BIOCHIP JOURNAL, Vol. 2, No. 4, 223-234, December 2008

Mini Review

Biosensor Arrays for Environmental Pollutants Detection

Ravindra P. Singh[1], Byung-Keun Oh[1,2], Kee-Kahb Koo[2], Jy-Young Jyoung[2], Siyoung Jeong[3] & Jeong-Woo Choi[1,2]

[1]Interdisciplinary program of Integrated Biotechnology,
[2]Department of Chemical and Biomolecular Engineering, and
[3]Department of Mechanical Engineering, Sogang University,
1 Sinsoo-Dong, Mapo-Gu, Seoul 121-742, Korea
Correspondence and requests for materials should be addressed to J.W. Choi (jwchoi@sogang.ac.kr)

Accepted 16 October 2008

Abstract

Sensors are an extremely broad field which impacts on many major industrial sectors. This review paper highlights the research carried out during the last 8 years based on recent advances in the development of enzyme and NA (nucleic acid) based biosensors for environmental detection including pollutants and toxic compounds in a wide range of samples. NA-based biosensors are also finding increasing use for the detection of environmental pollutants and their toxicity. Biosensors are now used in a wide variety of disciplines, so that the different types of biosensors are used with their advantages and limitations with different transducers forming the sensing devices, for the different environmental contaminants analysis. The general applications of the enzyme biosensors are highlighted related with an environmental monitoring application. Recent advances in the development and applications of biosensor arrays for environmental detection are highlighted in this review article with special emphasis on functional nucleic acid elements (aptamers, DNAzymes, aptazymes), widely useful in nanobiotechnology and also in lab-on-a-chip technology.

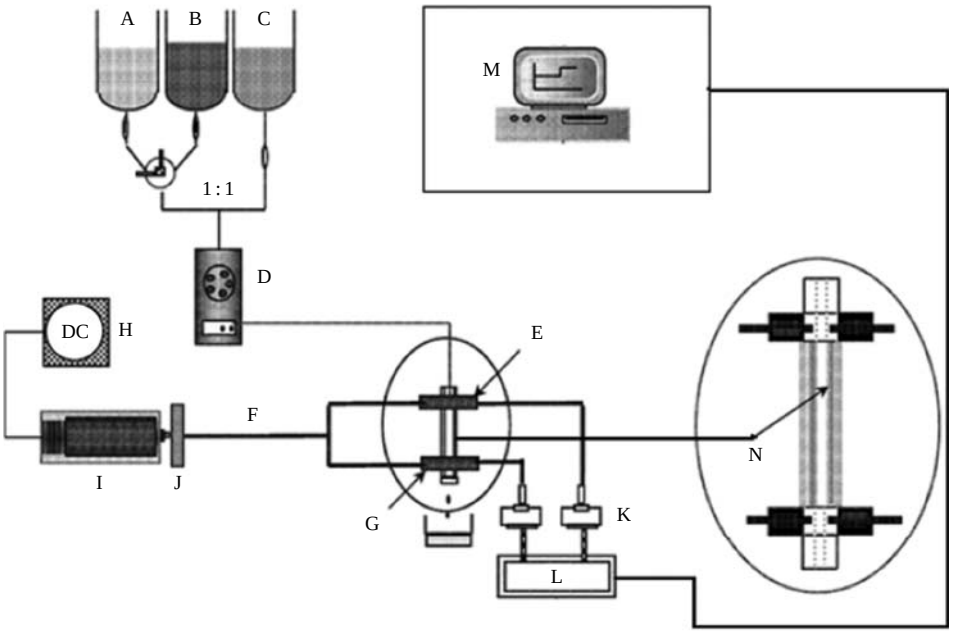
Keywords: Sensors, Biosensors, DNA, Toxicants, Pollutants

Introduction

Biosensors have recently been attracted much attention due to their applications in environmental pollution monitoring[1]. The last few years have seen great advances in the design of sensor architectures, the marriage of biological systems with monolithic silicon and optical technologies, the development of effective electron-transfer systems and the configuration of direct immunosensors[2]. Due to their performance capabilities including high specificity and sensitivity, rapid response, low cost, relatively compact size and user-friendly operation, they have become an important tool for detection of chemical and biological components[3]. The environmental detection of various diverse pollutants have been encouraged by the development of new technologies and more suitable methodologies like biosensor arrays to monitor as quickly and as cheaply as possible. The biosensors offer to determine not only specific chemicals but also their biological effects, like toxicity, cytotoxicity, genotoxicity. Despite these advantages, the application of biosensors in the environmental field is still limited in comparison to medical or pharmaceutical applications, where most research and development has converged[4].

