J Mater Sci: Mater Med (2009) 20:457–462
DOI 10.1007/s10856-008-3579-y

Enzyme-based logic systems and their applications for novel multi-signal-responsive materials

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Received: 16 July 2008 / Accepted: 29 August 2008 / Published online: 7 October 2008

Abstract Recent advances in biochemical logic systems and their integration with signal-responsive materials to yield "smart" hybrid systems are briefly outlined in the paper.

Recent research activity in unconventional chemical computing [1, 2] resulted in the development of various chemical systems processing information and performing Boolean logic operations in response to several chemical input signals [3–5]. Novel horizons were opened in the chemical computing research area upon introducing biochemical systems and formulating biomolecular computing (biocomputing) concepts [6, 7]. Biocomputing is an exciting new field which shows great promise, but at the same time faces substantial theoretical and experimental challenges. Biochemical systems composed of various biomolecules, such as enzyme, DNA or bioreceptors can perform different computing operations mimicking electronic units, e.g., logic gates, flip-flop memory units, keypad locks, etc. Biocomputing elements of moderate complexity could allow effective interfacing between complex physiological processes and nano-structured materials or/and electronic systems. In a short perspective such interface could be applicable in implantable devices, providing autonomous, individual, "upon-demand" medical care, which is the objective of the new nanomedicine concept. In the future this will result in novel human-computer interfaces providing direct coupling of brain (or at least physiological processes) with computers. On the conceptual level, development of biocomputing concepts might help us to understand how living organisms manage to control extremely complex and coupled biochemical reactions, i.e., interpret metabolic pathways in the language of information theory. The complexity of metabolic pathways is comparable with the complexity of computing electronic circuits, operating on internal physiological thresholds and enzymatic decision making much like the electrical thresholds and logic gates of a computer processor (Fig. 1).

The biocomputing systems benefit from highly specific biocatalytic or biomolecular-recognition reactions proceeding simultaneously in multi-component ensembles, where the individual steps are complementary and the reacting components are compatible. Recently a new kind of biomolecular computing systems based on enzyme-biocatalyzed reactions and performing various logic operations (such as AND, OR, XOR, InhibA, etc.,) was pioneered [8]. The enzyme-based logic gates are able to process biochemical input signals upon performing various Boolean operations and generate a single output signal as the result of the biocomputing process [9]. With the advent of biochemical computing, it has become important to explore scalability of biochemical logic gates. The individual logic gates were scaled up to biocomputing assemblies performing simple arithmetic operations (half-adder/half-subtractor) [10]. Further development of the enzyme-based logic gates allowed their assembly in biocomputing

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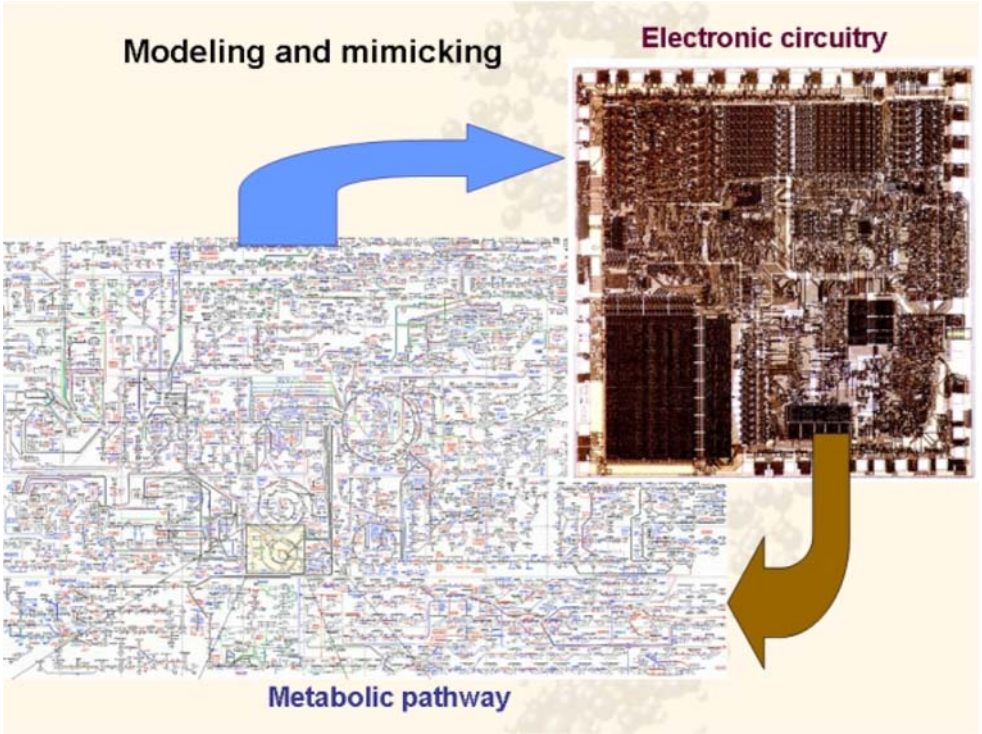

