# NANOBIOCATALYSTS FOR BIOFUEL CELLS AND BIOSENSOR SYSTEMS

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FIELD: Chemical Technology

*Abstract:*

*This overview summarizes the application of enzymes in the manufacture and design of biofuel cells and biosensors. The emphasis will be put on the protein engineering techniques used for improoving the properties of enzymes such as nanobiocatalysts, e.g. immobilization orientation, stability, activity and efficiency of electron transfer between immobilized enzymes and electrodes. Some possible applications in the military and some future designs of these electric devices will be discussed as well.*

*Key words: nanobiotechnology, enzyme logical gates, directed evolution, high throughput screening, microfluidics, glucose oxidase, army, cryptography.*

## Introduction

Ananobiocatalyst is a term reffering to a biocatalyst in the form of enzyme or cell immobilized or modified with nanostructured materials, such as nanoporous materials, nanoparticles, nanofibers and nanotubes [1].

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Recent advances in the nanoscale science and technology have brought a new wave in the field of biocatalysis. Nanoscale engineering of biocatalysts is greatly promising for the development of high performance biofuel cells and novel biosensor systems [2, 3]. In addition, advancements in protein enginee-ring techniques and bioinformatics are opening new possibilites for further im-provements of biocatalyst performances in these devices [4].

# Nanobiocatalysis in Biofuel Cells

Biofuel cells use biocatalysts to convert chemical energy into electrical. A biofuel cell requires an anode and a cathode with immobilized enzymes or cells, a supporting electrolyte medium to connect the two electrodes and an e-xternal circuit to use extractable power [5]. On the anode, organic compounds oxidise, helped by an enzyme/cell, giving electrones to the electrode and on the cathode another enzyme/cell receives these electrones from the electrode and transmits them to the oxygen or another oxidizer like hydrogen peroxide. One of the first enzymatic biofuel cells reported in the year 1963 was made using glucose oxidase on the anode [6]. Today most of enzymatic cells are obtained using glucose oxidase on the anode and cytochrome c oxidase on the cathode (Scheme 1).
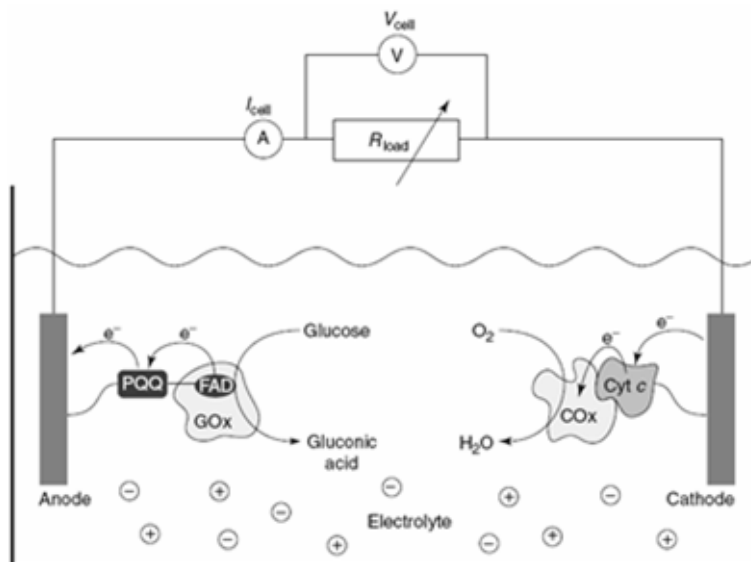


*Scheme 1* – An enzymatic biofuel cell. GOx- glucose oxidase; Cyt c-cytochrome c; COx-cytochrome c oxidase; PQQ-pyrroloquinoline, FAD-flavine adenine dinucleotide

*Šema 1* – Enzimska biogorivna ćelija. GOx-glukoza oksidaza; Cyt c-citohrom c; COx-citohrom c oksidaza; PQQ-pirolohinoline, FAD-flavin adenin dinukleotid

## *Biocatalysis on the anode*

Enzymes used on the anode are glucose oxidase [7, 8], formaldehyde & formate dehydrogenase [9, 10], alcohol dehydrogenases [10-12] and sugar dehydrogenases (fructose, glucose, cellobiose) [13-15]. Glucose oxidases (GOx) have been isolated from many organisms, but most often used forms in biofuel cells and biosensors are GOx from Aspergillus niger and Penicillium amagasakiense. Three different classes of dehydrogenases are used at the anodic compartment: quinoprotein-dehydrogenase, flavin dependent dehydrogenases and NAD$^+$ dependent dehydrogenase.

