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Biosensors and their applications – A review

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Abstract

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The various types of biosensors such as enzyme-based, tissue-based, immunosensors, DNA biosensors, thermal and piezoelectric biosensors have been deliberated here to highlight their indispensable applications in multitudinous fields.

Some of the popular fields implementing the use of biosensors are food industry to keep a check on its quality and safety, to help distinguish between the natural and artificial; in the fermentation industry and in the saccharification process to detect precise glucose concentrations; in metabolic engineering to enable in vivo monitoring of cellular metabolism. Biosensors and their role in medical science including early stage detection of human interleukin-10 causing heart diseases, rapid detection of human papilloma virus, etc. are important aspects. Fluorescent biosensors play a vital role in drug discovery and in cancer. Biosensor applications are prevalent in the plant biology sector to find out the missing links required in metabolic processes. Other applications are involved in defence, clinical sector, and for marine applications.

Keywords: Biosensors, Tissue based immunosensors, Enzyme based immunosensors

1. Introduction

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Biosensors are analytical devices that convert a biological response into an electrical signal. Quintessentially biosensors must be highly specific, independent of physical parameters such as pH and temperature and should be reusable. The term “biosensor” was coined by Cammann,[1] and its definition was introduced by IUPAC.[2, 3, 4]

Fabrication of biosensors, its materials, transducing devices, and immobilization methods requires multidisciplinary research in chemistry, biology, and engineering. The materials used in biosensors are categorized into three groups based on their mechanisms: biocatalytic group comprising enzymes, bioaffinity group including antibodies and nucleic acids, and microbe based containing microorganisms.

2. Material and methods

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The term “biosensors” was searched in Pubmed, Sciencedirect, and google, and all articles based on its applications were selected.

3. Review of literature

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3.1. Types of biosensors

Biosensors started in the 1960s by the pioneers Clark and Lyons. Various types of biosensors being used

are enzyme-based, tissue-based, immunosensors, DNA biosensors, and thermal and piezoelectric biosensors.

The first enzyme-based sensor was reported by Updike and Hicks in 1967. Enzyme biosensors have been devised on immobilization methods, i.e. adsorption of enzymes by van der Waals forces, ionic bonding or covalent bonding. The commonly used enzymes for this purpose are oxidoreductases, polyphenol oxidases, peroxidases, and aminooxidases.[5, 6, 7]

The first microbe-based or cell-based sensor was actualized by Diviès.[8] The tissues for tissue-based sensors arise from plant and animal sources. The analyte of interest can be an inhibitor or a substrate of these processes. Rechnitz[9] developed the first tissue based sensor for the determination of amino acid arginine. Organelle-based sensors were made using membranes, chloroplasts, mitochondria, and microsomes. However, for this type of biosensor, the stability was high, but the detection time was longer, and the specificity was reduced.

Immunosensors were established on the fact that antibodies have high affinity towards their respective antigens, i.e. the antibodies specifically bind to pathogens or toxins, or interact with components of the host's immune system.

The DNA biosensors were devised on the property that single-strand nucleic acid molecule is able to recognize and bind to its complementary strand in a sample. The interaction is due to the formation of stable hydrogen bonds between the two nucleic acid strands.[10]

Magnetic biosensors: miniaturized biosensors detecting magnetic micro- and nanoparticles in microfluidic channels using the magnetoresistance effect have great potential in terms of sensitivity and size.[11]

Thermal biosensors or calorimetric biosensors are developed by assimilating biosensor materials as mentioned before into a physical transducer.

Piezoelectric biosensors are of two types: the quartz crystal microbalance and the surface acoustic wave device. They are based on the measurement of changes in resonance frequency of a piezoelectric crystal due to mass changes on the crystal structure.

Optical biosensors consist of a light source, as well as numerous optical components to generate a light beam with specific characteristics and to beeline this light to a modulating agent, a modified sensing head along with a photodetector.[12]

Green fluorescent protein and the subsequent autofluorescent protein (AFP) variants and genetic fusion reporters have aided the development of genetically-encoded biosensors.[13, 14, 15, 16, 17, 18, 19, 20] This type of biosensor is user-friendly, easy to engineer, manipulate and transfer into cells. Single-chain FRET biosensor is another example. They consist of a pair of AFPs, which are able to transfer fluorescence resonance energy between them when brought close together. Different methods may be used to regulate changes in Förster resonance energy transfer (FRET) signals based on intensity, ratio or lifetime of AFPs. Peptide and protein biosensors are easily manufactured through synthetic chemistry followed by enzymatic labelling with synthetic fluorophores. Due to their independence of genetically-encoded AFPs, they are readily utilized to control target activity and constitute attractive alternatives and have an added advantage of being able to enhance signal-to-noise ratio and sensitivity of response through introduction of chemical quenchers and photoactivatable groups.