Fig. 1 Comparison between a biochemical metabolic pathway and an electronic circuitry fragment. Similar to the operational processing achieved using logic gates and other electrical components, the human metabolic system implements biochemical feedback via enzymes. The comparable complexity justifies modeling and mimicking of them using similar approaches

networks composed of several concatenated gates able to perform sequences of logic operations upon accepting many biochemical input signals. For example, a series of three concatenated logic gates (OR–AND–XOR) could accept 4 different biochemical signals in 16 different combinations processing them according to the built-in Boolean logic and generating one final output signal dependent on the combination of all input signals [11]. Enzyme-logic networks are able to perform complex logic operations, for example, an IMPLICATION function when the output signal is controlled not only by the values of the input signals, but also by their correct or incorrect order [12]. Enzymes were applied as input signals activating biocomputing systems and resulting in various logic operations [13]. The advantages of the enzyme-input-controlled logic systems are the application of the input signals in a low catalytic quantity and the possibility to use enzyme-inputs as immobilized materials. The enzyme-input-controlled logic gates could be scaled-up to at least 10 units in a logic network upon appropriate optimization of the system [14]. The increased complexity of the biocomputing systems composed of many concatenated logic gates, memory units, and signal transducing elements requires additional studies directed to digitalization and error suppression in the biochemical systems [15].

Achieve the interface between biochemical systems performing computing operations and electronic transducers is a challenging goal for future studies in the biocomputing area. Some experimental steps have already been done in this direction. For example, biomolecular functionalized magnetic nanoparticles were applied to assemble magneto-responsive logic gates with the electronically readable output signals [16]. Multi-enzyme systems electrically contacted with electrodes allowed easy switch between various logic operations depending on the potential applied on the electrode support [17] (Fig. 2). Biocomputing systems can be based on the existing chip technologies currently used for biosensing (particularly DNA chips, micro-fluidic lab-on-chip) [18]. Coupling of biocomputing systems, specifically enzyme-based logic

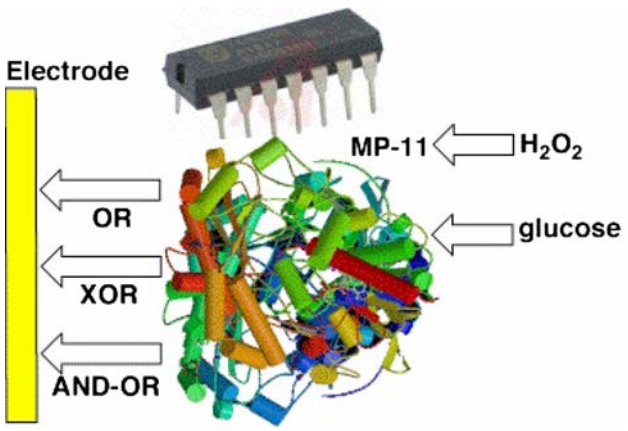

Fig. 2 Reconfigurable enzyme-logic gate based on electrically "wired" GOx and microperoxidase-11 (MP-11) performing OR, XOR, AND–OR logic operations upon application of different potentials on the electrode

gates or their networks, with signal-responsive biocatalytic interfaces would allow "smart" bioelectrochemical systems controlled by many biochemical signals coming simultaneously and processed logically according to the built-in Boolean program. Further scaling down of biocomputing devices to nano-size is possible. Computing problems can be solved at the level of a single molecule, resulting in dramatic miniaturization and allowing parallel computation performed by numerous molecules [19]. This approach will finally result in miniaturized multi-signal-responsive biosensors.

