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Biocomputing Security System: Concatenated Enzyme-Based Logic Gates Operating as a Biomolecular Keypad Lock

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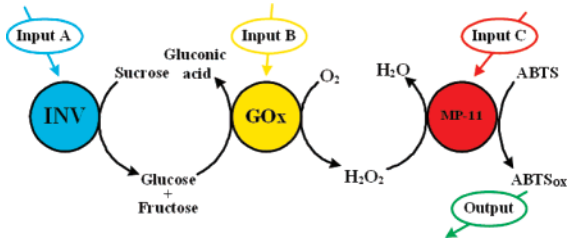
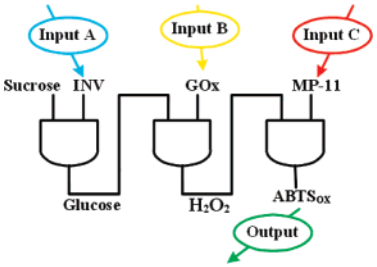
Molecular computing based on chemical reactions performed in solutions or at functionalized interfaces became an important part of research in the area of modern unconventional computing.[1] Numerous chemical systems mimicking different gates performing Boolean logic operations and responding to a large variety of activating input signals (e.g., light, electrical, magnetic, and chemical) were developed in the past decade.[2] Scaling-up these systems allowed assembly of simple computing networks capable of operating as molecular computing devices performing arithmetic functions.[3] Molecular systems mimicking other components of electronic devices performing digital operations (e.g., memory units,[4] comparator,[5] demultiplexer[6]) were reported recently. Further progress in the molecular computing systems resulted in the development of single-molecule-based logic gates[7] and nanosize molecular computing systems.[8] Despite the fact that a very promising future is expected for unconventional chemical computing systems,[9] the development of these systems is limited by their synthetic complexity and difficulty to scale them up for assembling large networking systems. The latter problem originates from incompatibility of most chemical systems performing individual computing operations. This limitation can be solved by the application of biomolecular systems designed by Nature to perform highly specific catalytic or recognition reactions in large ensembles where the individual steps are complementary and the reacting components are compatible. Biomolecular computing (biocomputing) became an important step forward in chemical computing allowing for the solution of complex mathematic problems[10] and assembling large networking systems.[11] Recently developed logic gates based on enzyme-catalyzed reactions[12] allowed assembly of the logic gates in concatenated systems[13] as easily as putting together pieces of a puzzle. The enzyme-based logic gates can be connected with electronic transducers allowing interfacing between biomolecular and electronic systems.[14] The computing networks composed of the enzyme-based concatenated logic gates can be used for mimicking various electronic devices. A ***molecular*** keypad lock was designed recently using a system of fluorescent complexes.[15] The present paper reports on a novel approach to the assembly of the ***biomolecular*** keypad lock using the enzyme-based networking system.

Natural biochemical paths include concert operation of multienzyme systems biocatalyzing chain reactions. Taking out one of the biocatalytic units effectively inhibits the whole chain of the biochemical reactions. This property was used to assemble the enzyme-based biomolecular keypad lock. We designed a model biochemical reaction chain, which included hydrolysis of sucrose to glucose, oxidation of glucose by oxygen to yield hydrogen peroxide, and then oxidation of a synthetic dye, 2,2′-azino-*bis*(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), by H_2O_2 resulting in the formation of a colored product $ABTS_{ox}$, Scheme 1. These reaction steps were biocatalyzed by invertase, Inv (E.C. 3.2.1.26), glucose oxidase, GOx (E.C. 1.1.3.4), and microperoxidase-11, MP-11, respectively, and they proceeded only in the presence of these enzymes. The enzymes were immobilized on glass beads (1 mm diameter) and used in the following amounts: 0.7 nmol of Inv, 0.6 nmol of GOx, and 30 nmol of MP-11 (see the characterization of the immobilized biocatalysts in the Supporting Information).

Scheme 1. Biocatalytic Reactions in the Biomolecular Keypad Lock System



Scheme 2. Representation of the Biomolecular Keypad Lock System as a Network of Three Concatenated **AND** Gates



The initial reaction system included 10 mM sucrose, oxygen (in equilibrium with air), and 0.1 mM ABTS dissolved in 0.1 M phosphate buffer, pH 5.0. The enzymes activating the reaction steps served as input signals for the system, which can be presented as the network composed of three concatenated **AND** gates. Each **AND** gate was activated by two input signals: one of them a simple chemical (sucrose, glucose, and H_2O_2) and the second is a biocatalyst (Inv, GOx, and MP-11), Scheme 2. The chemical input signals for the system were considered as "**1**" when they are present and "**0**" if they are absent. The output signal of the concatenated gates system was measured as the absorbance change ($\lambda = 415$ nm) of the biocatalytically oxidized dye, $ABTS_{ox}$, being "**1**" when $\Delta A > 0.3$ and "**0**" when $\Delta A < 0.2$ (the output signals in the range of $0.2 < \Delta A < 0.3$ were considered undefined similarly to the definitions in electronic logic gates). It should be noted that the sucrose input signal for the first **AND** gate was always "**1**", while glucose and H_2O_2 input signals "**1**" for the second and the third **AND** gates were produced *in situ* upon the biocatalytic reactions.

