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

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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 and biomolecular 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 in the context of chemical and biomolecular therapeutics.

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,\textsuperscript{12} ion concentration,\textsuperscript{13} enzyme reactions,\textsuperscript{11,12,14} and charged macromolecules (DNA, polyelectrolytes).\textsuperscript{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\textsubscript{2}--Ta\textsubscript{2}O\textsubscript{5} structures (30 nm thermally grown SiO\textsubscript{2}; 60 nm Ta\textsubscript{2}O\textsubscript{5}; 300 nm Al as rear-side contact layer) with chip size of 10 mm x 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\textsubscript{2}O\textsubscript{5} films was in the range of 55–59 mV/pH that is in good agreement with results reported for a Ta\textsubscript{2}O\textsubscript{5} layer previously.\textsuperscript{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 Ta2O5 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 Ta2O5 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 (VDC) 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 VAC=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).12 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 O2, 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 mutarotate has been used to mutarotate (interconvert) the α-D-glucose to β-D-glucose, followed by the oxidation of β-D-glucose by GOx in the presence of dissolved O2. 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 $\Delta \text{pH}=1.47$. 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 $\Delta \text{pH}=2.7$ 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).

The glucose oxidation 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 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, 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.

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### Notes and references


