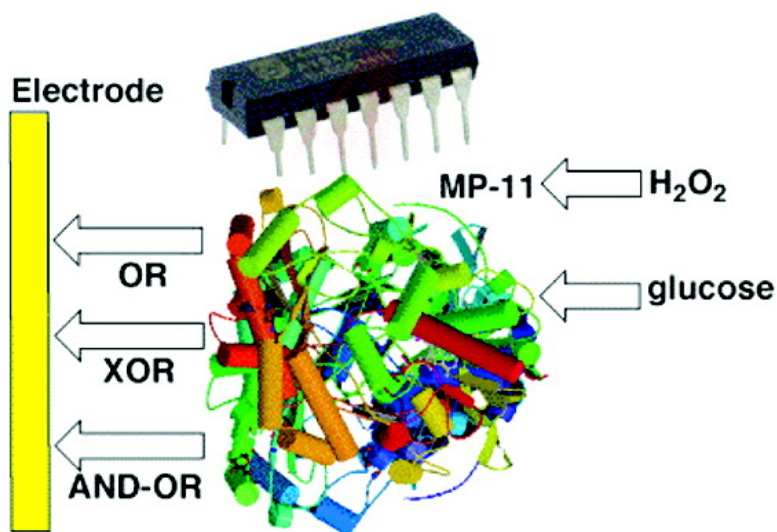


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*J. Am. Chem. Soc.*, **2008**, 130 (1), 36-37 • DOI: 10.1021/ja077908a

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## Multiple Logic Gates Based on Electrically Wired Surface-Reconstituted Enzymes

Marcos Pita and Evgeny Katz\*

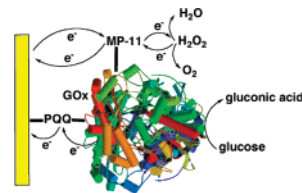
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In the past decade, much research interest was directed to unconventional chemical computing<sup>1</sup> based on chemical systems performing various computing operations in homogeneous solutions or at interfaces functionalized with molecular or supramolecular moieties.<sup>2</sup> The state-of-the-art methods in this challenging research area allows solution of computing problems at the level of a single molecule or a catalytic site,<sup>3</sup> resulting in the nanoscale determination of the computing elements and allowing parallel computation to be done by numerous molecular elements performing different reactions.<sup>4</sup> Various molecule and supramolecule chemical systems were developed to mimic logic operations utilizing a large variety of input signals, such as light,<sup>5</sup> electrical potential/current,<sup>6</sup> or pH changes<sup>7</sup> to activate the computing systems. Chemical systems with simultaneous operation of different gates allow simple arithmetic operations, for example, operating as a half-adder<sup>8</sup> or a half-subtractor.<sup>9</sup> Despite the fact that numerous chemical systems mimicking different computing operations were developed, the field is still in a very early experimental and theoretical stage, but a great future potential is expected.<sup>10</sup> The biggest problems with the chemical logic systems are interference between the reactions resulting in “cross-talking” of the computing units and incompatibility of various chemical gates operating under different conditions. This makes assembling multicomponent/multifunctional chemical logic circuitries very difficult.<sup>11</sup> This problem can be solved by means of a biochemical computation (biocomputing) performed by DNA,<sup>12</sup> enzymes,<sup>13</sup> or complex biochemical systems,<sup>14</sup> performing very specific biochemical transformations and allowing simultaneous operation of several concatenated logic gates in the same homogeneous system.<sup>15</sup> Recently reported logic gates based on enzyme-catalyzed reactions were operating in solutions with the optical read of the output signals.<sup>13,15</sup> The present paper is the first report on the electrically wired enzyme system assembled on an electrode surface and performed with various logic operations depending on the applied potential. This approach allows electrical interfacing between biomolecular computing systems and “normal” electronics.