## *Biocatalysis on the cathode*

On the cathode, most often used enzymes are multicopper oxidases such as laccases and bilirubin oxidase [16, 17], peroxidases such as horseradish, microperoxidase [18] and cytochrome c oxidase [19]. Laccases and bilirubin oxidase contain four Cu centers in one protein molecule and in a four-electrone reduction process, the final electrone acceptor oxygen is reduced to water. Fungal laccases have broad substrate specificities and are able to oxidize a wide range of organic compounds. Peroxidases from plants like horseradish contain heme and they can transfer electrones to water and generate hydrogen peroxide [20].

## *Miniature biofuel cells*

Due to recent developments in nanotechnology, biofuel cells can be miniaturized and used as power sources for electric medical devices such as implantable sensors, pacemakers and insulin pumps [21, 22]. They use glucose and oxygen from blood as a source of electric energy. The choice of enzymes for the nanobiocatalyst preparation in implantable miniature biofuel cells is limited by the blood composition and physiological conditions (e.g. pH, ions, glucose concentrations). Mainly cytochrome c oxidase, laccase or bilirubin oxidase have been employed on the cathode, while glucose oxidase from A.niger and glucose dehydrogenase from A.calcoaceticus are preferentially used on the anode.

# Nanobiocatalysis in Biosensor Systems

## *Biosensors*

A nanobiocatalyst can also be used for manufacturing biosensors, devices that convert a chemical signal into an electrical one. The main components of biosensors are a biological component, a transducer and electronics, Scheme 2.
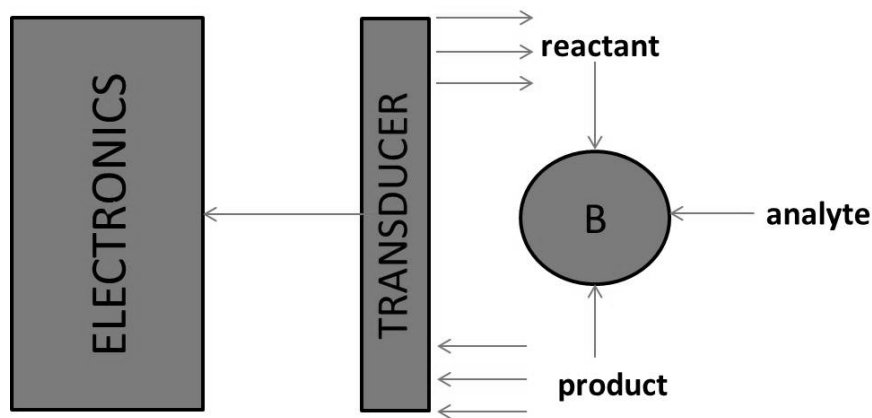
*Scheme 2* – Biosenosor with a bioactive element (B)
*Šema 2* – Biosenzor sa bioaktivnim elementom (B)

A transducer senses a biochemical event and converts it to a potential change, electron transfer, light emitted or adsorbed by a product or reactant, heat or mass change. Based on transducers, biosensors can be: electrochemical, optical, piezoelectrical or thermal. The most often used electrochemical biosensors can be based on amperometric, potentiometric and impendance detection.

Bioactive components are cells or biological macromolecules such as: enzymes, monoclonal antibodies, nucleic acids, and lipids. The most often used biological components for biosensor manufacturing are enzymes (glucose oxidase), usually in an immobilized form [23].

## *Enzyme Logical Gates*

For injured civilians or soldiers at an accident spot, a rapid and reliable diagnosis of physiological conditions would allow immediate medical intervention. Since the majority of battlefield deaths occur within the first half an hour after injury, a rapid diagnosis and treatment are crucial for a survival rate. In order to determine the type and the extent of an injury, it is usually necessary to monitor several physiological parameters. A multiple biosensor device (enzymatic reactions) connected similarly to electronic circuits in computer logical gates can perform biocomputing and process various biochemical information received from body fluids to determine the injury type [24, 25]. Various Boolean logic gates such as AND, OR, XOR, NOR, NAND, INHIB and XNOR were made using biomolecular switchable systems (proteins/enzymes, DNA, RNA, whole cells) [26-29]. An example of the AND logical gate is shown in Scheme 3.
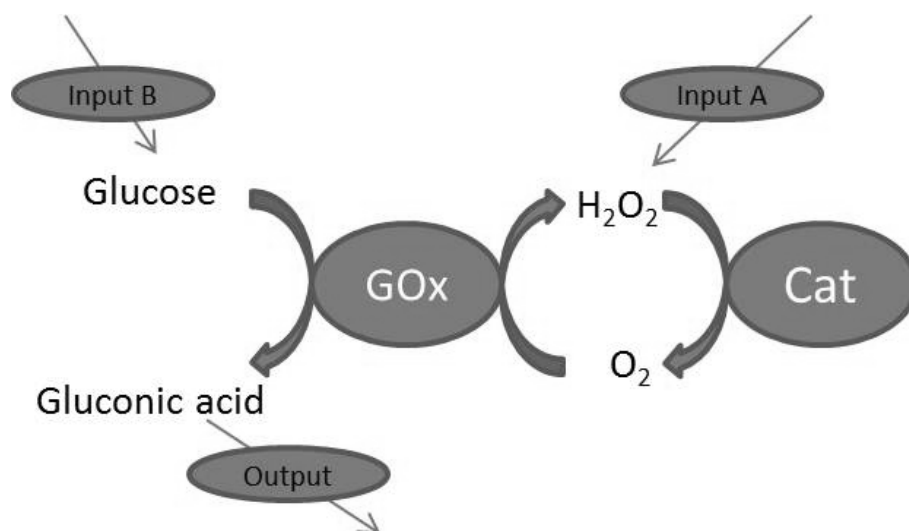
*Scheme 3* – AND logical gate made from glucose oxidase (GOx) and catalase (CAT)
*Šema 3* – AND logičko kolo napravljeno od glukoza oksidaze (GOx) i katalaze (CAT)

