Multisignal digital biosensors for medical applications

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Abstract. Biocomputing has recently gained a place beneath other computing paradigms. Utilizing the natural rules of interaction between biochemical substances mimicking conventional computer systems, new ways and techniques of solving computing problems have been developed. This review covers the concept of biosensors, based on enzyme logic networks, that can process multiple biochemical input signals. This concept of utilizing enzymes as the "machinery" of biochemical logic networks, aims to create complex analytical biosensors for medical application.

Keywords: Multi-input Biosensors · Biosensors · enzyme-based logic gates · logic networks · biomedical applications!!!!!!

1 Introduction

In the medical field biosensors (analytic devices to convert the result of a biochemical reaction into an electric signal) are an essential tool for the monitoring and detection of a wide range of medical conditions. Biochemical substances are chemical substances that occur in natural environments like enzyme or glucose. The currently available sensors work as single-input devices, that can analyse only one specific substance. The new concept aim to create devices, that can analyse multiple inputs and transform those into a single output signal. This change allows for a variety of possible applications.

Once realized, those biosensors are expected to revolutionize a great number of medical treatments and therapies. Through the analysis of multiple inputs, the sensors could scan specific injuries or diseases. More complex models could create sense-act-treat devices, that immediately apply the correct treatment after recognizing a specific result of the analysis. Especially in medical emergencies, where rapid reactions are of paramount importance, these developments would mark a ground-breaking change. As a further application the device could oversee the complete therapy process and analyse the impact and adjust the treatment if necessary. This repeatable mechanism is called a feedback-loop. A similar setup is already in use for Diabetes treatment. Even personalized medical therapy, which could autonomously adjust the treatment tailored to a patients individual needs, is imaginable.

The basic principle of biosensors are its two layers: the receptor, that processes the biochemical input substances and the transducer, that translates the received signal into a yes/no-output. The main innovation of the new concept is the replacement of the current one-input receptor with a biocomputing network, that allows a re-creatable analysis of multiple inputs, which are then transformed into one binary output signal. The logic network, consisting of enzyme-based logic gates mimicking Boolean operations, can process biochemical information.

!!!!!!!As the controlled reactions provide a reliable result, this result can be transformed into a yes/no signal.

This document is structures as follows. The concept of biosensors with enzyme-based logic is introduced in Section 2. Subsection 2.1 describes the principle of enzyme-based logic gates, while Subsection 2.2 covers the topic of logic gate networks and their combination with transducers. Section 3 presents a practical concept of a relevant medical application. The most common considerations for future development are listed in section 4, while section 5 summarizes this article.

2 Concept

The reviewed concept presents biosensors that rely on complex enzyme-based logic networks. Those networks consist of biochemical logic gates that mimic Boolean operations. The concept relies on an classic two layer structure with a biocomputing layer, containing the logic network, and a transducer converting biochemical interaction results into a signal that is observable.(e.g an electric current). This allows the device to process several biochemical input signals in the biocomputing layer before transducing the result of the logic network.

2.1 Enzyme-based logic gates

In biocomputing enzymes are used as logic gate constituents. Through reaction with chemical inputs they create an always-the-same end product, which is used to mimick Boolean logic gates such as AND and OR. Two concentration levels of the input chemicals are defined as the Boolean 0 and 1 values. 0 is usually considered as the absence of a substrate, but it can be altered. 1 equals a significantly difference to the as 0 defined concentration. The concentration of the desired target substance that results out of the logic gate reaction is defined with the Boolean value 0 and 1 as well. [5]

Logic gate example AND In Fig 1 the enzymes glucose oxidase (short Gox; catalyses the oxidation of glucose and is often used for analysing free sugar in blood) and catalase (catalyzes the decomposition of hydrogen peroxide to water and oxygen) operate as the logic gate machinery. The two input signals are H_2O_2 (Hydrogen peroxide) and glucose (blood sugar). If both substrates are present the reaction produces gluconic acid (a mild organic acid), which results in an optical absorbance change. This change was defined as the target output signal of the enzyme logic gate. The optical absorbance change will only appear in the presence of both inputs, mimicking the Boolean operation AND.