Another research direction related to the enzyme-logic systems is the fabrication of "smart" multi-signal-responsive materials equipped with built-in Boolean logic. The systems will be capable of switching physical properties (such as optical, electrical, magnetic, wettability, permeability, etc.,) upon application of some incoming chemical signals and according to the build-in logic program. Chemical reactions biocatalyzed by the enzyme-based systems will respond to the incoming chemical signals according to the Boolean logic and transfer the outgoing signal to the responsive polymeric support. The chemical coupling between the enzymatic systems and the polymeric supports can be based on electron or proton exchange between them. The electron exchange between the redox-active polymeric support and the enzymatic system will result in the reduction or oxidation of the polymer. The proton exchange between the polymeric support (polyelectrolyte in this case) and the enzyme-system will yield different ionic states (protonated or unprotonated) of the polyelectrolyte support. The expected changes of the polymeric support upon oxidated or protonated states will result in the changes of the composition/structure of the polymeric matrix support. This will result in the corresponding changes of the matrix specific properties to be considered as final outgoing signals (Fig. 3). It should be noted that two distinct parts of the integrated systems will be responsible for the process: (i) The biochemical (enzymatic) systems responsive to the external chemical incoming signals will provide the variation of the system composition/structure according to the build-in Boolean logic. (ii) The polymeric supports will transduce the composition/structure changes to the changes of the matrices properties, thus providing the transduction of the incoming chemical signals (addition of chemicals) into outgoing physical signals (change of the optical, electrical, magnetic properties, change of wettability, permeability, etc., of the matrix). It should be emphasized that this transduction process will follow the logic operations provided by the biochemical systems.

Recent studies have shown that chemical transformations occurring at various interfaces and in polymeric matrices (induced by photochemical [20], electrochemical [21], magnetic [22], or chemical/biochemical means [23]) can result in the substantial changes of the properties of the materials (optical density, reflectivity, electrical conductivity, porosity/permeability, density/volume, wettability, etc.,). Mixed-polymer systems (specifically polymer "brushes") were shown as highly efficient responsive systems substantially changing their physical properties upon reconfiguration of the components included in the mixed system [24, 25]. Incorporation of enzymes, which operate as logic gates [8–13], in the polymeric matrices would allow Boolean treatment of the incoming (input) chemical signals and the respective changes of the material properties. Two different input signals coming to the system will

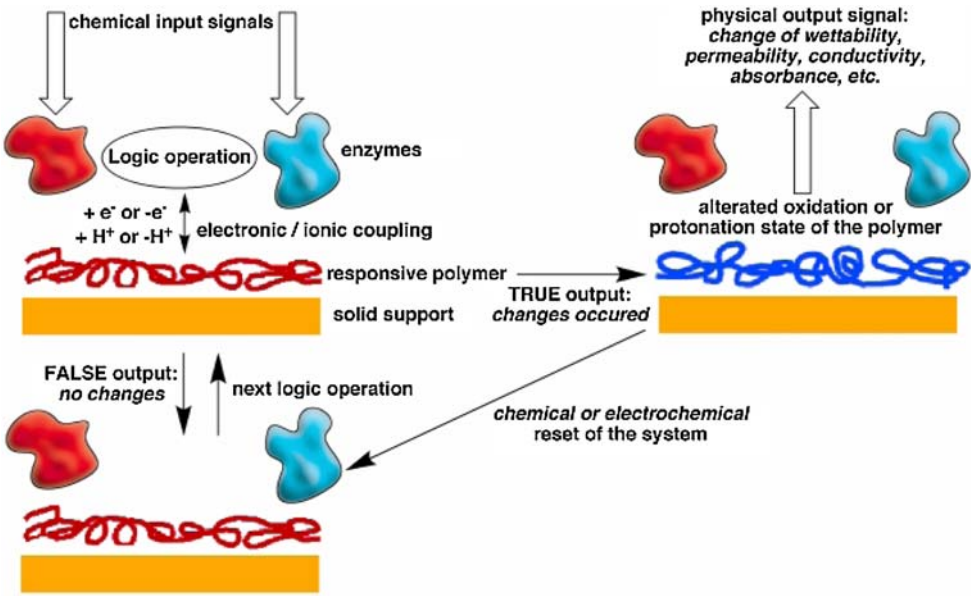

Fig. 3 Scheme showing the general concept of interfacing enzyme-based logic systems with signal-responsive polymers operating as "smart" chemical actuators controlled by the gate output signals

change the material properties according to the Boolean treatment of the incoming signals and upon electronic or ionic coupling between the enzymes and the responsive polymer (exchange of electrons or protons resulting in the alteration of the oxidation or protonation state of the polymer). It should be noted that the changes schematically shown in Fig. 3 will occur only when the output signal of the logic gate is "TRUE" (1), while the "FALSE" (0) will not result in any changes in the system. If the system is changed (upon a TRUE outcome signal), it will be reset to the original state by chemical or electrochemical means (addition of chemicals to the solution or application of a potential on the conductive solid support). Then, the system will be ready to response to the next incoming signals that may (or may not) change the polymer state according to the Boolean treatment of the new input signals.