# Protein Engineering for Nanobiocatalysis

Biocatalysts are not optimized for the application in bioelectrocatalysis. Nonoptimal operating conditions, radical formation during electrone transfer and a poor electric contact with the electrode diminish the power output and the operational life of these devices. Immobilization of biocatalysts on nanostructured materials, rational design and directed evolution have successfully been used to improve the nanobiocatalyst properties such as activity towards artificial cofactors or electron mediators, electrical contact between the enzyme and the electrode, stability in the presence of an organic solvent, thermostability and stability in the presence of oxidizing reagens like hydrogen peroxide [4].

The rational protein design requires the knowledge of the protein structure and the understanding of structrure-function relatioships. Due to the lack of deeper understanding of these relations, directed enzyme evolution is becoming increasingly important in protein design. Directed evolution does not require a detailed knowledge of protein structures and uses the principles of the Darwinian evolution from the nature and applies them in the lab. In the iterative process of mutation and selection, biocatalysts are „forced" to evolve in the direction needed for a better performance on the electrode, Scheme 4.
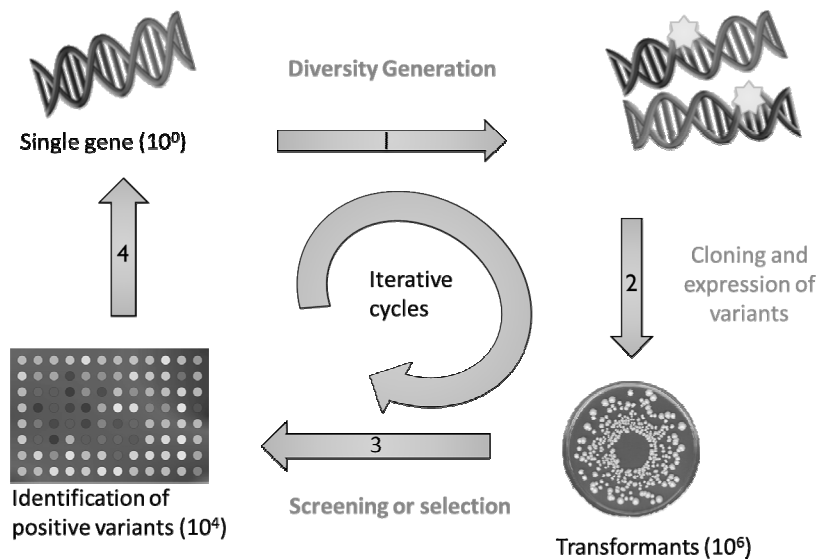
*Scheme 4* – Basic steps of directed evolution.
*Šema 4* – Osnovni koraci dirigovane evolucije.

The most limiting step in directed evolution is a screening process and there is an enormous effort to develop high throughput screening systems for gene libraries generated in directed evolution experiments. These screening systems are usually performed in aqueous microdrpolets of water in oil emulsions where in 1 mL of an emulsion it is possible to perform $10^{10}$ different reactions and screen libraries with sizes of up to $10^8$. For screening and sorting these microcompartments, scientists use flow cytometry [30] or microfluidic devices [31, 32].

In the literature there is a growing number of articles with examples of successful applications of protein engineering and directed evolution in developing more efficient biocatalysts like glucose oxidase [30, 33], glucose dehydrogenase [34], formaldehyde dehydrogenase [35], lactate dehydrogenase [36], horseradish peroxidase [37] and laccase [38, 39] for applications in biofuel cells and biosensor systems.