3.2. Applications of biosensors

Biosensors have been applied in many fields namely food industry, medical field, marine sector etc., and they provide better stability and sensitivity as compared with the traditional methods.

3.2.1. In food processing, monitoring, food authenticity, quality and safety) An arduous quandary in food processing industry is of quality and safety, maintenance of food products and processing. Traditional techniques performing chemical experiments and spectroscopy have shortcomings due to human fatigue, are expensive and time consuming. Alternatives for food authentication and monitoring with

objective and consistent measurement of food products, in a cost effective manner, are desirable for the food industry. Thus development of biosensors in response to the demand for simple, real-time, selective and inexpensive techniques is seemingly propitious.[21]

Ghasemi-Varnamkhasti et al.[22] worked on the monitoring of ageing of beer using enzymatic biosensors, based on cobalt phthalocyanine. These biosensors evinced a good capability to monitor the ageing of beer during storage.

Biosensors are used for the detection of pathogens in food. Presence of *Escherichia coli* in vegetables, is a bioindicator of faecal contamination in food.[23, 24] *E. coli* has been measured by detecting variation in pH caused by ammonia (produced by urease–*E. coli* antibody conjugate) using potentiometric alternating biosensing systems. Washing the vegetables such as sliced carrots and lettuce with peptone water provides us with the liquid phase. It is then separated by amalgamating it in a sonicator, to disaffiliate bacterial cells from food items.[25]

Enzymatic biosensors are also employed in the dairy industry. A biosensor, based on a screen-printed carbon electrode, was integrated into a flow cell.[26] Enzymes were immobilized on electrodes by engulfment in a photocrosslinkable polymer. The automated flow-based biosensor could quantify the three organophosphate pesticides in milk.

One of the popular food additives extensively used today are sweeteners, which are adversely causing undesirable diseases including dental caries, cardiovascular diseases, obesity and type-2 diabetes. It is believed that artificial sweeteners are addictive and coax us to eat more high-energy food unconsciously, inadvertently causing weight gain. Thus their detection and quantification are of prime importance. Traditional methods to distinguish the two types of sweeteners are ion chromatographic methods, which are complicated and laborious.

A more efficacious method, which combined lipid films with electrochemical techniques as biosensors for speedy and sensitive screening of sweeteners has been explored by multi-channel biosensor, which detect the electrophysiological activities of the taste epithelium. The signals are analyzed using spatiotemporal techniques, on MATLAB, where glucose and sucrose represent natural sugars while saccharin and cyclamate comprise artificial sweeteners. Since all sweeteners are mediated by heterodimeric G-protein coupled receptors in Type-II cells in the bud, they have a plurality of binding sites to identify sweet stimuli of different structures respectively. Studies suggest two types of sweet stimuli: cyclic adenosine monophosphate pathway which utilizes natural sugars such as sucrose, and the second is, inositol triphosphate and diacylglycerol pathway exploited by artificial sweeteners for purpose of signal transduction. The response to artificial sweeteners greatly depends on residues in the amino terminal domains of taste receptors as ligand binding sites. The signal responses of taste receptor cells towards natural and artificial sweeteners are discrete. The taste epithelium biosensor delivered sparse signals with positive waveforms, when glucose was applied whereas sucrose sustained signals with negative spikes. The taste epithelium responded to artificial sweeteners with more intensive signals, showing that the responses to artificial sweeteners were quite different from those of natural sugars, in both time and frequency domains.

3.2.2. In fermentation processes In fermentation industries, process safety and product quality are crucial. Thus effective monitoring of the fermentation process is imperative to develop, optimize and maintain biological reactors at maximum efficacy. Biosensors can be utilized to monitor the presence of products, biomass, enzyme, antibody or by-products of the process to indirectly measure the process conditions. Biosensors precisely control the fermentation industry and produce reproducible results due to their simple instrumentation, formidable selectivity, low prices and easy automation. Nowadays, several kinds of commercial biosensors are accessible; capable of detecting biochemical parameters (glucose, lactate, lysine, ethanol etc.) and are widely used in China, occupying about 90% of its market.

In fermentation process, saccharification was monitored by traditional Fehiing's method. Since this method involves titration of reducing sugar, its outcomes were inaccurate. However, since the launch of glucose biosensor commercially in 1975, the fermentation industries have been benefited. Now the factories successfully use glucose biosensors to control production in the saccharification and

fermentation workshop and utilize the bioenzymatic method to produce glucose.

