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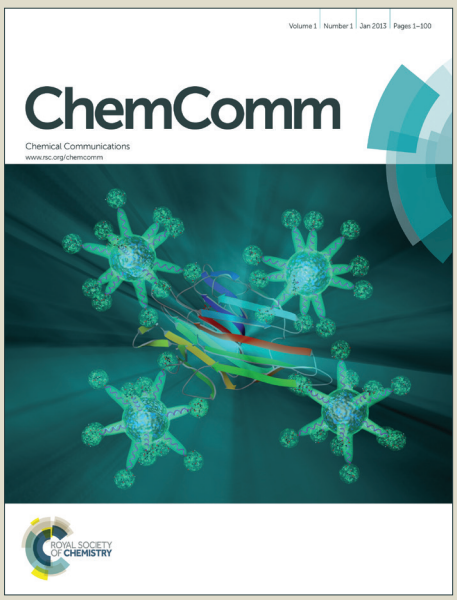

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Enzyme logic AND-Reset and OR-Reset gates based on a field-effect electronic transducer modified with multi-enzyme membrane

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

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Capacitive field-effect sensors modified with a multi-enzyme membrane have been applied for an electronic transduction of biochemical signals processed by enzyme-based AND-Reset and OR-Reset logic gates. The local pH change at the sensor surface induced by the enzymatic reaction was used for the activation of the Reset function for the first time.

Building a computer out of ions, biomolecules (e.g., proteins, DNA (deoxyribonucleic acid), enzymes) or even living cells capable for working and computing inside biological systems may sound like science fiction. However, recent developments in the field of chemical[1] and biomolecular[2,3] logic gates (e.g., AND, OR, XOR, NOR, NAND, INHIB, etc.) demonstrate their ability to mimic the operation of electronic logic gates and show a great potential of this new technology. These devices are capable of receiving multiple biochemical signals to generate a logic output, opening a new research avenue in advanced diagnostics, therapeutics and drug-release systems.[4-6] On the other hand, because the operation of these logic gates is often based on changes in some parameters of the bulk solution (e.g., composition or pH value) induced by the biochemical reaction, the practical realization of multiple logic elements or complex logic systems in the same solution seems to be problematic. Therefore, future of chemical and molecular logic systems is strongly related to their successful transfer from the fundamental research and proof-of-principle experiments to functioning biomedical devices, their integration with electrodes[7-9] and electronic chips, realization of gate-to-gate communications as well as the possibility of their addressing and switching on/off externally. In this context, an interfacing of biomolecular logic principles with field-effect electrolyte-insulator-semiconductor (EIS) devices, which represent an electrochemical analog of the basic element of conventional electronic logic gates and computing, is one of the most promising approaches for the transformation of molecular logic outputs into electrical signals. The feasibility of this approach has recently been demonstrated by realizing enzyme-based AND and OR gates using pH-sensitive capacitive field-effect EIS sensors consisting of an Al-p-Si-SiO_2 structure functionalized with gold nanoparticles and an Al-p-Si-SiO_2-Ta_2O_5 structure modified with a multi-enzyme membrane.[10,11]

To achieve reversible functioning, the logic gates, however, should provide a so-called Reset function, by which the whole logic system or defined individual logic gate is returned to its initial state. To complete the reversible working cycle of the enzyme logic gate, the Reset function is often activated by returning the bulk pH value of the solution to its initial value (e.g., by addition of urea in the solution containing the enzyme urease or by changing the solution and exposing the logic transducers to the initial buffer solution again).[10,11] As a consequence, such approaches make the realization of large logic networks as well as the addressing and switching of individual logic gates very problematic.

In the present communication, we report on application of capacitive field-effect EIS sensors modified with a multi-enzyme membrane (further referred as electrolyte-membrane-insulator-semiconductor (EMIS) sensor) for electronic transduction of biochemical signals processed by enzyme-based **AND-Reset** and **OR-Reset** logic gates. In contrast to other works, the local pH change induced by the enzymatic reaction was used for the activation of the **Reset** function for the first time, while the pH value of the bulk solution remains practically unchanged.

