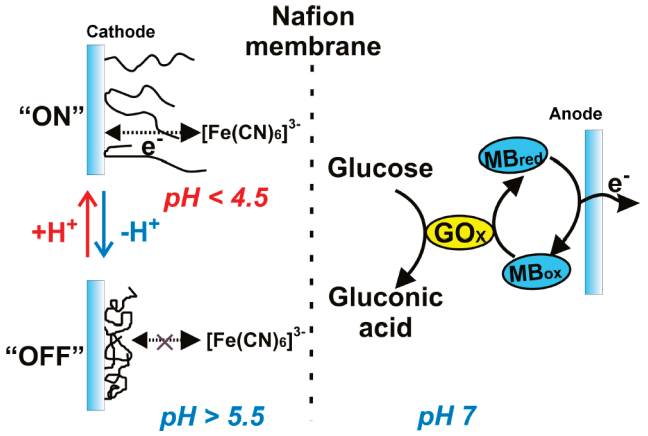
Scheme 2. The Biofuel Cell with the Cathode Switchable by pH Changes[a]



[a] MB_{ox} and MB_{red} are the oxidized and reduced forms of methylene blue redox mediator.

a background solution composed of 0.1 M Na_2SO_4. When pH < 4.5 is applied, the pyridine groups in the polymer-brush are protonated, yielding the positively charged swollen hydrophilic thin-film permeable for anionic redox species, $[Fe(CN)_6]^{3-}$, thus allowing their electrochemical reaction.[38] The polymer brush in the nonprotonated hydrophobic shrunk state at pH > 5.5 is impermeable for $[Fe(CN)_6]^{3-}$, thus inhibiting the cathodic reaction.[38] The anode was an unmodified ITO electrode operating in the presence of soluble glucose oxidase (GOx, type X-S from *Aspergillus niger*, E.C. 1.1.3.4, 250 units·mL^{-1}) which oxidized the glucose fuel (0.1 M) with the help of a diffusional redox mediator methylene blue, 0.1 mM in 100 mM phosphate buffer, pH 7.0, under Ar. The electrodes were separated with a Nafion membrane (0.09 mm thick). The oversimplified design of the biofuel cell was specially selected to clearly demonstrate the power control by the immune-based keypad lock system without any complications from secondary effects related to the bioelectrocatalytic reactions. The experiment was started at pH 4.2 in the cathodic solution when the switchable cathode was in the "ON" state and the entire biofuel cell was active demonstrating a high current−voltage output (Figure 3A, curve a) and high power release (Figure 3B, curve a). Addition of the solutions with almost unchanged pH produced by the immune-system upon "wrong" permutations of the antibody-signals did not affect the biofuel cell performance preserving its power output. The solution with the increased pH value of ca. 6.6 obtained upon application of the only correct sequence of the antibody-signals (**1**,**2**,**3**) resulted in the restructuring of the pH-switchable polymer brush at the modified cathode inhibiting the penetration of the $[Fe(CN)_6]^{3-}$ redox species to the cathode surface. This resulted in the decrease of the biofuel cell activity reflected by the smaller current−voltage output (Figure 3A, curve b) and lower generated power (Figure 3B, curve b). The power release measured at the external load resistance of 180 kΩ, corresponding to the maximum power produced by the biofuel cell in the active state, was significantly decreased only when the "correct"

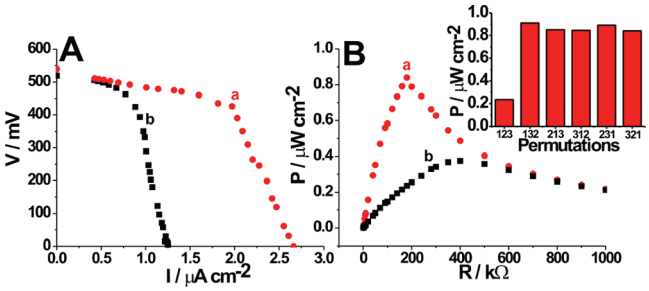


Figure 3. Polarization curves (A) and power output (B) generated by the biofuel cell (a) in the initial active state (pH 4.2 in the cathodic compartment), and (b) after addition of the solution (pH ca. 6.6) generated by the correct sequence (**1**,**2**,**3**) of the antibody-signals finished with the urease-labeled antibody. Inset: The power output measured on the load resistance of 180 kΩ upon application of different permutations of the antibody signals and addition of the produced solution to the cathodic compartment.

sequence of the antibodies was applied (**1**,**2**,**3**) and the obtained solution was added to the biofuel cell (Figure 3B, inset). This was considered as the "YES" output corresponding to the "opening" of the "lock" upon the correct application of the **1**,**2**,**3** password.

The designed immune-based keypad lock system demonstrated the **IMPLICATION** logic function, generating the final output "YES" only when the correct order of the antibody-input signals is applied (the **1**,**2**,**3** "password"). The "answer" "YES" was obtained in the form of the decreased electrical power produced by the biofuel cell. All other permutations of the antibody inputs did not result in the inhibition of the biofuel cell, preserving it in the initial active state, thus "denying" access to the locked information. The designed security system operated without the need of any external power source, producing electrical power itself. It should be noted that the present example device is aimed at the concept demonstration only, while the real operating system should be based on a microfluidic system (lab on a chip) allowing for its miniaturized design.

SUPPORTING INFORMATION AVAILABLE

Chemical composition, assembling and operation of the immune-system, pH-switchable electrode preparation, and the biofuel cell assembling and operation. This material is available free of charge via the Internet at http://pubs.acs.org.

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