Biosensors are also employed in ion exchange retrieval, where detection of change of biochemical composition is carried out. For instance, glutamate biosensor has been used to conduct experiments on ion exchange retrieval of an isoelectric liquor supernatant of glutamate. The fermentation process is a byzantine process with multiple pivotal variables, most of which are laborious to measure in real-time. On-line monitoring of critical metabolites is essential to facilitate quick optimization and to control biological processes. In past years, biosensors have attracted a lot of attention in online monitoring in fermentation process due to its simplicity and quick response.[27]

3.2.3. Biosensing technology for sustainable food safety The term food quality refers to the appearance, taste, smell, nutritional value, freshness, flavour, texture and chemicals.[11] Smart monitoring of nutrients and fast screening of biological and chemical contaminants are of paramount importance, when it comes to food quality and safety. Material science, nanotechnology, electromechanical and microfluidic systems are striding in to make sensing technology imminent for use in market. Efforts are being made for developing control systems ensuring food quality and safety and, as a consequence, human health.

Glucose monitoring becomes indispensable as during storage the food content and composition may get altered.[28] German[29] studied the electrochemistry of glucose oxidase immobilized on a graphite rod, altered by gold nanoparticles (AuNPs), which improved its sensitivity.

Glutamine is the nitty-gritty of crucial functions such as (signalling, transport and precursor in biosynthesis of nucleic acids, amino sugars and proteins). Patients deficient in glutamine suffer from pathologies such as malabsorptive disorders and have to be supplemented, to improve immune functions, preserve intestinal functionality and lessen bacterial translocation.[30] Glutaminase-based microfluidic biosensor chip with a flow-injection analysis for electrochemical detection has been used for detection in fermentation process.[31]

Biosensors are being employed to perceive general toxicity and specific toxic metals, due to their capability to react with only the hazardous fractions of metal ions.[32] Pesticides pose grave threats to the environment. The common pesticides used are organophosphates and carbamic insecticide species. Immunosensors have proved their merit as sensitive, high-speed agrifood and environmental monitoring. AChE and butyrylcholinesterase biosensors have been devised for aldicarb, carbaryl, paraoxon, chlorpyrifosmethyl etc. Oxon utilizing screen-printed electrodes was developed by Arduini and colleagues.[33] A similar type of biosensor is used to detect pesticides in wine and orange juice.[5, 34, 35] Arsenic can be measured with the help of bacteria-based bioassays.[36]

3.2.4. In medical field In the discipline of medical science, the applications of biosensors are growing rapidly. Glucose biosensors are widely used in clinical applications for diagnosis of diabetes mellitus, which requires precise control over blood-glucose levels.[37] Blood-glucose biosensors usage at home accounts for 85% of the gigantic world market.[38]

Biosensors are being used pervasively in the medical field to diagnose infectious diseases. A promising biosensor technology for urinary tract infection (UTI) diagnosis along with pathogen identification and anti-microbial susceptibility is under study.

Identifying end-stage heart failure patients, prone to adverse outcomes during the early phase of left ventricular assisted device implantation, is important. A novel biosensor, based on hafnium oxide (HfO_2), has been used for early stage detection of human interleukin (IL)-10.[39] Interaction between recombinant human IL-10 with corresponding monoclonal antibody is studied for early cytokine detection after device implantation. Fluorescence patterns and electromechanical impedance spectroscopy characterize the interaction between the antibody–antigen and bio-recognition of the protein is achieved by fluorescence pattern. Chen et al. applied HfO_2 as a greatly sensitive bio-field-effect transistor.[40] HfO_2 biosensor has been functionalized for antibody deposition with detection of a human antigen by electrochemical impedance spectroscopy.

The biggest dilemma faced today is of heart failure with about one million people suffering from it.

Techniques for detection of cardiovascular diseases include immunoaffinity column assay, fluorometric, and enzyme-linked immunosorbent assay.[41, 42, 43, 44, 45] These are laborious, require qualified personnel and are time consuming. Biosensors established on electric measurement employ biochemical molecular recognition for desired selectivity with a particular biomarker of interest.

The various other biosensors applications include: quantitative measurement of cardiac markers in undiluted serum, microfluidic impedance assay for controlling endothelin-induced cardiac hypertrophy, immunosensor array for clinical immunophenotyping of acute leukemias, effect of oxazaborolidines on immobilized fructosyltransferase in dental diseases; histone deacylase (HDAC) inhibitor assay from resonance energy transfer, biochip for a quick and accurate detection of multiple cancer markers and neurochemical detection by diamond microneedle electrodes.