## *Glucose Oxidase*

Glucose oxidase (GOx) from A.niger is the most studied enzyme in electrochemistry for applications in biofuel cells and biosensors. It has a molecular mass of around 155-160 kDa in its glycosylated form and consists of two identical subunits. The Km value for β-D-glucose has been reported between 11.0 and 41.8 mM. GOx catalyzes oxidation of glucose

by molecular oxygen to gluconic acid and hydrogen peroxide. In order to be better suitable for the application in miniature biofuel cells, glucose oxidase has been evolved for higher activity and stability at physiological conditions (pH 7.4, 4 mM glucose) and better activity with artificial electron mediators like ferrocene. A ferrocene-based assay for glucose oxidase was used for screening approximatelly 2000 GOx mutants. A double mutant of GOx (T30S, I94V) has increased $k_{cat}$ comapred to wt (69.5 1/s WT; 137.7 1/s T30S I94V) and increased pH and thermal resistance [33]. In another directed evolution experiment, an ultrahigh throughput screening system based on emulsion technology and FACS (fluorescent activated cell sorter) was used for screening a high error prone PCR GOx gene library containing $10^5$ different mutants (30). Mutant M12 contained five mutations (N2Y, K13E, T30V, I94V, K152R) and 3.3 times increased specificity constant compared to wt (2.49 mM/s WT; 8.26 mM/s M12).

## Glucose Dehydrogenase

Using directed evolution techniques (gene shuffling approach), glucose dehydrogenase from Bacillus megaterium was improved in its thermal stability. The improved mutant contained two amino acid substitutions, Glu170Lys and Gln252Leu [34]. To select active variants high-throughput screening was performed in two steps: a filter-based prescreen of clones grown on agar plates using as a redox system 5-ethylphenazinium ethylsulfate and tetrazolium salt and an NADH based quantification assay in microtiter plates. A crystal structure of the double mutant showed that these two residues strengthen the subunit-subunit interactions by stabilizing a hydrophobic cavity.

## Formaldehyde Dehydrogenase

By substituting only one residue Ser 318 to Gly, the formaldehyde dehydrogenase from Pseudomonas putida was improved in activity by 1.7 times [35]. The enzymatic assay was done by placing nylon membranes soaked with detergent and p-nitroblue tetrazolium on diformazan agar plates. The positive colonies that developed blue colour were further analysed by the NADH-based assay in microtiter plates. The obtained mutant unfortunatelly decreased its thermal residence.

## Lactate Dehydrogenase

The lactate dehydrogenase from Bacillus stearmophillus is a thermostable L-2-hydroxyacid dehydrogenase used in biofuel cells. It is allosterically activated by fructose 1,6-bisphosphate (FBP) resulting in a 100-fold drop in $K_m$ and 2.5-fold drop in $k_{cat}$ due to the tetramerization of the dime-

ric form. After three rounds of family shuffling and screening 3000 clones variants of lactate dehydrogenase, a mutant was found that could form a tetrameric oligomer in the absence of FBP (36). The obtained mutant had three substitutions (Arg118Cys, Gln203Leu, Asn307Ser) and $K_m$ for pyruvate was reduced to 0.07 mM.

## *Horseradish Peroxidase*

A plant horseradish contains several peroxidase (HRP) isoforms and C isoenzyme is the most abundant. Thermal stability and resistance to peroxide inactivation was improved for this enzyme using the directed evolution [*37*]. Incresead activity and changed specificity and enantiose-lectivity were also obtained by directed evolution [*37, 40, 41*]. Using the emulsion technology and microfluidic devices, HRP also evolved for 7-fold increase in activity [*31*]. Such high increase in activity was achieved due to the screening of big gene libraries $10^7$ in multiple rounds of directed evolution. This is one of the best examples that can be done by directed evolution if there is a good high throughput screening system.

## *Laccase*

Laccases belong to the group of copper containing enzymes that can oxidize various phenols by oxygen. It has been successfully expressed in yeasts for directed evolution [*42*]. Using *in vivo* the recombination approach, the thermostability of versatile (VL) and high redox potential laccases (HRPL) was improved [*39*]. The activity at $65^oC$ for VP was improved 3-fold while at $75^oC$ the improvement was over 10-fold. The HRPL with evolved theromstabi-lity was subjected to further rounds of directed evolution and the activity of the best mutant OB-1 had 34000-fold enhanced activity [*39*].