Enzymatic biosensors are based on the selective inhibition of specific enzymes by different classes of compounds as target analyte for their quantification. Whole living cells like bacteria, yeast, fungi, plant and animal cells, or even tissue slices, have been used for the determination of the toxicity of certain compounds to the cells of choice. Genetically engineered bacteria are often used in cell-based biosensors. DNA biosensors are applied to detect pollutants by one is the hybridisation detection of nucleic acid sequences from infectious microorganisms, and the other is the monitoring of small pollutants interacting with the immobilized DNA layer with drugs, mutagenic pollutants, etc.[5].

Nowadays monitoring of toxicants, contaminants or pollutants in the air, water and soil are very important to save from the risks pose to human health and ecosystems. There is need have fast, cost-effective, less time consuming analytical techniques to be used for analyze environmental samples. Recently, nucleic acids (NAs) have been incorporated into a wide range of biosensors and bioanalytical assays, due to their wide range of physical, chemical and biological activities for a specific identification of animal and vegetal species, genetically-modified organisms, bacteria, viruses, toxins, etc.[6,7]. A nucleic acid is a macromolecule composed of nucleotide chains but many NA molecules are known as aptamers with high affinity and specificity[8,9] can be recognized by small organic molecules, proteins, cells and even intact viral particles. The catalytic DNA molecules are also called



A. Sample
B. Distilled water
C. Substrate
D. Peristaltic pump
E. Reference
F. Optic fiber
G. Detector
H. Power supply
I. Xe-lamp
J. Optical filter (400 nm)
K. Photodiode
L. Multiplier
M. Computer
N. Enzyme immobilized

Figure 1. Experimental set-up of biosensor system based on flow type SPR for the detection of captan and organophosphorous compounds,

DNAzymes (DNA enzymes, deoxyribozymes or catalytic DNA), aptamers and aptazymes are collectively called functional DNAs, whose functions extend beyond the Watson-Crick base pair recognition of complementary strands. These naturally occurring NAs as well as DNA-templated materials that are responsive to chemical stimuli have been used to assemble biosensors[10].

Enzyme-based Biosensors for Environmental Pollutants Detection

Biosensors are analytical devices which tightly combine biorecognition elements and physical transducers for detection of the pollutant compounds to inhibit the activity of certain enzymes, their activity and the resulting product concentration. The development of biosensors based on immobilized enzymes solve several problems including loss of enzyme, maintenance of enzyme stability and shelf life and additionally to reduce the time of the enzymatic response and offer disposable devices which can be easily used in stationary or in flow systems[11]. The interaction between the enzyme and the toxic compound involved inhibition/inactivation of enzyme depends on the substrate concentration and exposure time.

The limit of the detection of biosensors depends on the several parameters such as pH, temperature, the enzyme loading the substrate concentration, the immobilization matrix and the time of reaction between the enzyme and the inhibitor. Besides these, the choice of organic solvent needs to be considered as part of the method development in order to avoid undesirable effects as well as minimize the enzyme inhibition/inactivation[12,13].

Biosensors based on the immobilization of the biochemical component for the detection of various toxicants. Sensor devices coupled with reactors which contain an immobilized enzyme matrix. The inhibitor passes through the reactor and inhibits the enzyme as shown in Figure 1, which illustrated experimental set-up of biosensor system. The residual activity of the enzyme is evaluated by measuring the enzymatic product before and after the inhibition and also reported captan in real contaminated samples up to 2 ppm and the response time to steady sensor signal was about 15 min[14]. Furthermore, they have also been reported the fiber-optic biosensor consisting of AChE-immobilized LB film for simple and direct detection of orgarnophosphorus compounds in contaminated water up to 2 ppm and the response time to steady signal of the sensor was about 10 min[15].

The biosensor systems for the environmental detection of various toxic chemical entities are based on

Table 1. The list of few enzyme biosensors for the determination of inhibitors.

Inhibitors	Enzymes used	Methods	Sources	Limit of detection	References
Oxalic acid	AChE	Amerometric	–	0.5-20 $mmol^{-1}$	1
Nitric oxide	Xanthine oxidase	Amerometric	–	0-2 $mmol^{-1}$	40
Nitric oxide	HRP	Amerometric	–	$2.7\times10^{-6}-1.1\times10^{-5}$ mol L^{-1} LOD=2×10^{-6} mol L^{-1}	41
Benzoic acid	Tyrosinase	Amerometric	Soft drinks	9×10^{-7} mol L^{-1}	1
Cyanide	Tyrosinase	Amerometric	–	LOD=0.1 nmol L^{-1}	19
Methyl isothiocynate	AIDH	Amerometric	–	100-1,000 ppb, LOD=100 ppb	1
Anatoxin-a(s)	AChE	Amerometric	Fresh water	–	2
Captan and pesticides	GST	Optical	Contaminated water	Upto 2 ppm	14, 15, 17

Table 2. The list of enzyme biosensors for the determination of heavy metals.