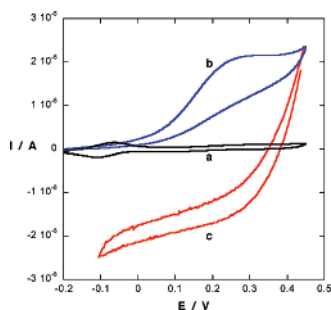
Glucose oxidase (GOx) was reconstituted on an FAD-cofactor-modified electrode surface to yield an electrically wired biocatalytic interface.<sup>16</sup> A Au wire electrode (ca. 0.2 cm<sup>2</sup> area) was modified by a pyrroloquinoline quinone (PQQ) monolayer covalently bound to a cystamine monolayer self-assembled on the electrode surface. Carboxylic groups of the PQQ monolayer were used to bind covalently 1 × 10<sup>-3</sup> M 3-aminophenylboronic acid using 5 × 10<sup>-3</sup> M 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) as a coupling reagent in 0.1 M HEPES buffer, pH 7.3. The phenylboronic acid groups were coupled with vicinal hydroxyl groups of the FAD resulting in the immobilization of the cofactor. Apo-GOx was reconstituted on the FAD-functionalized surface, yielding the electrically wired enzyme, which communicates with the conductive support through the electron transfer mediated by the co-immobilized PQQ monolayer. The lysine residues of the reconstituted

**Scheme 1.** Electrode Functionalized with the Reconstituted GOx and Co-immobilized MP-11 Biocatalyzing Oxidation of Glucose or Reduction/Oxidation of H<sub>2</sub>O<sub>2</sub> Depending on the Applied Potential

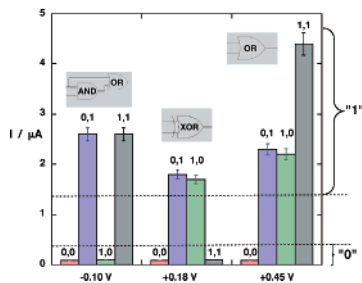


GOx were reacted with microperoxidase-11 (MP-11) in the presence of EDC, resulting in the covalent immobilization of MP-11 at the top of the GOx layer (see the modification procedure in Supporting Information). The modification steps were monitored by electrochemical and quartz-crystal microbalance methods, giving results similar to the published modification scheme, thus ensuring the similar modified electrode structure and activity of the biocatalytic elements.<sup>16</sup> The surface concentration of the reconstituted GOx, 2 × 10<sup>-12</sup> mol cm<sup>-2</sup>, was derived from the QCM measurements, while the surface concentration of the co-immobilized MP-11, 5 × 10<sup>-10</sup> mol cm<sup>-2</sup>, was derived from the cyclic voltammogram of the GOx/MP-11-modified electrode and independently confirmed by QCM measurements (GOx/MP-11 ratio is ca. 1:250 and assumes a multilayer coverage of the electrode with MP-11). The GOx/MP-11-modified electrode was able to catalyze three different reactions: oxidation of glucose and reduction or oxidation of H<sub>2</sub>O<sub>2</sub> depending on the applied potential (Scheme 1). A cyclic voltammogram obtained in the absence of glucose and H<sub>2</sub>O<sub>2</sub> shows only a reversible redox process for the PQQ species attached to the electrode surface (Figure 1, curve a). Upon addition of 5 mM glucose, the modified electrode develops an anodic current corresponding to the glucose oxidation biocatalyzed by the reconstituted GOx and mediated by the PQQ monolayer<sup>16</sup> (Figure 1, curve b). The same electrode upon addition of 5 mM H<sub>2</sub>O<sub>2</sub> generates a cathodic current at *E* < 0.38 V and an anodic current at *E* > 0.38 V originating from MP-11-biocatalyzed reduction and oxidation of H<sub>2</sub>O<sub>2</sub>, respectively<sup>17</sup> (Figure 1, curve c). It should be noted that both bioelectrocatalytic systems, based on GOx and MP-11, are electrically contacted with the conductive support and are independently biocatalytically active. Thus, the addition of both substrates, glucose and H<sub>2</sub>O<sub>2</sub>, results in the activation of both biocatalysts and generates the current corresponding to their bioelectrocatalytic activity upon application of the appropriate potentials. The biocatalytic system was stable enough to operate for several days without significant decay in the activity, and multiple resets upon washing the electrode and addition of new chemical inputs were possible.

In order to perform logic operations with the enzyme-based system, we consider the addition of 5 mM glucose and 5 mM H<sub>2</sub>O<sub>2</sub> as input signals “1”, while the absence of the respective substrate was considered as input “0”. We also defined the currents produced



**Figure 1.** Cyclic voltammograms of the GOx/MP-11-modified electrode in the presence of (a) the background solution, (b) 5 mM glucose, (c) 5 mM H<sub>2</sub>O<sub>2</sub>. The experiments were performed in 0.1 M phosphate buffer, pH 7.0, under Ar, scan rate = 5 mV s<sup>-1</sup>.