# Military Application of Nanobiocatalysts

## *Enzyme Logic Systems for Battlefield Injuries*

There is a growing interest in the armies worldwide for developing a field hospital-on-a—chip that could monitor a soldier's injuries and administer medications. The Office of Naval Research is funding a program entitled „Integrated Sense and Treat Enzyme Logic Systems for Battlefield Injuries" that if successful would provide U.S. soldiers with a wearable device to constantly monitor vital signs and help treat wounds. These microfluidic laboratories on-a-chip would allow unskilled personnel to perform specialized tests in the field. The chip would fluids like blood and sweat for the „biomarkers" of common battlefield

injuries like schock or fatigue and then automatically inject the appropriate drugs. Preliminary results in these field with enzymatic logical gates are making this apporach quite realistic [24, 43].

## *Information Security: Biomolecular keypad lock systems, steganography and encrypting*

The computing networks composed of enzyme logical gates can be also used for mimicking a biomolecular keypad lock [*44, 45*]. A designed biochemical reaction chain was composed of several enzymatic reactions: hydrolysis of sucrose to glucose, oxidation of glucose to oxygen and then oxidation of ABTS dye to green product [*46*]. These reaction steps were catalysed by invertase, glucose oxidase and microperoxidase. The enzymes were immobilized on glass beads. The experiment was performed when the order of the enzyme-encoded input signals varied in 6 different combinations. Only one correct order of the input signals resulted in output 1 (generation of green product). A similar enzyme-based keypad lock was inegrated with the biofuel cell where only a correct „password" (specific order of adding enzymes) resulted in the activation of the biofuel cell, while all other „wrong" permutations of the enzyme inputs preserved the „OFF" state of the biofuel cell [*45*].

## *Keypad Lock Security, Steganography and Encrypting Based on Immunochemical Systems*

A keypad lock device can also be made using an immuno-based biorecognition system. Such a device was integrated with a switchable biofuel cell that was giving power output only after the correct input of the „password" encoded in the antibody-sequence.

Steanography and encrypting was also demonstrated by using immunochemistry systems. IgG antibodies were used as invisible ink developed with complementary antibodies labelled with enzymes producing color spots [47]. This approach in the future could provide information protection and watermark-technology and scaling down the encoded text to a micro size is also feasible with the use of nanotechnology.

# Conclusion

The development of nanotechnology, protein engineering and novel concepts in molecular biocomputing have opened new possibilites in the manufacture and design of biofuel cells and biosensor systems. These

technologies will help in continuos monitoring of human health conditions and increase survival rate under shock conditions. Novel concepts in molecular biocomputing could also provide new ways of information storage and protection.

## *Literature*

[1] Kim, J., Kim, B. C., Lopez-Ferrer, D., Petritis, K., and Smith, R. D. (2010) Nanobiocatalysis for protein digestion in proteomic analysis, Proteomics 10, 687-699.

[2] Lost, R. M., Madurro, J. M., Brito-Madurro, A. G., Nantes, I. L., Caseli, L., and Crespilho, F. N. (2011) Strategies of Nano-Manipulation for Application in Electrochemical Biosensors, Int J Electrochem Sc 6, 2965-2997.

[3] Wang, P., Kim, J. B., Jia, H. F., and Zhao, X. Y. (2005) Nanobiocatalysts for biofuel cells, Abstr Pap Am Chem S 230, U1662-U1663.

[4] Schwaneberg, U., Guven, G., and Prodanovic, R. (2010) Protein Engineering - An Option for Enzymatic Biofuel Cell Design, Electroanal 22, 765-+.

[5] Akers, N., Gellett, W., Kesmez, M., Schumacher, J., and Minteer, S. D. (2010) Biofuel Cells for Portable Power, Electroanal 22, 727-731.

[6] Davis, J. B., and Yarbrough, H. F., Jr. (1962) Preliminary Experiments on a Microbial Fuel Cell, Science 137, 615-616.

[7] Lee, J. H., Kim, H., Lee, I., Kwon, Y., Kim, B. C., Ha, S., and Kim, J. (2011) Immobilization of glucose oxidase into polyaniline nanofiber matrix for biofuel cell applications, Biosens Bioelectron 26, 3908-3913.

[8] Moon, S. H., Shim, J., and Kim, G. Y. (2011) Covalent co-immobilization of glucose oxidase and ferrocenedicarboxylic acid for an enzymatic biofuel cell, J Electroanal Chem 653, 14-20.

[9] Kar, P., Wen, H., Li, H. Z., Minteer, S. D., and Barton, S. C. (2011) Simulation of Multistep Enzyme-Catalyzed Methanol Oxidation in Biofuel Cells, J Electrochem Soc 158, B580-B586.

[10] Minteer, S. D., Addo, P. K., and Arechederra, R. L. (2010) Evaluating Enzyme Cascades for Methanol/Air Biofuel Cells Based on NAD(+)-Dependent Enzymes, Electroanal 22, 807-812.