3.2.5. Fluorescent biosensors Fluorescent biosensors are imaging agents, for use in cancer and drug discovery. They have enabled insights into the role and regulation of enzymes at cellular level. GFP-based and genetically encoded FRET biosensors play a vital role.

Fluorescent biosensors are small scaffolds onto which one or several fluorescent probes are mounted (enzymatically, chemically or genetically) through a receptor. The receptor identifies a specific analyte or target, thereby transducing a fluorescent signal which can be readily detected and measured.[46, 47] Fluorescent biosensors can probe ions, metabolites, and protein biomarkers with great sensitivity and can also report the presence, activity or status of the target (serum, cell extracts) in complex solution. They are employed in probing gene expression, protein localization, and conformation in fields such as signal transduction, transcription, cell cycle and apoptosis. Indication of arthritis, inflammatory diseases, cardiovascular and neurodegenerative diseases, viral infection, cancer and metastasis is done using these sensors.

Fluorescent biosensors are used in drug discovery programmes for the identification of drugs by high throughput, high content screening approaches, for postscreening analysis of hits and optimization of leads. These are considered potent tools for preclinical evaluation and clinical validation of therapeutic potential, biodistribution and pharmacokinetics of candidate drugs.[48, 49, 50, 51, 52] Fluorescent biosensors are effectively employed for early detection of biomarkers in molecular and clinical diagnostics, for monitoring disease progression and response to treatment/therapeutics, for intravital imaging and image guided surgery.[53]

A genetically-encoded FRET biosensor developed for detection of Bcr-Abl kinase activity was used on cancer patient cells to assess Bcr-Abl kinase activity and to establish an interrelation with the disease status in chronic myeloid leukaemia. This probe was further employed to regulate response to therapy, and to observe the onset of drug-resistant cells, permitting prediction for alternative therapeutics.[54, 55, 56]

3.2.6. Biodefense biosensing applications Biosensors can be used for military purposes at times of biological attacks. The main motive of such biosensors is be to sensitively and selectively identify organisms posing threat in virtually real time called biowarfare agents (BWAs) namely, bacteria (vegetative and spores), toxins and viruses. Several attempts to device such biosensors has been done using molecular techniques which are able to recognize the chemical markers of BWAs.

Nucleic acid-based sensing systems are more sensitive than antibody-based detection methods as they provide gene-based specificity, without utilizing amplification steps to attain detection sensitivity to the required levels.

The human papilloma virus HPV (double stranded DNA virus) has been categorized into two types: HPV 16 and 18; and is related to invasive cervical cancer. HPVs can be rapidly detected using a novel leaky surface acoustic wave peptide nucleic acid biosensor with double two-port resonators. This probe directly detects HPV genomic DNA without polymerase chain reaction amplification, and can also bind to the target DNA sequences with a lot of efficacy and precision.

3.2.7. In metabolic engineering Environmental concerns and lack of sustainability of petroleum-derived products are gradually exhorting need for development of microbial cell factories for synthesis of

chemicals. Researchers view metabolic engineering as the enabling technology for a sustainable bioeconomy.[57] They have also envisioned that a substantial fraction of fuels, commodity chemicals and pharmaceuticals will be produced from renewable feedstocks by exploiting microorganisms rather than relying on petroleum refining or extraction from plants. The high capacity for diversity generation also requires efficient screening methods to select the individuals carrying the desired phenotype. The earlier methods were spectroscopy-based enzymatic assay analytics however they had limited throughput. To circumvent this obstacle genetically encoded biosensors that enable in vivo monitoring of cellular metabolism were developed which offered potential for high-throughput screening and selection using fluorescence-activated cell sorting (FACS) and cell survival, respectively.

FRET sensors comprised a pair of donor and acceptor fluorophores, and a ligand-binding peptide was sandwiched between the two. When it was bound by a ligand of interest the peptide underwent a conformational change thereby a FRET change.[58, 59] Though they had high orthogonality, temporal resolution, and ease of construction, FRET sensors were merely able to report the copiousness of metabolites concerned and were unable to exert downstream regulation to the signal.[60]

Transcription factors are natural sensory proteins evolved to regulate gene expression in response to changes in environment for high throughput screening.[61] It is accomplished by hacking into host transcription system and employing a synthetic condition specific promoter to drive the expression of a reporter gene. These exhibit poor orthogonality and background noise.[62, 63]

The third class of biosensors comprises riboswitches; the regulatory domain of a mRNA that can selectively bind to a ligand and thereupon change its own structure, consequently regulating transcription of its encoded protein. As opposed to TF based biosensors, they are comparatively faster as the RNA has already been transcribed, also they do not rely on protein–protein or protein–metabolite interactions. In the recent decades ribosomes have been extensively engineered in bacterial systems.[64, 65]

3.2.8. Biosensors in plant biology Revolutionary new technologies in the areas of DNA sequencing and molecular imaging, have lead to advancements in plant science. Traditional methods of mass spectroscopy for gauging insights into cellular and subcellular localization, and measure of ion and metabolite levels had unprecedented precision but lacked the key information regarding location and dynamics of enzyme substrates, receptors and transporters. However, this information can be easily successfully tapped using biosensors. To measure a dynamic process under physiological conditions, we need to device tactics to visualize the actual process, for instance, the conversion of one metabolite into another or triggering of signalling events. This visualization can be done by sensors which respond dynamically.