**Figure 2.** Amperometric responses (output signals) of the system upon applying different combinations of the chemical input signals and application of various potentials on the electrode.

by the enzyme-modified electrode as the output signal “1” when it is higher than 1.4 μA and the output signal “0” if it is below 0.4 μA (independently on the anodic or cathodic direction of the current). It should be noted that the current range from 0.4 to 1.4 μA was considered to be an undefined output signal (similarly to the approach used in electronics).

The output signals were measured as the currents generated by the enzyme-modified electrode upon application of constant potentials 0.45, 0.18, and -0.10 V in the absence or presence of glucose and H<sub>2</sub>O<sub>2</sub>, corresponding to the combinations of the input signals “0,0”; “0,1”; “1,0”, and “1,1” (Figure 2). At the applied potential of 0.45 V, both biocatalytic units, GOx and MP-11, are activated for the oxidation of glucose and H<sub>2</sub>O<sub>2</sub>, respectively. However, the system demonstrates only a low background current (output signal “0”) in the absence of both substrates (input signals “0,0”). In the presence of any substrate, glucose or H<sub>2</sub>O<sub>2</sub> (input signals “0,1” or “1,0”) or both of them (input signals “1,1”), the modified electrode generates the anodic currents corresponding to the oxidation of glucose or H<sub>2</sub>O<sub>2</sub> or both of them (output signal “1”). Thus, the system performs **OR** logic operation corresponding to the Boolean logic addition:  $A + B$ . At the applied potential of 0.18 V, the reconstituted GOx can oxidize glucose, yielding an anodic current, but co-immobilized MP-11 is capable of reducing H<sub>2</sub>O<sub>2</sub> to produce a cathodic current. This potential was specifically selected to compensate the oxidative and reductive processes and to generate a low resulting current in the presence of both substrates. The modified electrode demonstrates a low background current (output signal “0”) at the potential of 0.18 V in the absence of both substrates (input signals “0,0”). Addition of either 5 mM glucose or 5 mM H<sub>2</sub>O<sub>2</sub> (input signals “0,1” or “1,0”) results in a bioelectrocatalytic current, anodic or cathodic, respectively (output signal “1”). Simultaneous addition of 5 mM glucose and 5 mM H<sub>2</sub>O<sub>2</sub> (input signals “1,1”) results in a low current (output signal “0”) because the oxidative and reductive processes are compensated in the system. Thus, the system performs **XOR** (Exclusive-OR)

logic operation corresponding to the Boolean logic “inequality” function:  $A \oplus B$ . At the applied potential of -0.10 V, the reconstituted GOx is not active for the glucose oxidation and only MP-11 is capable of generating a cathodic electrocatalytic current in the presence of H<sub>2</sub>O<sub>2</sub>. The modified electrode demonstrates a low background current (output signal “0”) in the absence of both substrates or in the presence of 5 mM glucose and absence of H<sub>2</sub>O<sub>2</sub> (input signals “0,0” or “1,0”). However, the system generates a high cathodic electrocatalytic current (output signal “1”) in the presence of 5 mM H<sub>2</sub>O<sub>2</sub> and in the absence or presence of glucose (input signals “0,1” or “1,1”). This behavior of the system corresponds to the equivalent circuitry made of **AND-OR** logic gates performing the following Boolean logic operations:  $AB + A$ .

To conclude, the GOx/MP-11 biocatalytic electrode performs different logic operations upon additions of various combinations of the chemical inputs (glucose and H<sub>2</sub>O<sub>2</sub>) and application of different potentials. The output signals are electrochemically readable, thus allowing the electrical coupling of the enzyme-based biocomputing systems and electronic devices. The enzyme-based system can be adopted to various chemical signals and their combinations to perform logic operations of different kinds and complexity by integrating different enzymes with the electrochemical interface. Integration of several enzymes with the electrode support will allow scaling up of the biocomputing network where the connections between the enzymes will be maintained through the exchange of substrates/products of the biochemical reactions, and the final output signal will be transduced into the electrical current.

**Acknowledgment.** This research was supported by NSF Grants DMR-0706209 and CCF-0726698.

**Supporting Information Available:** Procedure for the chemical functionalization and electrochemical characterization of the modified electrode. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA077908A