[11] Ramanavicius, A., Kausaite, A., and Ramanaviciene, A. (2008) Enzymatic biofuel cell based on anode and cathode powered by ethanol, Biosens Bioelectron 24, 761-766.

[12] Treu, B. L., and Minteer, S. D. (2005) Improving the lifetime, simplicity, and power of an ethanol biofuel cell by employing ammonium treated Nafion membranes to immobilize PQQ-dependent alcohol dehydrogenase., Abstr Pap Am Chem S 229, U1120-U1121.

[13] Zhang, Y. H. P., Zhu, Z. G., Wang, Y. R., and Minteer, S. D. (2011) Maltodextrin-powered enzymatic fuel cell through a non-natural enzymatic pathway, J Power Sources 196, 7505-7509.

[14] Gorton, L., Tasca, F., Harreither, W., Ludwig, R., and Gooding, J. J. (2011) Cellobiose Dehydrogenase Aryl Diazoniunn Modified Single Walled Carbon Nanotubes: Enhanced Direct Electron Transfer through a Positively Charged Surface, Anal Chem 83, 3042-3049.

[15] Willner, I., Yehezkeli, O., Tel-Vered, R., and Reichlin, S. (2011) Nano-engineered Flavin-Dependent Glucose Dehydrogenase/Gold Nanoparticle-Modified Electrodes for Glucose Sensing and Biofuel Cell Applications, Acs Nano 5, 2385-2391.

[16] Vazquez-Duhalt, R., Martinez-Ortiz, J., and Flores, R. (2011) Molecular design of laccase cathode for direct electron transfer in a biofuel cell, Biosens Bioelectron 26, 2626-2631.

[17] Kokoh, K. B., Habrioux, A., Napporn, T., Servat, K., and Tingry, S. (2010) Electrochemical characterization of adsorbed bilirubin oxidase on Vulcan XC 72R for the biocathode preparation in a glucose/O(2) biofuel cell, Electrochim Acta 55, 7701-7705.

[18] Ferapontova, E. E., Gomez, C., and Shipovskov, S. (2010) Peroxidase biocathodes for a biofuel cell development, J Renew Sustain Ener 2.

[19] Su, L. Y., Hawkridge, F. M., and Rhoten, M. C. (2004) Electroreduction of oxygen by cytochrome c oxidase immobilized in electrode-supported lipid bilayer membranes, Chem Biodivers 1, 1281-1288.

[20] Radotic, K., Laketa, D., Bogdanovic, J., Prodanovic, R., and Kalauzi, A. (2010) The effect of pH on the activity of soluble peroxidase in needles of Serbian spruce (Picea omorika (Panc.) Purkinye): application of a mathematical model, Gen Physiol Biophys 29, 122-128.

[21] Mano, N., Mao, F., and Heller, A. (2004) A miniature membrane-less biofuel cell operating at +0.60 V under physiological conditions, Chembiochem 5, 1703-1705.

[22] Mano, N., Mao, F., and Heller, A. (2002) A miniature biofuel cell operating in a physiological buffer, J Am Chem Soc 124, 12962-12963.

[23] Yu, E. H., Prodanovic, R., Guven, G., Ostafe, R., and Schwaneberg, U. (2011) Electrochemical Oxidation of Glucose Using Mutant Glucose Oxidase from Directed Protein Evolution for Biosensor and Biofuel Cell Applications, Appl Biochem Biotechnol.

[24] Halamek, J., Windmiller, J. R., Zhou, J., Chuang, M. C., Santhosh, P., Strack, G., Arugula, M. A., Chinnapareddy, S., Bocharova, V., Wang, J., and Katz, E. (2010) Multiplexing of injury codes for the parallel operation of enzyme logic gates, Analyst 135, 2249-2259.

[25] Manesh, K. M., Halamek, J., Pita, M., Zhou, J., Tam, T. K., Santhosh, P., Chuang, M. C., Windmiller, J. R., Abidin, D., Katz, E., and Wang, J. (2009) Enzyme logic gates for the digital analysis of physiological level upon injury, Biosens Bioelectron 24, 3569-3574.

[26] Halamek, J., Bocharova, V., Chinnapareddy, S., Windmiller, J. R., Strack, G., Chuang, M. C., Zhou, J., Santhosh, P., Ramirez, G. V., Arugula, M. A., Wang, J., and Katz, E. (2010) Multi-enzyme logic network architectures for assessing injuries: digital processing of biomarkers, Mol Biosyst 6, 2554-2560.

[27] Melnikov, D., Strack, G., Zhou, J., Windmiller, J. R., Halamek, J., Bocharova, V., Chuang, M. C., Santhosh, P., Privman, V., Wang, J., and Katz, E. (2010) Enzymatic AND logic gates operated under conditions characteristic of biomedical applications, J Phys Chem B 114, 12166-12174.