The capacitive EIS sensor is the simplest field-effect (bio-)chemical device and has been applied for the detection of pH,[12] ion concentration,[13] enzymatic reactions,[11,12,14] and charged macromolecules (DNA, polyelectrolytes).[15,16] A schematic of the EMIS sensor and the measurement setup for recording of the output signal generated by the enzyme logic gate is shown in Fig. 1. The capacitive Al–p-Si–SiO_2–Ta_2O_5 structures (30 nm thermally grown SiO_2; 60 nm Ta_2O_5; 300 nm Al as rear-side contact layer) with chip size of 10 mm × 10 mm were prepared from Si wafer (p-Si, ρ=1–10 Ωcm, Si-Mat, Germany). For the details of chip preparation, see Ref. 16. The pH sensitivity of the prepared Ta_2O_5 films was in the range of 55–59 mV/pH that is in good agreement with results reported for a Ta_2O_5 layer previously.[12,17]

The **AND-Reset** logic gate composes of four enzymes (invertase (Inv; EC 3.2.1.26), mutarotase (Mut; EC 5.1.3.3), glucose oxidase (GOx; EC 1.1.3.4) and urease (Ur; EC 3.5.1.5)), while the **OR-Reset**

logic gate consists of three enzymes (GOx, esterase (Est; EC 3.1.1.1) and Ur). The enzymes were immobilized on the Ta_2O_5 surface by crosslinking with bovine serum albumin (BSA) and glutaraldehyde according to the procedure described in Ref. 11,18. To prepare a membrane solution, the enzyme cocktails consisting of GOx (5.9 kU/ml), Inv (20 kU/ml), Mut (1.3 kU/ml), and BSA (40 mg/ml) (for the **AND** gate) and GOx (5.9 kU/ml), Est (0.4 kU/ml), and BSA (40 mg/ml) (for the **OR** gate) were mixed in the ratio of 1:1 v/v with 1% v/v glutaraldehyde solution comprising 10% v/v glycerol. The multi-enzyme membrane was obtained via drop-coating method by applying 2 µl of the particular membrane solution onto the Ta_2O_5 surface, followed by drying in air at room temperature for 30 min and rinsing in ultrapure water to remove non-immobilized components. For the Ur immobilization**,** 1 µl of the solution containing Ur (70 kU/ml) in phosphate saline buffer (PBS, 1 mM, pH 7.5) mixed in the ratio of 1:1 with 1% aqueous glutaraldehyde solution comprising 10% glycerol was applied onto the surface of the EMIS structure of both the **AND** and **OR** gates. The enzymes Inv, Est, GOx, Mut and Ur were acquired from Sigma–Aldrich and Sinus Biochemicals. Other reagents or chemicals (pH buffer solutions, KCl, BSA, glutaraldehyde, glycerol, sucrose, β-D-glucose, ethyl butyrate, urea) were purchased from Fluka and Sigma–Aldrich.

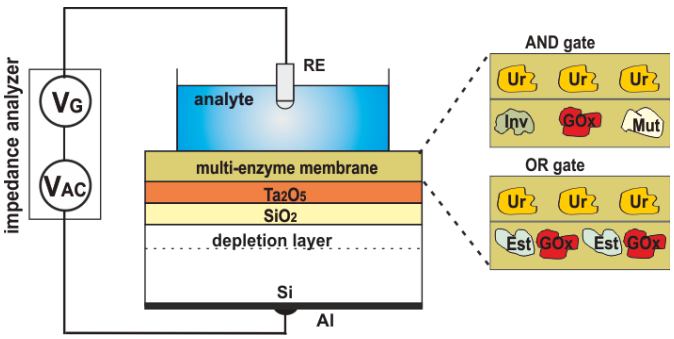

Fig. 1. Multilayer structure of the capacitive field-effect EMIS sensor with multi-enzyme membrane and measurement setup for monitoring the sensor output signal generated by the particular biochemical logic input. For measurements, a direct-current polarization voltage (V_G) was applied between the conventional liquid-junction Ag/AgCl reference electrode (Metrohm) and the rear-side Al contact in order to set the working point of the field-effect transducer in the depletion region of the capacitance-voltage curve, and a small alternating voltage of V_{AC}=20 mV was superimposed to measure the capacitance of the EMIS structure. RE: reference electrode.

The operation of enzyme logic **AND-Reset** and **OR-Reset** gates realized in this study is based on local pH changes near the gate surface of the EMIS sensor induced by the enzymatic reactions. As a result, the pH-sensitive capacitive field-effect transducer generates an electronic signal corresponding to the logic output produced by the enzymes. Therefore, the pH sensitivity of the EMIS structure has been measured before the logic-gate experiments. The pH sensitivity of the EMIS sensor with immobilized glucose oxidase, invertase, mutarotase and urease (**AND-RESET** gate) and with glucose oxidase, esterase and urease (**OR-RESET** gate) was 34 mV/pH and 48 mV/pH, respectively.