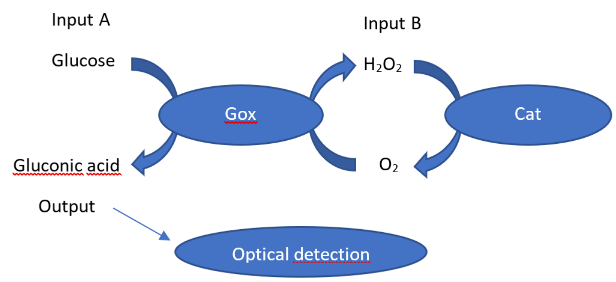

Fig. 1. Network diagramm

Glucose	H_2O_2	Output
0	0	0
0	1	0
1	0	0
1	1	1

2.2 Networks with electrochemical transduction

By assembling multiple logic gates, each mimicking a Boolean operation, it is possible to create small logic networks (e.g. half-adder/ half-subtractor). To convert the physical change of the final network output into an electric signal a transducer is added.

Figure 1: A combination of different enzym-based logic gates, functioning as AND or OR. The cascade of reactions, when all four input chemicals are present, results in ph changes. Ph measures the degree to which a solution is acidic or alkaline. The scale is defined from 0 (acid solution) to 14 (lye solution). The logic network is composed of four concatenated logic gates. Those gates contain the enzymes alcohol dehydrogenase (ADH, disassembling toxic alcohols), glucose dehydrogenase(catalyst for the oxidation of glucose) and glucose oxidase act as logic gates the four specific different input signals NADH (resulting through reduction from Nicotinamid-Adenin-Dinukleotid NAD, which is a coenzyme found in all living cells), actealdehyde (also named ethanal, which is an organic chemical compound, for example naturally occuring in coffee, bread, and ripe fruit), glucose and oxygen (O). When all inputs are present the reaction yields to an acid medium, lowering the original pH value of the solution from initial 6-7 to approximately 4.

Fig. 4 a Multigate/multisignal processing enzyme logic system producing in situ pH changes as the output signal. **b** The equivalent logic circuitry for the biocatalytic cascade. *ADH* alcohol dehydrogenase, *GDH* glucose dehydrogenase (From [53] with permission. Copyright American Chemical Society, 2009)

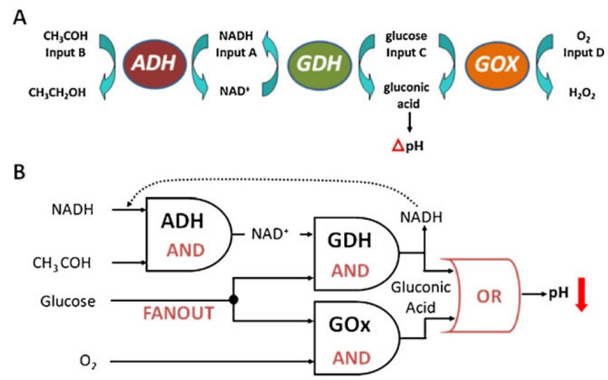

Fig. 2. Network diagramm

The lower pH value resulted in switching to ON of an electrochemical interface that functioned as a transducer which makes the electrical signal readable (with low voltage as the 0-value and a defined higher voltage as 1). While 16 different combinations of input signals were possible (being present or absent) only four of those resulted in an ON state.

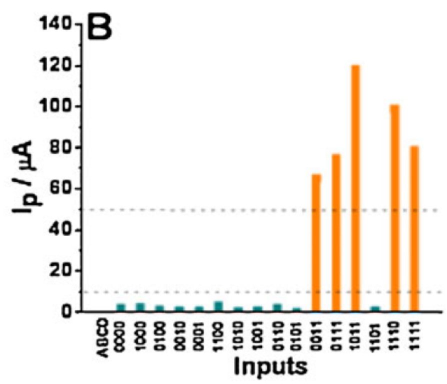

Fig. 3. Network diagramm

This combination is an example for a multisignal processing enzyme logic system coupled with an electrochemical transduction readout of the output signal (with pH change).