Heavy metals	Enzymes	Methods	Sources	Limit of detection	References
Hg^{+2}, Cu^{+2}, Cd^{+2}	Urease	Optical	Tap and river water	LOD=10 nM, 50 μM, 500 μM	28, 29
Hg^{+2}, Cu^{+2}	GOx	Amperometric	–	2 5 μmol^{-1}-0 2 μmol^{-1} and 2 5 μmol^{-1}-0.2 μmol^{-1}	24
$Hg(NO_3)_2$, $HgCl_2$, $Hg_2(NO_3)_2$	Urease	Potentiometric	Water	0.05-1.0/0.2, 0.05-1.0/ 0.2, 0.05-1.0/0.1 μmol^{-1}	30, 31
Cd^{+2}	Urease	Optical (SPR)	–	0-10 mg L^{-1}	1
Hg^{+2}, Ag^{+}, Cu^{+2}, Pb^{+2}, Ni^{+2}, Zn^{+2}, Co^{+2}	Urease	Optical fibre	–	1×10^{-9}-1×10^{-5}, 1×10^{-8}-1×10^{-5} 1×10^{-7}-1×10^{-5}, 1×10^{-6}-1×10^{-5} 2×10^{-5}-1×10^{-3}, 2×10^{-5}-1×10^{-3} 1×10^{-4}-1×10^{-3} mol L^{-1}	18
Mercury(II), mercury(I), methylmercury, Hg-GSH complex, Methyl mercury, Hg^{+} and Hg^{+2}	HRP	Amperometric	–	LOD=0.1, 0.1, 1, 7 ng mL^{-1}	1
Ag^{+}, Ni^{+2}, Cu^{+2}	Urease	Potentiometri pH-SFET	–	LOD=3.5×10^{-8}, 7×10^{-5}, 2×10^{-6} mol L^{-1}	27
Cu^{+2}	AChE	Amperometric	–	0.05-4.0 $mmol^{-1}$	2
Hg^{+2}	GOx	Amperometric	Spiked water	2.5-12 ng mL^{-1} LOD=1 ng mL^{-1}	2
Methyl and Phenyl mercury	Invertase	Amperometric	–	I_{50}=0.27, 0.032, 0.27, 0.34, 0.12 ppm	25, 26
Chromium (vi)	GOx	Amperometric	Soil samples	0.49 μg L^{-1} to 8.05 mg L^{-1}, LOD=0.49 μg L^{-1}	2
Nitrate	Recomb. *E. coli*, nitrite reductase	Optical	Water	–	60

the principle of enzyme inhibition including pesticides, heavy metals and chemical toxins. In general, the development of these biosensing systems relies on a quantitative measurement of the enzyme activity before and after exposure to a target analyte basically the percentage of inhibited enzyme that results quantitatively related to the inhibitor (i.e. analyte) concentration and the incubation time[16,17]. Kuswandi has developed a simple optical biosensor based on immobilized urease for the monitoring of heavy metals and the detection limit depends on the incubation time (5 min) of the enzyme with the inhibitor[18]. Shan *et al.* have been reported specific electrostatic interaction of the host matrix that may induce an accumulation of the inhibitor within the anionic clay and this phenomenon improved the sensitivity of the amperometric