Roger Tsien's lab was the first to develop protein prototype sensors to measure caspase activity and control levels of calcium in live cells.[66] These sensors were based on FRET (FRET) between two spectral variants of GFP.[67, 68] In vivo application of biosensors involves high temporal resolution imaging of calcium oscillations using cameleon sensors.

Biosensors can be utilized to identify missing components pertinent to metabolism, regulation, or transport of the analyte. FRET sensor for sucrose, responsible for the identification of proteins, performs a transport step in phloem loading-sucrose efflux from the mesophyll. Fluorimeter-based assays with FRET sugar sensors successfully recognize sugar transporters that can function immediately after exposure of starved yeast cells to glucose.[69, 70] Similar assays identify genes that affect cytosolic or vacuolar pH in yeast,[71, 72] and justify that biosensors can be applied in genetic screens provided imaging technologies of suitable throughput are available.[73]

4. Future scope

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Cell and tissue-based biosensors consist of genetically engineered proteins that are infused into cells ex vivo or in vivo. They allow the researcher to sense levels of hormones, drugs, or toxins, continuously and noninvasively, using biophotonics or other physical principles. The scope in this regard could be of value in ageing research.

Biosensors are used for marine applications for detection of eutrophication using nitrite and nitrate sensors. Various sensors based on nucleic acid hybridization detection have been developed for organism detection; “Environmental Sample Processor” is under process at the Monterey Bay Aquarium Research Institute whose goal is the automated detection of toxic algae in situ from moorings using ribosomal RNA probes is a promising development in this field. Also detection of pollutants, heavy metal and pesticides through biosensors is one of the prime goals.

Applications of nanomaterials in biosensors provide opportunities for building up a new generation of biosensor technologies. Nanomaterials improve mechanical, electrochemical, optical and magnetic properties of biosensors and are developing towards single molecule biosensors with high throughput biosensor arrays. Biological molecules possess special structures and functions, and determining how to fully use the structure and function of nanomaterials and biomolecules to fabricate single molecule multifunctional nanocomposites, nanofilms, and nanoelectrodes, is still a great challenge. The processing, characterization, interface problems, availability of high quality nanomaterials, tailoring of nanomaterials, and the mechanisms governing the behaviour of these nanoscale composites on the surface of electrodes are also great challenges for the presently existing techniques. Ways to enhance the signal to 116 noise ratio, how to enhance transduction and amplification of the signals, are also major impediments.

Future work should focus on clarifying the mechanism of interaction between nanomaterials and biomolecules on the surface of electrodes or nanofilms and using novel properties to fabricate a new generation of biosensors. Nevertheless, nanomaterial-based biosensors show great attractive prospects, which will be broadly applied in clinical diagnosis, food analysis, process control, and environmental monitoring in the near future.

Conflicts of interest

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The author has none to declare.

References

Go to:

1. Cammann K. Biosensors based on ion-selective electrodes. Fresen Z Anal Chem. 1977;287:1–9.

2. Thevenot D.R., Toth K., Durst R.A., Wilson G.S. Electrochemical biosensors: recommended definitions and classification. Pure Appl Chem. 1999;71:2333–2348.

3. Thevenot D.R., Toth K., Durst R.A., Wilson G.S. Electrochemical biosensors: recommended definitions and classification. Biosens Bioelectron. 2001;16:121–131. [PubMed]

4. Thevenot D.R., Toth K., Durst R.A., Wilson G.S. Electrochemical biosensors: recommended definitions and classification. Anal Lett. 2001;34:635–659. [PubMed]

5. Wang J. Electrochemical glucose biosensors. Chem Rev. 2008;108:814–825. [PubMed]

6. Akyilmaz E., Yorganci E., Asav E. Do copper ions activate tyrosinase enzyme? A biosensor model for the solution. Bioelectrochemistry. 2010;78:155–160. [PubMed]

7. Venugopal V. Biosensors in fish production and quality control. Biosens Bioelectron. 2002;17:147–157. [PubMed]

8. Diviès C. Remarques sur l’oxydation de l’éthanol par une electrode microbienne d’acetobacter zylinum. Ann Microbiol. 1975;126A:175–186. [PubMed]

9. Rechnitz G.A. Biochemical electrodes uses tissues slice. Chem Eng News. 1978;56:16–21.

10. Wang J. DNA biosensors based on peptide nucleic acid (PNA) recognition layers. A review. Biosens Bioelectron. 1998;13:757–762. [PubMed]

11. Scognamiglio V., Arduini F., Palleschi G., Rea G. Biosensing technology for sustainable food safety. Trends Anal Chem. 2014;62:1–10.