For the **AND-Reset** and **OR-Reset** logic-gate experiments, the particular analyte solution (1 mM ethyl butyrate, glucose or sucrose, 10 mM urea, pH 7.5) was applied to the EMIS sensor surface as biochemical input, and the logic output signals have been recorded by means of the dynamic constant-capacitance (ConCap) method using an impedance analyzer (Zahner Elektrik, Germany).[11] The presence of the respective analytes in the solution corresponds to the input signal **1**, while absence of analytes is considered as the input signal **0**.

Fig. 2 shows the schematic of the enzyme-based **AND-Reset** logic gate (a) and the ConCap logic output signal (b) during consecutive exposing of the enzyme-modified EMIS sensor to buffer (pH 7.5) and 1 mM sucrose solution in the presence of dissolved oxygen and its absence. To remove dissolved O_2, nitrogen was bubbled through the solution. The operation of the **AND** logic gate is based on the cascade of enzymatic reactions and was activated by sucrose and dissolved oxygen (input signal **1,1**). The enzyme Inv catalyzes the hydrolytic conversion of sucrose to α-D-glucose and fructose. It should be noted, that because GOx reacts with the α-D-glucose at only 0.64% of the rate that it reacts with the β-D-glucose, the α-D-glucose is a much less effective substrate for GOx.[7,19] Therefore, an additional enzyme mutarotase has been used to mutarotate (interconverse) the α-D-glucose to β-D-glucose, followed by the oxidation of β-D-glucose by GOx in the presence of dissolved O_2. The final product of these biochemical reactions is gluconic acid, yielding to a local decrease of the pH value at the sensor surface.

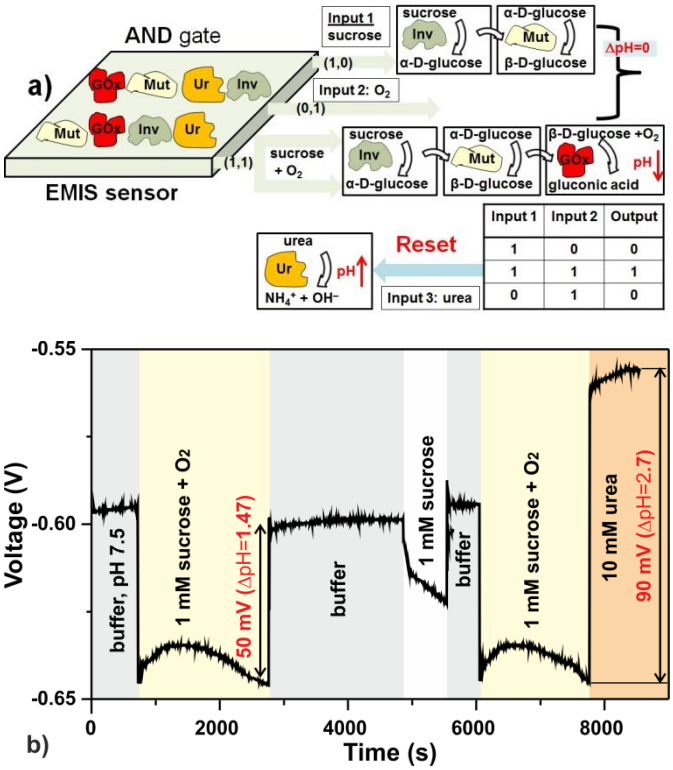

Fig. 2. Schematic of an enzyme-based **AND-Reset** logic gate integrated with the capacitive field-effect transducer (a) and ConCap response of the EMIS sensor with multi-enzyme membrane containing glucose oxidase, invertase, mutarotase and urease measured in 1 mM PBS (pH 7.5) and in analyte solution (1 mM sucrose with and without dissolved oxygen, 10 mM urea) (b). All potential values are referred to the reference electrode.

As can be seen from Fig. 2b, only if both analytes (sucrose and oxygen) are present in the solution (input **1,1**), the cascade of enzymatic reactions is completed, resulting in a reproducible large signal change of about 50 mV towards negative voltage values. Since the pH sensitivity of the EMIS sensor with the multi-enzyme **AND-Reset** gate was about 34 mV/pH, these signal changes

correspond to a local pH decrease at the EMIS surface of ΔpH≈1.47. After each measurement in the sucrose solution, the sensor signal was recorded in a buffer solution demonstrating the reproducibility of the sensor response. The reaction cascade cannot start if sucrose is missing (input signal **0,1**) or it cannot be completed if oxygen is missing (input signal **1,0**). The truth table with respective input signal combinations is presented in Fig. 2a.