Since many polymer systems are pH-sensitive and switchable, we designed enzyme-based logic gates using enzymes as biocatalytic input signals, processing information according to the Boolean operations AND or OR, and generating pH changes as the output signals from the gates. The AND gate performed a sequence of biocatalytic reactions (Fig. 4): sucrose hydrolysis was biocatalyzed by invertase (Inv) yielding glucose, which was then oxidized by oxygen in the presence of glucose oxidase (GOx). The later reaction resulted in the formation of gluconic acid and therefore lowered the pH value of the solution. The absence of the enzymes was considered as the input signals "0", while the presence of them in the optimized concentrations was interpreted as the input signals "1". The biocatalytic reaction chain was activated only in the presence of both enzymes (Inv and GOx) (input signals "1,1") resulting in the decrease of the solution pH value. The absence of any of two enzymes (input signals "0,1" or "1,0") or both of them (input signals "0,0") resulted in the inhibition of the gluconic acid formation and thus no pH changes were produced. Thus, the biocatalytic chain mimics the AND logic operation expressed by the standard truth table corresponding to AND Boolean operation. Another gate operating as Boolean OR logic function was composed of two parallel reactions (Fig. 5): hydrolysis of ethyl butyrate and oxidation of glucose biocatalyzed by

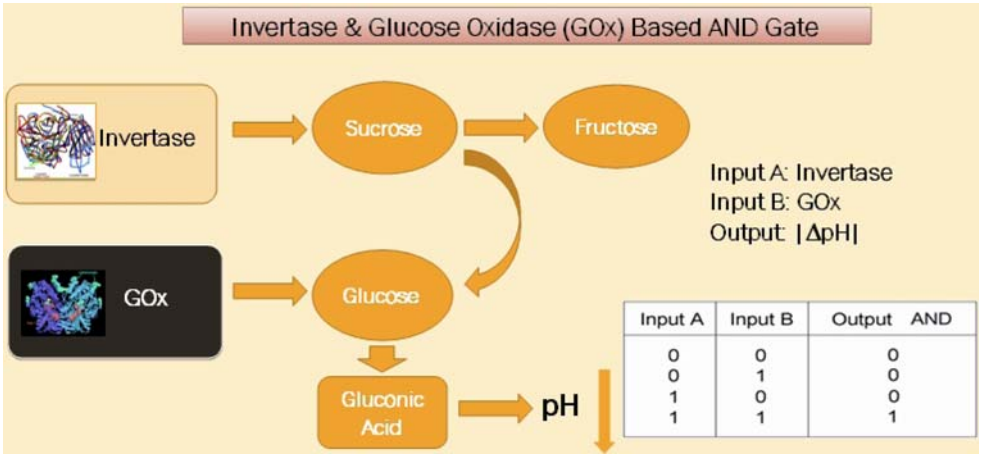

Fig. 4 Enzyme-based AND logic gate producing pH changes as the output signal

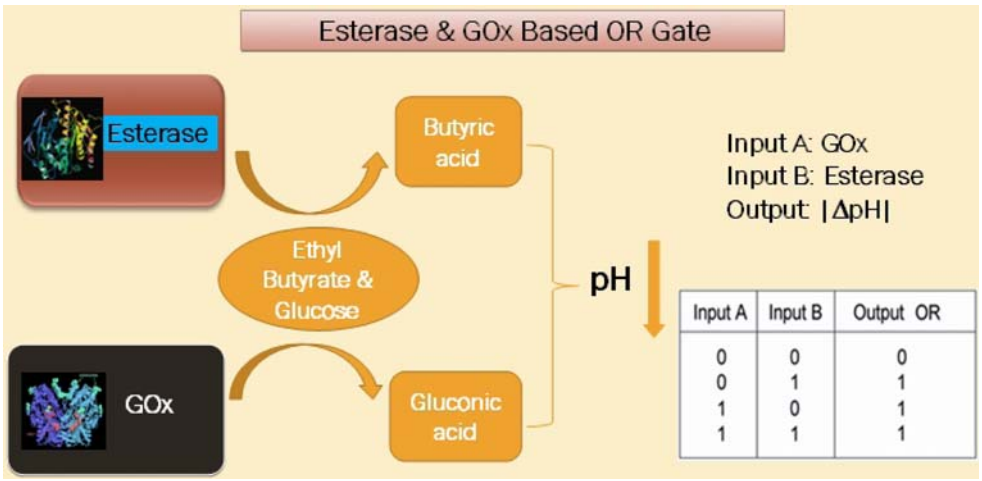

Fig. 5 Enzyme-based **OR** logic gate producing pH changes as the output signal

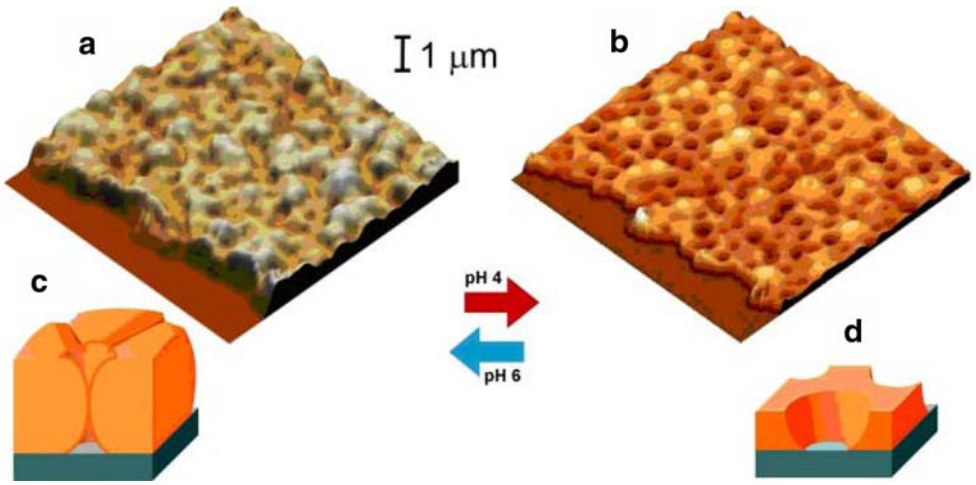

Fig. 6 "Smart" membrane coupled with the enzyme-logic systems to open and close pores upon processing biochemical information. Scanning probe topography (SPM) images (10×10 μm^2) of a swollen, pH 4 (**a**), and shrunken, pH 6 (**b**), polymer membrane with the corresponding schematic representations of a single pore in the closed (**c**) and open (**d**) states