12. Leatherbarrow R.J., Edwards P.R. Analysis of molecular recognition using optical biosensors. Curr Opin Chem Biol. 1999;3:544–547. [PubMed]

13. Zhang J., Campbell R.E., Ting A.Y., Tsien R.Y. Creating new fluorescent probes for cell biology. Nat Rev Mol Cell Biol. 2002;3:906–918. [PubMed]

14. Lippincott-Schwartz J., Patterson G.H. Development and use of fluorescent protein markers in living cells. Science. 2003;300:87–91. [PubMed]

15. Shaner N.C., Steinbach P.A., Tsien R.Y. A guide to choosing fluorescent proteins. Nat Methods. 2005;2:905–909. [PubMed]

16. Tsien R.Y. Breeding and building molecules to spy on cells and tumors. FEBS Lett. 2005;579:927–932. [PubMed]

17. Giepmans B.N., Adams S.R., Ellisman M.H., Tsien R.Y. The fluorescent toolbox for assessing protein location and function. Science. 2006;312:217–224. [PubMed]

18. Ibraheem A., Campbell R.E. Designs and applications of fluorescent protein-based biosensors. Curr Opin Chem Biol. 2010;14:30–36. [PubMed]

19. Wu B., Piatkevich K.D., Lionnet T., Singer R.H., Verkhusha V.V. Modern fluorescent proteins and imaging technologies to study gene expression, nuclear localization, and dynamics. Curr Opin Cell Biol. 2011;23:310–317. [PubMed]

20. Aye-Han N.N., Qiang N., Zhang J. Fluorescent biosensors for real-time tracking of post-translational modification dynamics. Curr Opin Chem Biol. 2009;13:392–397. [PubMed]

21. Scognamiglio V., Arduini F., Palleschi G., Rea G. Bio sensing technology for sustainable food safety. Trends Anal Chem. 2014;62:1–10.

22. Ghasemi-Varnamkhasti M., Rodriguez-Mendez M.L., Mohtasebi S.S. Monitoring the aging of beers using a bioelectronic tongue. Food Control. 2012;25:216–224.

23. Arora P., Sindhu A., Dilbaghi N., Chaudhury A. Biosensors as innovative tools for the detection of food borne pathogens. Biosens Bioelectron. 2011;28:1–12. [PubMed]

24. Ercole C., Del Gallo M., Mosiello L., Baccella S., Lepidi A. *Escherichia coli* detection in vegetable food by a potentiometric biosensor. Sens Actuators B: Chem. 2003;91:163–168.

25. Torun O., Boyaci I., Temur E., Tamer U. Comparison of sensing strategies in SPR biosensor for rapid and sensitive enumeration of bacteria. Biosens Bioelectron. 2012;37:53–60. [PubMed]

26. Mishra R., Dominguez R., Bhand S., Munoz R., Marty J. A novel automated flow-based biosensor for the determination of organophosphate pesticides in milk. Biosens Bioelectron. 2012;32:56–61. [PubMed]

27. Yan C., Dong F., Chun-yuan B., Si-rong Z., Jian-guo S. Recent progress of commercially available biosensors in china and their applications in fermentation processes. J Northeast Agric Univ. 2014;21:73–85.

28. Monosik R., Stredansky M., Tkac J., Sturdik E. Application of enzyme biosensors in analysis of food and beverages enzyme and microbial technology. Food Anal Methods. 2012;5:40–53.

29. German N., Ramanaviciene A., Voronovic J., Ramanavicius A. Glucose biosensor based on graphite electrodes modified with glucose oxidase and colloidal gold nanoparticles. Mikrochim Acta. 2010;168:221–229.