3 designing for biomedical analytic applications

To recognize various medical relevant conditions, it is necessary to use characteristic biochemical substances as inputs for the logic network. Physiological conditions resulting of a injury lead to a change in the concentration of some biochemical substances in the body, that are typical for this condition. This substances can be used as inputs for the enzyme-based network, created for the analysis.

brain injury and hemorrhagic shock The following logical system, composed AND and IDENTITY gates, has been designed to process information related to brain injuries and hemorrhagic shock. The logic gates include the three enzymes lactate oxidase(!!!!!!), horseradish peroxidase(!!!!) and glucose dehydrogenese(!!!!!). As inputs it receives the three substrates glucose, lactate(!!!!) and norepinephrine()!!!!!!!! related to conditions originating from traumatic brain injury and hemorrhagic shock().

A increased glucose concentration could relate to a hemorrhagic shock, while a higher lactate concentration could either indicate a hemorrhagic shock or a brain trauma and norepinephrine can be a indicative of any traumatic injury. For the inputs 0 has been defined with the normal physiologically concentration and 1 as a abnormally increased concentration.

Optical and electrochemical means were used as transducer

Especially the following combinations of input signals appeared to correspond the the two different conditions

condition	norepinquinone	NADH	glucose	lactate	norepinephrine
traumatic brain injury	1	0	0	1	1
hemorrhagic shock	1	1	1	1	1

binary output

4 considerations

Being only a concept yet, there are plenty of challenges, that must be solved until a successful realization of complex applicable biosensors. The most current are as follows.

Surface confinement In recent research, the success of enzymatic reactions was growingly dependant on the immobilization of the reagent layer. Early experiments with enzyme logic dissolved the gate constituents and chosen inputs in a solution. With the aim of complex networks that require multiple different gate constituents and inputs, each section needs to be differentiated and isolated, to prevent cross-reactions. To achieve multiple and stackable logic gates, the biocomputing layer needs to be immobilized. Agglomerations of multiple logic systems also induce further challenges. All reacting components need to be carefully evaluated to prevent cross-reactions, while the outside coating of the device must simultaneously protect the reagents within the device but be permeable to the desired inputs. The impact of an immobilization on the performance of biosensing devices has yet to be examined.

Optimal transduction More complex setups require better and more versatile transducers. With the universal goal of processing an arbitrary number of inputs into only one output signal, the development of versatile transducers becomes crucial. WHAT The transducer must be able to process multiple logic-gate results and yet translate those into one (optical or electrical) output signal. Current research had to apply variant transducing strategies for complex devices, but aims to develop a multipurpose transducer, which can be generally utilized.

Defining the Boolean 0- and 1-values In the biochemical logic system as well as for the transductiors, there has to be a defined 0 and a respectively defined 1 value. 0 should mark the biological standard value, while the state of the art is to define it as the absence of signals(!!!!!), 1 the critical value. The definition of the 1-value is also problematic. In research, the 1 value has mostly been defined as a random convenient value, instead of using applicable medically critical values. Within the same field, the difference between a relevant 0 and a relevant 1 can be minimal. This leads to difficulties with the transduction. An offered solution would be a sigmoidal signal translation rather than the usual linear, to emphasise the difference an allow more reliable reading. Scalability One central aim of research is to achieve a maximal flexibility of sensors. In the end, it is highly expected to be able to scale every parameter of the sensor, from the complexity of the logic system to the specificity of the transducer. The aim is, to potentially combine any given number of logic gates into networks, that create more and more complex logic systems and respectively allow new applications.

Relevant inputs A majority of laboratory projects worked with biomedical irrelevant substances for their proof of concept. A crucial step is to make the concept work with relevant inputs. In recent studies, scientists realized networks with biomedically relevant substances as inputs. The used logic networks yet did not depict a senseful logic context to the substances(WHAT). Thus not only the work with relevant substances needs to be developed further, but also the logic networks need to be adjusted to depict relevant ???

Challenges of drug delivery One slightly more distant concern is the functionality of drug delivery devices. One of the key goals of research is to develop autonomous devices, that analyse certain physiological parameters and offer an immediate reaction. Experiments have shown a need for different methods and technology to distribute the correct treatment within the cycle of those devices.