We have studied the logic responses of the system on the external signals encoded by the added immobilized enzymes: Inv, GOx, and MP-11 (input signals **A**, **B**, and **C**, respectively, considered as "**1**" in the presence and "**0**" in the absence of the enzymes). The experimental absorbance spectra obtained upon eight different

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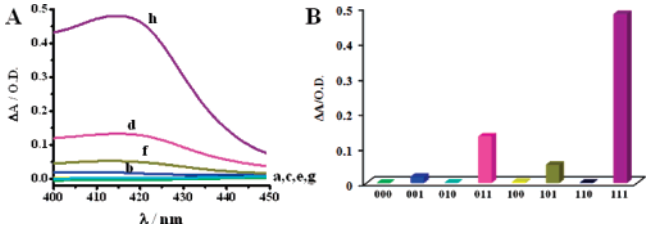

Figure 1. (A) Spectral features of the biomolecular keypad lock system measured 25 min after the enzyme input signals: (a) **0**,**0**,**0**; (b) **0**,**0**,**1**; (c) **0**,**1**,**0**; (d) **0**,**1**,**1**; (e) **1**,**0**,**0**; (f) **1**,**0**,**1**; (g) **1**,**1**,**0**; (h) **1**,**1**,**1**. (B) Bar presentation of the output signals derived from the absorbance spectra, $\lambda = 415$ nm.

Table 1. Truth Table for the Network Composed of Three Biomolecular Concatenated **AND** Gates

Input A	Input B	Input C	Output
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

Table 2. Truth Table for the Biomolecular Keypad Lock System upon Varying the Order of the **A**,**B**,**C** Input Signals

Input 1	Input 2	Input 3	Output
A	B	C	1
A	C	B	0
B	A	C	0
B	C	A	0
C	A	B	0
C	B	A	0

combinations of the three (**A**, **B**, **C**) input signals "**0**" or "**1**" are shown in Figure 1. The responses obtained from the system correspond to the truth table expected for the sequence of the concatenated **AND** gates, Table 1.

However, the most important feature of the keypad lock system is the dependence of the output signal on ***the correct order of the input signals***. Thus, we performed the experiment when the order of the enzyme-encoded input signals was varied in six different combinations, Table 2. Only one correct order of the input signals (**ABC**) resulted in the **TRUE** output signal "**1**" while all others produced the **FALSE** output signal "**0**", Figure 2. Thus, the developed system represents the **implication** logic operation. The **TRUE** output signal "**1**" can be used to "open" the lock while the **FALSE** signal "**0**" can result in the "alarm" signal indicating the wrong password.

The advantage of the used biocomputing approach is an easy reconfiguration of the keypad system with the possibility to introduce many additional biochemical steps/inputs to increase the complexity of the security system. Any fragment of a natural or artificial biochemical path with a different number of the reacting steps activated by various enzymes can operate in a similar way providing a high security of the lock. The approach does not require

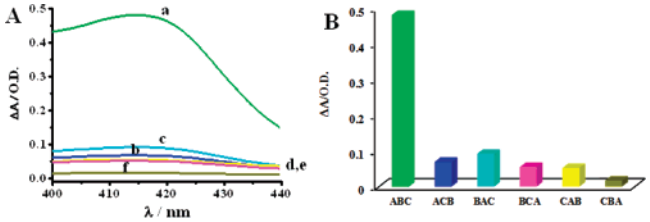

Figure 2. (A) Spectral features of the biomolecular keypad lock system measured 25 min after the enzyme input signals "**1**" applied in a different order: (a) **ABC**; (b) **ACB**; (c) **BAC**; (d) **BCA**; (e) **CAB**; (f) **CBA**. (B) Bar presentation of the output signals derived from the absorbance spectra, $\lambda = 415$ nm.

any complex synthetic materials, and it is based on all naturally available materials providing "green" chemistry. The enzyme input signals are used in catalytic quantities, and they are not consumed upon reactions allowing their multiple use. The present study demonstrates the possibility to scale-up biocomputing elements for assembly in biocomputing networking systems. In addition to the biomolecular security applications, the enzyme-based **implication** logic networks will be extremely important for making autonomous decisions on the use of specific tools/drugs in various implantable medical systems.

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Supporting Information Available: The characterization of the immobilized enzymes and the measurements details. This material is available free of charge via the Internet at http://pubs.acs.org.

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