30. Chen Q.H., Yang Y., He H.L. The effect of glutamine therapy on outcomes in critically ill patients: a meta-analysis of randomized controlled trials. Crit Care. 2014;18:R8. [PubMed]

31. Backer D., Rakowski M., Poghossiana A., Biselli M., Wagner P., Schoning M.J. Chip-based amperometric enzyme sensor system for monitoring of bioprocesses by flow-injection analysis. J

Biotechnol. 2013;163:371–376. [PubMed]

32. Amaro F., Turkewitz A.P., Martin-Gonzalez A., Gutierrez J.C. Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from *Tetrahymena thermophila*. Microb Biotechnol. 2011;4:513–522. [PubMed]

33. Arduini F., Ricci F., Tuta C.S., Moscone D., Amine A., Palleschi G. Detection of carbamic and organophosphorus pesticides in water samples using cholinesterase biosensor based on Prussian blue modified screen printed electrode. Anal Chim Acta. 2006;58:155–162. [PubMed]

34. Ivanov I., Younusov R.R., Evtugyn G.A., Arduini F., Moscone D., Palleschi G. Cholinesterase sensors based on screen-printed electrodes for detection of organophosphorus and carbamic pesticides. Anal Bioanal Chem. 2003;377:624–631. [PubMed]

35. Suprun E., Evtugyn G., Budnikov H., Ricci F., Moscone D., Palleschi G. Acetylcholinesterase sensor based on screen-printed carbon electrode modified with Prussian blue. Anal Bioanal Chem. 2005;383:597–604. [PubMed]

36. Diesel E., Schreiber M., van der Meer J.R. Development of bacteria-based bioassays for arsenic detection in natural waters. Anal Bioanal Chem. 2009;394 [PubMed]

37. Scognamiglio V., Pezzotti G., Pezzotti I. Biosensors for effective environmental and agrifood protection and commercialization: from research to market. Mikrochim Acta. 2010;170:215–225.

38. Rea G., Polticelli F., Antonacci A. Structure-based design of novel *Chlamydomonas reinhardtii* D1-D2 photosynthetic proteins for herbicide monitoring. Protein Sci. 2009;18:2139–2151. [PubMed]

39. Lee M., Zine N., Baraket A. A novel biosensor based on hafnium oxide: application for early stage detection of human interleukin-10. Sens Actuators B. 2012;175:201–207.

40. Chen Y.W., Liu M., Kaneko T., McIntyre P.C. Atomic layer deposited hafnium oxide gate dielectrics for charge-based biosensors. Electrochem Solid State Lett. 2010;13:G29–G32.

41. Ooi K.G.J., Galatowicz G., Towler H.M.A., Lightman S.L., Calder V.L. Multiplex cytokine detection versus ELISA for aqueous humor: IL-5, IL-10, and IFN profiles in uveitis. Investig Ophthalmol Vis Sci. 2006;47:272–277. [PubMed]

42. Caruso R., Trunfio S., Milazzo F. Early expression of pro- and anti-inflammatory cytokines in left ventricular assist device recipients with multiple organ failure syndrome. Am Soc Art Int Org J. 2010;56:313–318. [PubMed]

43. Caruso R., Verde A., Cabiati M. Association of pre-operative interleukin-6 levels with interagency registry for mechanically assisted circulatory support profiles and intensive care unit stay in left ventricular assist device patients. J Heart Lung Transplant. 2012;31(6):625–633. [PubMed]

44. Watson C.J., Ledwidge M.T., Phelan D. Proteomic analysis of coronary sinus serum reveals leucine-rich 2-glycoprotein as a novel biomarker of ventricular dysfunction and heart failure. Circulation: Heart Fail. 2011;4:188–197. [PubMed]

45. Maurer M., Burri S., de Marchi S. Plasma homocysteine and cardiovascular risk in heart failure with and without cardiorenal syndrome. Int J Cardiol. 2010;141:32–38. [PubMed]

46. Wang H., Nakata E., Hamachi I. Recent progress in strategies for the creation of protein-based fluorescent biosensors. Chem BioChem. 2009;10:2560–2577. [PubMed]

47. Morris M.C. Fluorescent biosensors of intracellular targets from genetically encoded reporters to modular polypeptide probes. Cell Biochem Biophys. 2010;56:19–37. [PubMed]

48. Giuliano K.A., Taylor D.L. Fluorescent-protein biosensors: new tools for drug discovery. Trends Biotechnol. 1998;16:135–140. [PubMed]

49. Wolff M., Wiedenmann J., Nienhaus G.U., Valler M., Heilker R. Novel fluorescent proteins for

high-content screening. Drug Discov Today. 2006;11:1054–1060. [PubMed]

50. Lang P., Yeow K., Nichols A., Scheer A. Cellular imaging in drug discovery. Nat Rev Drug Discov. 2006;5:343–356. [PubMed]

51. El-Deiry W.S., Sigman C.C., Kelloff G.J. Imaging and oncologic drug development. J Clin Oncol. 2006;24:3261–3273. [PubMed]