In order to provide the reversible operation of the **AND** gate, the local pH value should be increased again. This has been achieved via activation of the **Reset** function by exposing the sensor surface to 10 mM urea solution, resulting in a rapid change in the sensor signal for about 90 mV in the direction of less negative (or more positive) voltage values. This signal change corresponds to a local pH increase at the EMIS surface of ΔpH≈2.7 and is caused due to the formation of hydroxyl ions induced by the enzymatic reaction of urea with immobilised Ur. The local pH at the EMIS gate surface/electrolyte interface was estimated to be pH≈8.7, while the pH value of the bulk solution remains practically unchanged (pH 7.5).

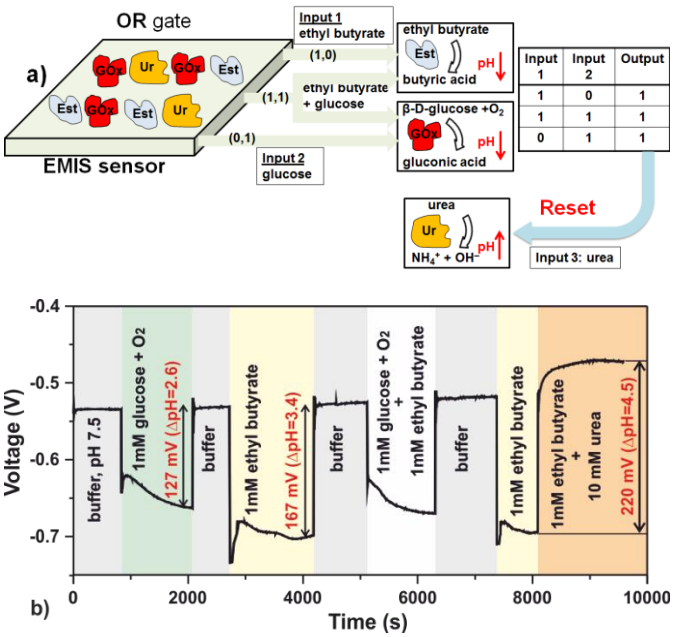

Fig. 3. Schematic of the enzyme-based **OR-Reset** logic gate (a) and the ConCap logic output signal (b) of the enzyme-modified EMIS sensor consecutively exposed to buffer (pH 7.5), 1 mM glucose, 1 mM ethyl butyrate, mixture of 1 mM glucose/1 mM ethyl butyrate or mixture of 1 mM ethyl butyrate/10 mM urea solutions.

The **OR** gate was activated by glucose or/and ethyl butyrate. Fig. 3 demonstrates the schematic of the enzyme-based **OR-Reset** logic gate (a) and the ConCap logic output signal (b) of the enzyme-modified EMIS sensor consecutively exposed to buffer (pH 7.5), 1 mM glucose (input signal **0,1**), 1 mM ethyl butyrate (input signal **1,0**) or mixture of glucose (1 mM)/ethyl butyrate (1 mM) solutions (input signal **1,1**).

The glucose oxidation catalyzed by GOx (in the presence of dissolved oxygen) or hydrolysis of ethyl butyrate catalysed by Est or both of these reactions will result in the formation of acids (gluconic acid, butyric acid, or both), yielding to lower local pH values at the EMIS sensor surface. Signal changes of 127 mV and 167 mV in the direction corresponding to lower pH values have been observed during the exposure of the EMIS sensor to 1 mM glucose and 1 mM ethyl butyrate solution, respectively. Taking into account that the pH sensitivity of the EMIS structure with immobilized enzymes of GOx, Est and Ur was about 48 mV/pH, these signal changes correspond to a local pH lowering at the EIS surface by ΔpH≈2.6 and ΔpH≈3.4 when exposed to glucose and ethyl butyrate solution, respectively. Similar to the **AND-Reset** gate, the **Reset** function was realized by exposing the EMIS sensor to 10 mM urea solution, resulting in a signal change of 220 mV that corresponds to a local pH increase at the EMIS surface of ΔpH≈4.5.

In summary, experiments performed in this study demonstrate for the first time the successful realization of the **Reset** function for both **AND** and **OR** gates via local pH change at the EMIS sensor surface induced by enzymatic reactions. Since the pH value of the bulk solution remains practically unchanged, this approach could enable the realization of large logic networks as well as the addressing and switching of individual logic gates via local pH change. It should be noted that the present device represents a novel interface between biochemical logic systems and electronics. Although, there is an optimistic vision of future use of biomolecular logic systems for real computational applications,[20] the present level of technology and limited complexity of the biomolecular logic systems allow their use rather in biosensing and bioactuating systems logically processing multiple input signals and producing binary YES/NO responses.[21]

The authors thank K. Malzahn, P. Mehndiratta for technical support and M. Weil for valuable discussion.

Notes and references

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