[28] Bi, S., Yan, Y., Hao, S., and Zhang, S. (2010) Colorimetric logic gates based on supramolecular DNAzyme structures, Angew Chem Int Ed Engl 49, 4438-4442.

[29] Katz, E., and Privman, V. (2010) Enzyme-based logic systems for information processing, Chem Soc Rev 39, 1835-1857.

[30] Schwaneberg, U., Prodanovic, R., Ostafe, R., and Scacioc, A. (2011) Ultrahigh Throughput Screening System for Directed Glucose Oxidase Evolution in Yeast Cells, Comb Chem High T Scr 14, 55-60.

[31] Agresti, J. J., Antipov, E., Abate, A. R., Ahn, K., Rowat, A. C., Baret, J. C., Marquez, M., Klibanov, A. M., Griffiths, A. D., and Weitz, D. A. (2010) Ultrahigh-throughput screening in drop-based microfluidics for directed evolution (vol 170, pg 4004, 2010), P Natl Acad Sci USA 107, 6550-6550.

[32] Weitz, D. A., Agresti, J. J., Antipov, E., Abate, A. R., Ahn, K., Rowat, A. C., Baret, J. C., Marquez, M., Klibanov, A. M., and Griffiths, A. D. (2010) Ultrahigh-throughput screening in drop-based microfluidics for directed evolution, P Natl Acad Sci USA 107, 4004-4009.

[33] Zhu, Z., Wang, M., Gautam, A., Nazor, J., Momeu, C., Prodanovic, R., and Schwaneberg, U. (2007) Directed evolution of glucose oxidase from Aspergillus niger for ferrocenemethanol-mediated electron transfer, Biotechnol J 2, 241-248.

[34] Baik, S. H., Ide, T., Yoshida, H., Kagami, O., and Harayama, S. (2003) Significantly enhanced stability of glucose dehydrogenase by directed evolution, Appl Microbiol Biot 61, 329-335.

[35] Fujii, Y., Yamasaki, Y., Matsumoto, M., Nishida, H., Hada, M., and Ohkubo, K. (2004) The artificial evolution of an enzyme by random mutagenesis: The development of formaldehyde dehydrogenase, Biosci Biotech Bioch 68, 1722-1727.

[36] Allen, S. J., and Holbrook, J. J. (2000) Production of an activated form of Bacillus stearothermophilus L-2-hydroxyacid dehydrogenase by directed evolution, Protein Eng 13, 5-7.

[37] Khajeh, K., Asad, S., and Ghaemi, N. (2011) Investigating the Structural and Functional Effects of Mutating Asn Glycosylation Sites of Horseradish Peroxidase to Asp, Appl Biochem Biotech 164, 454-463.

[38] Farinas, E. T., Gupta, N., and Lee, F. S. (2010) Laboratory evolution of laccase for substrate specificity, J Mol Catal B-Enzym 62, 230-234.

[39] Alcalde, M., Mate, D., Garcia-Burgos, C., Garcia-Ruiz, E., Ballesteros, A. O., and Camarero, S. (2010) Laboratory Evolution of High-Redox Potential Laccases, Chem Biol 17, 1030-1041.

[40] Klibanov, A. M., Antipov, E., Cho, A. E., and Wittrup, K. D. (2008) Highly L and D enantioselective variants of horseradish peroxidase discovered by an ultrahigh-throughput selection method, P Natl Acad Sci USA 105, 17694-17699.

[41] Tidor, B., Lipovsek, D., Antipov, E., Armstrong, K. A., Olsen, M. J., Klibanov, A. M., and Wittrup, K. D. (2007) Selection of horseradish peroxidase variants with enhanced enantioselectivity by yeast surface display, Chem Biol 14, 1176-1185.

[42] Alcalde, M., Bulter, T., Zumarraga, M., Garcia-Arellano, H., Mencia, M., Plou, F. J., and Ballesteros, A. (2005) Screening mutant libraries of fungal laccases in the presence of organic solvents, J Biomol Screen 10, 624-631.

[43] Zhou, J., Halamek, J., Bocharova, V., Wang, J., and Katz, E. (2011) Bio-logic analysis of injury biomarker patterns in human serum samples, Talanta 83, 955-959.

[44] Pu, F., Liu, Z., Yang, X., Ren, J., and Qu, X. (2011) DNA-based logic gates operating as a biomolecular security device, Chem Commun (Camb) 47, 6024-6026.

[45] Halamek, J., Tam, T. K., Strack, G., Bocharova, V., Pita, M., and Katz, E. (2010) Self-powered biomolecular keypad lock security system based on a biofuel cell, Chem Commun (Camb) 46, 2405-2407.