52. Willmann J.K., van Bruggen N., Dinkelborg L.M., Gambhir S.S. Molecular imaging in drug development. Nat Rev Drug Discov. 2008;7:591–607. [PubMed]

53. Morris M.C. Fluorescent biosensors – probing protein kinase function in cancer and drug discovery. Biochim Biophys Acta. 2013;1834:1387–1395. [PubMed]

54. Mizutani T., Kondo T., Darmanin S., Tsuda M., Tanaka S., Tobiume M. A novel FRET-based biosensor for the measurement of BCR-ABL activity and its response to drugs in living cells. Clin Cancer Res. 2010;16:3964–3975. [PubMed]

55. Lu S., Wang Y. Fluorescence resonance energy transfer biosensors for cancer detection and evaluation of drug efficacy. Clin Cancer Res. 2010;16:3822–3824. [PubMed]

56. Tunceroglu A., Matsuda M., Birge R.B. Real-time fluorescent resonance energy transfer analysis to monitor drug resistance in chronic myelogenous leukemia. Mol Cancer Ther. 2010;9:3065–3073. [PubMed]

57. Woolston B.M., Edgar S., Stephanopoulos G. Metabolic engineering: past and future. Annu Rev Chem Biomol Eng. 2013;4:259–288. [PubMed]

58. Peroza E.A., Ewald J.C., Parakkal G., Skotheim J.M., Zamboni N. A genetically encoded Förster resonance energy transfer sensor for monitoring in vivo trehalose-6-phosphate dynamics. Anal Biochem. 2015;474:1–7. [PubMed]

59. Mohsin M., Ahmad A. Genetically-encoded nanosensor for quantitative monitoring of methionine in bacterial and yeast cells. Biosens Bioelectron. 2014;59:358–364. [PubMed]

60. Bermejo C., Haerizadeh F., Takanaga H., Chermak D., Frommer W.B. Optical sensors for measuring dynamic changes of cytosolic metabolite levels in yeast. Nat Protoc. 2011;6:1806–1817. [PubMed]

61. Zhang J., Jensen M.K., Keasling J.D. Development of biosensors and their application in metabolic engineering. Curr Opin Chem Biol. 2015;28:1–8. [PubMed]

62. Becker K., Beer C., Freitag M., Kuck U. Genome-wide identification of target genes of a mating-type a-domain transcription factor reveals functions beyond sexual development. Mol Microbiol. 2015;96(5):1002–1022. [PubMed]

63. Lefrançois P., Euskirchen G.M., Auerbach R.K. Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. BMC Genomics. 2009;10:37. [PubMed]

64. Berens C., Suess B. Riboswitch engineering – making the all-important second and third steps. Curr Opin Biotechnol. 2015;31:10–15. [PubMed]

65. Groher F., Suess B. Synthetic riboswitches – a tool comes of age. Biochim Biophys Acta. 2014;1839(10):964–973. [PubMed]

66. Okumoto S. Quantitative imaging using genetically encoded sensors for small molecules in plants. Plant J. 2012;70:108–117. [PubMed]

67. Topell S., Glockshuber R. Circular permutation of the green fluorescent protein. Methods Mol Biol. 2002;183:31–48. [PubMed]

68. Tian L., Hires S.A., Looger L.L. Imaging neuronal activity with genetically encoded calcium indicators. Cold Spring Harb Protoc. 2012:647–656. [PubMed]

69. Bermejo C., Ewald J.C., Lanquar V., Jones A.M., Frommer W.B. In vivo biochemistry: quantifying ion and metabolite levels in individual cells or cultures of yeast. Biochem J. 2011;438:1–10. [PubMed]

70. Bermejo C., Haerizadeh F., Takanaga H., Chermak D., Frommer W.B. Dynamic analysis of cytosolic glucose and ATP levels in yeast using optical sensors. Biochem J. 2010;432:399–406. [PubMed]

71. Brett C.L., Kallay L., Hua Z. Genome-wide analysis reveals the vacuolar pH-state of *Saccharomyces cerevisiae*. PLoS ONE. 2011;6:e17619. [PubMed]

72. Orij R., Urbanus M.L., Vizeacoumar F.J. Genome-wide analysis of intracellular pH reveals quantitative control of cell division rate by pHc in *Saccharomyces cerevisiae*. Genome Biol. 2012;13:R80. [PubMed]

73. Jones A.M., Grossmann G., Danielson J.A.H. In vivo biochemistry: applications for small molecule biosensors in plant biology. Curr Opin Plant Biol. 2013;16:389–395. [PubMed]