esterase (Est) and GOx, respectively, and resulting in the formation of butyric acid and gluconic acid. Any of the produced acids and both of them together resulted in the formation of the acidic solution. Thus, in the absence of both enzymes (Est and GOx) (input signals "0,0") the both reactions were inhibited and the pH value was unchanged. When either enzyme (Est or GOx; input signals "0,1" or "1,0") or both of them are together (input signals "1,1") one or both of the reactions proceeded and resulted in the acidification of the solution. The features of the system correspond to the OR logic operation and can be expressed by the standard truth table. The enzyme-logic systems producing pH changes were coupled with many pH-sensitive polymer-functionalized systems: membranes, polymer-stabilized nanoparticle suspensions or water/oil emulsions, polymer-modified electrodes, etc. For example, the pH changes produced by the AND/OR enzyme-logic gates shown in Figs. 4 and 5 were coupled with a pH-responsive membrane, resulting in the opening/closing of the membrane pores, thus transducing the biochemical logic operation into the bulk material property change (Fig. 6). All studied systems revealed Boolean logic operations encoded in the enzyme systems.

A variety of applications could be envisaged for the developed hybrid systems, including for example signal-controlled drug delivering. A diverse range of 'smart' (stimuli-responsive) materials, with switchable physical properties, has been developed for in vivo drug delivery [26]. The new hybrid materials with the built-in Boolean logic will be capable of switching physical properties in response to the output of the enzyme-logic system towards autonomous on-demand drug delivery. The gate output signals will activate "smart" chemical actuators, resulting, for example, in the opening of a membrane releasing a drug (such as mannitol, if the logic states dictated need for a reduced intracranial pressure [27]).

The demonstrated approach for the interfacing of biomolecular computing systems with signal-responsive materials enables the use of various biocatalytic reactions to control transition of the properties of responsive materials and systems with built-in Boolean logic. This approach would be an efficient way to fabricate "smart" multi-signal-responsive drug delivery systems, sensors, miniaturized switchers, microfluidic devices, etc., which can serve without communication to an external computer.

Acknowledgments This research was supported by the NSF grants "Signal-Responsive Hybrid Biomaterials with Built-in Boolean Logic" (DMR-0706209) and "Biochemical Computing: Experimental and Theoretical Development of Error Correction and Digitalization Concepts" (CCF-0 726698).

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