[46] Strack, G., Ornatska, M., Pita, M., and Katz, E. (2008) Biocomputing security system: concatenated enzyme-based logic gates operating as a biomolecular keypad lock, J Am Chem Soc 130, 4234-4235.

[47] Kim, K. W., Bocharova, V., Halamek, J., Oh, M. K., and Katz, E. (2011) Steganography and encrypting based on immunochemical systems, Biotechnol Bioeng 108, 1100-1107.

NANOBIOKATALIZATORI ZA BIOGORIVNE ĆELIJE
I BIOSENZORNE SISTEME

OBLAST: hemijske tehnologije

*Rezime:*

*U ovom preglednom članku je sumirana primena enzima u proizvodnji i dizajnu biogorivnih ćelija i biosenzora. Naglasak u pregledu literature je stavljen na tehnike proteinskog inžinjeringa, koje se koriste za poboljšanje osobina enzima u nanobiokatalizatorima kao što su orijentacija kod imobilizacije, stabilnost, aktivnost i efikasnost transfera elektrona između imobilizovanog enzima i elektrode. Na kraju pregleda je dato nekoliko primera moguće primene u vojsci.*

*Nanobiokatalizatori su biokatalizatori u obliku enzima ili ćelija imobilizovani na nanomaterijalima.*

*Koriste se kao sastavni elementi gorivnih ćelija u vidu imobilizovanih oksidoreduktaza na elektrodama. Na anodi se uz pomoć enzima oksiduju hemijska jedinjenja i elektroni predaju elektrodi, dok se na katodi elektroni uz pomoć druge oksidoreduktaze prebacuju sa elektrode na vodu ili kiseonik. Enzimi koji se koriste na anodi su glukoza oksidaza, formaldehid dehidrogenaza, alkohol dehidrogenaza i druge oksidaze šećera. Na katodi se uglavnom koriste lakaze, bilirubin oksidaza, peroksidaze i citohrom c oksidaza. Zahvaljujući razvoju nanotehnologije razvijaju se i minijaturne biogorivne ćelije koje proizvode električnu energiju za implantirane medicinske uređaje (insulinske pumpe, pejsmejkere, biosenzore) koristeći glukozu i kiseonik iz ljudske krvi.*

*Biosenzori predstavljaju uređaje koji se sastoje iz biološke komponente, transducera i električne komponente. Oni pretvaraju koncen-*

*traciju hemijske supstance u električni signal i koriste se za analitiku. Kao biološka komponenta se mogu koristiti enzimi, monoklonska antitela,nukleinske kiseline i lipidi.*

*Enzimska logička kola predstavljaju kombinaciju različitih biosenzora (enzimskih reakcija) koji mere nekoliko ulaznih parametara i na osnovu njih daju odgovarajući izlazni signal. Koristeći znanja kompjuterske tehnologije enzimskim logičkim kolima mogu se simulirati AND, OR, XOR, NOR, NAND, INHIB i XNOR logička kola.*

*Za poboljšanje osobina biokatalizatora u cilju efikasnije primene u bioelektrokatalizi koriste se tehnike proteinskog inžinjeringa kao što su racionalni dizajn i dirigovana evolucija. Dirigovana evolucija koristi iterativne korake mutiranja i selekcije, kako bi biokatalizator evoluirao u pravcu koji nam je potreban. Najsporiji stupanj u ovoj tehnologiji predstavlja „skrining", te se u novije vreme pomoću protočne citometrije i mikrofluidike pokušavaju razviti nove metode visoko propusnog skrininga. U literaturi opisani primeri dirigovane evolucije glukoza oksidaze, glukoza dehidrogenaze, formaldehid dehidrogenaze, laktat dehidrogenaze, peroksidaze i lakaze.*

*Kombinacijom enzimskih logičkih kola i mikrofluidne tehnologije se pokušavaju napraviti laboratorije na čipu koje bi omogućile kontinuirano praćenje zdravstvenog stanja vojnika na bojnom polju i u slučaju šoka (ranjavanja) primenu odgovarajuće terapije u toku prvih 30 minuta od povrede. To bi obezbedilo veći stepen preživljavanja vojnika u ratu. Takođe upotrebom enzimskih logičkih kola i antitela moguće je postići uskladištenje i šifrovanje informacija, kao i zaštitu lozinkom, odgovarajućih elektronskih uređaja kao što su biogorivne ćelije*

*Razvoj nanotehnologije, proteinskog inžinjeringa i molekularnog računarstva otvara vrata novim mogućnostima u proizvodnji i dizajnu biogorivnih ćelija i bisenzorskih sistema, kao i u skladištenju i zaštiti informacija.*