5 Conclusion

- design of biosensoric systems with logically processes signals represented by varous biomarkers characteristic for different abnormal physiological conditions
- analysis of the chemical output signals generated by biomolecular logic systems is often limited.
- Enzyme logic system: multiassemblies to perform simple arithmetic functions
- idea: applicatoin of biomolecular logic system for analytical purpose new class of biosensors that accept many input signals and produce binary outputs in form yes/no
- example analyse protein libraties associated with muliple sclerosis(58)

- not just chronic but also ....
- state of the art
- feedback loop currently devoted to management of diabetes through integration of an electrochemical glucose sensing element with an insulin-delivery feedback loop for the optimal dose of insulin (69-71)
- example analyse protein libraties associated with muliple sclerosis(58) ENzyme logic system recognizing various injury-related physiological conditions
  - types of injuries result in concentrations of chemical substances in the body
  - example: lactate axidase, horeserasish peroxidase and glucose dehydrogenase = designed to process biochemical information related to pathophysiological conditions from brain injury
  - markers: glucose(hemorrhagic shock),lactate(rhagic shock or traumic brain injury) and norepinephrine(traumatic injury)
  - logic 0 = normal concentrations
  - change results into different numbers 1,2,3 - convenient
  - = biocomputing logic system
  - challenge: difference between normal and unnormal minimal =¿ not linear, should be sigmoidal

- optimal surface confinement of the biocomputing layer
- engineering enzyme microenvironment (transducer layer)
- contact between biocomputing layer and transducing surface
- combine individual logic-gates and maintain high enzymatic stability and reataining individual reagents
- leakage of cosubstrate
- no cross-reactions
- surface confinement? layer-by layer? more efficient and rational
- level of the surface confined reagents tailored for account of different input concentrations /enzyme activities
- coating: optimized for transport and excluding potential interfernece and protecting the surface

5.1 optimal transduction of biocomputing signal processes

- simultaneous measurements of multiple outputs require different transduction strategies (common: fixed potential)

- Requires:interface of biocomputing systems + electronic transducer Therefore
- scalability (increasing nuber of logic gates, assembling into complex networks)
- complexity(coupling of gates abd non boolean elements)
- composition, preparation and immobilization of the biocomputing surface layer
- layer by layer
- optimal surface confinement
- careful engineering of the enzyme microenvironment(on transducer surface) for performance
- biocomputing layer + transducing layer + combine individual logic-gate elements

good but needs lot of work
sums up bla
Profound impact

Through processing automatically several biochemical inputs(physiological information), it can provide a rapid and reliable assessment of overall physiological conditions. This can help a optimal timley therapeutic intervention. They will realize sense/delivery feedback loops by coupling signal processing with chemical actuators to revolutionize patient monitoring and drug delivery.

In the Biosensors processing multiple biochemical signal, the core idea is to add a biocomputing layer that produces a final output in form of a yes/no response. Kapitel 1.2

Chances:

In contrast to recent biosensors, those with a 11111111111 logic promise a higher fidelity, a greater range of processable inputs, more complex applications such as sense-act-treat loops and rapid assessment of the respective substances.(mehr ausformulieren)

5.2 Biosensors logic systems

Biosensors logically processing multiple biochemical signals
-such procassed information produces a final output yes/no
- boolean logic networks composed of biomolecular systems

- multiple target analytes(inputs) for enzyme gates
- high-fidelity compared

- closed loop/feedback loops possible (sense/act/treat)
- rapid and reliable assessment of overall physiological condition
- could initiate optimal timely therapeutic intervention
- biosensors + enzyme logic gates
- allows direct coupling of signal processing with chemical actuators
- application og biomolecular logic systems for analystic purposes could yield a novel class of biosensors: many input signals and binary outputs
- logically processed feedback between drug appl. and physiological conditions can signifacntly imprive drug targeting and efficiency
- difficulties: complexity by assembling individual logic gates into complex logic networks (intelligent by molecular logic) (43-34-67)
- new approach for the sensor design and operation, interfach biocomputing system and electronic transducers

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