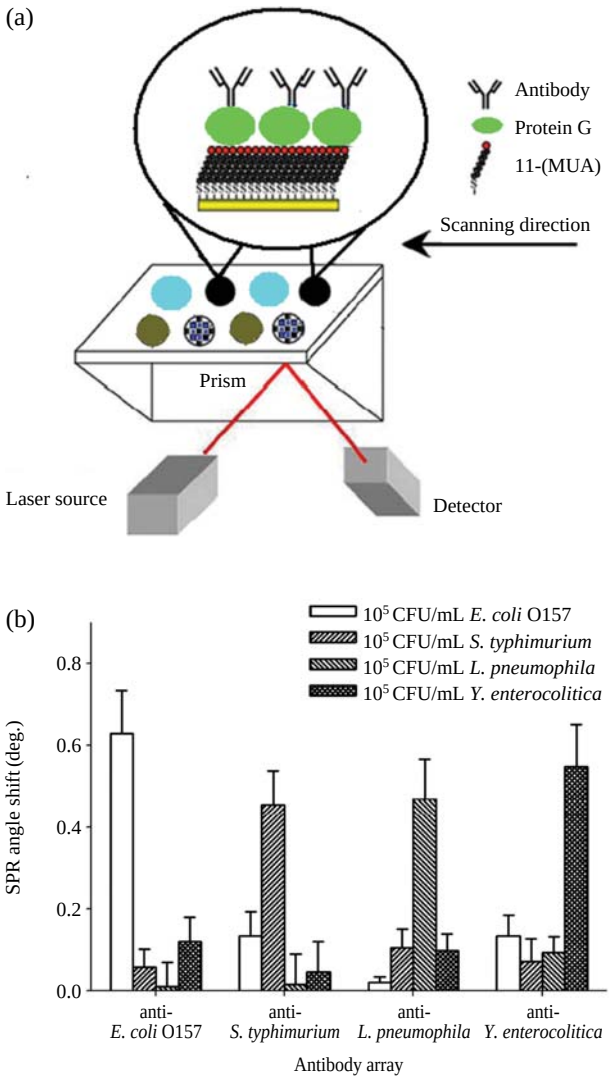


Figure 2. (a) Schematic illustration of the proposed protein chip based on SPR and (b) The response of Mab against four pathogens spot of protein chip based on SPR for ca. 10^5 CFU/mL of four pathogens, such as *E. coli* O157 : H7, *S. typhimurium*, *L. pneumophila*, and *Y. enterocolitica*.

biosensor toward cyanide (0.1 nM)[19]. Mazzei *et al.* have been reported the use of a bioelectrochemical system for the determination of pesticides by alkaline phosphatase inhibition[20]. Simonian *et al.* have been reported direct detection of OP neurotoxins using OPH enzyme attached gold nanoparticle[21]. The inhibition of tyrosinase has been investigated for the determination of carbaryl using amperometric[22].

Table 1 and 2 highlight enzyme based biosensors for the detection of various inhibitors (pesticides and other toxic pollutants) and heavy metals respectively including tyrosinase[22], GST[15], glucose oxidase[23], alcohol oxidase[24], invertase[25,26], Urease[27-32], AChE[33-37], butyrilcholinesterase (BChE)[38], Lactate dehydrogenase (LDH)[39], cellobiose dehydrogenase[40], nitric oxide (NO)[41] and superoxide radicals[42]. Lee *et al.* presented a cell-based array technology that uses 20 recombinant bioluminescent bacteria to detect and classify environmental toxicity[43]. Photosynthesis inhibition is an interesting indicator that rapidly reflects the toxic effect of certain pollutants. Taking advantage of this feature, some biosensors based on Photosystem II (PSII) have been reported to be able to detect herbicides[44]. Recently, a label-free direct piezoelectric immunosensor built on a flow-through cell was used for the determination of 2,4-D in water. Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants, considered as carcinogenic and a potential threat to human health has been determined by SPR based biosensor[45]. Bisphenol A is an environmental pollutant determined by immunosensors based on bacterial magnetic particle; a SPR; total internal reflection fluorescence and has also been reported based on a tyrosinase-carbon paste electrode of an optical biosensor for phenolic EDCs including bisphenol A, nonylphenol and diethylstilbestrol[46]. Amperometric biosensors for naphthalene found in contaminated soils were constructed using *Sphingomonas yanoikuyae* B1 and a recombinant *Escherichia coli*-based biosensor for benzene determination in air[47]. Hansen and Sorensen have been presented the choice of three different recombinant cells modified with a tetracycline-inducible promoter in the development of three corresponding whole-cell biosensors[48].

The detection of toxins especially bacterial and mycotoxin in environmental and clinical samples are very important. Oh *et al.* have been reported protein chip based on SPR for the simultaneous detection of pathogens existing in contaminated environment, such as *Escherichia coli* O15 : H7, *Salmonella typhimurium*, *Legionella pneumophila*, and *Yersinia enterocolitica* as shown in Figure 2a. They have detected 4 pathogens by SPR spectroscopy. The proposed protein chip, based on SPR for the simultaneous detection of pathogens, could also be applied to develop other protein chips with a high efficiency[49] as shown in Figure 2b. Choi *et al.* have been investigated biosurface fabrication based on synthetic cysteine terminated oligopeptide on gold surface for the application to cell chip platform with *E. coli* O157 : H7 for the biological toxicity detection such as phenol exposure as shown in Figure 3. The detection limit was determined to be 5 ppm of phenol. The proposed cell immobilization method using self-assembly technique can be applied to construct the cell chip for the diagnosis, drug detection, and on-site monitoring[50,51].

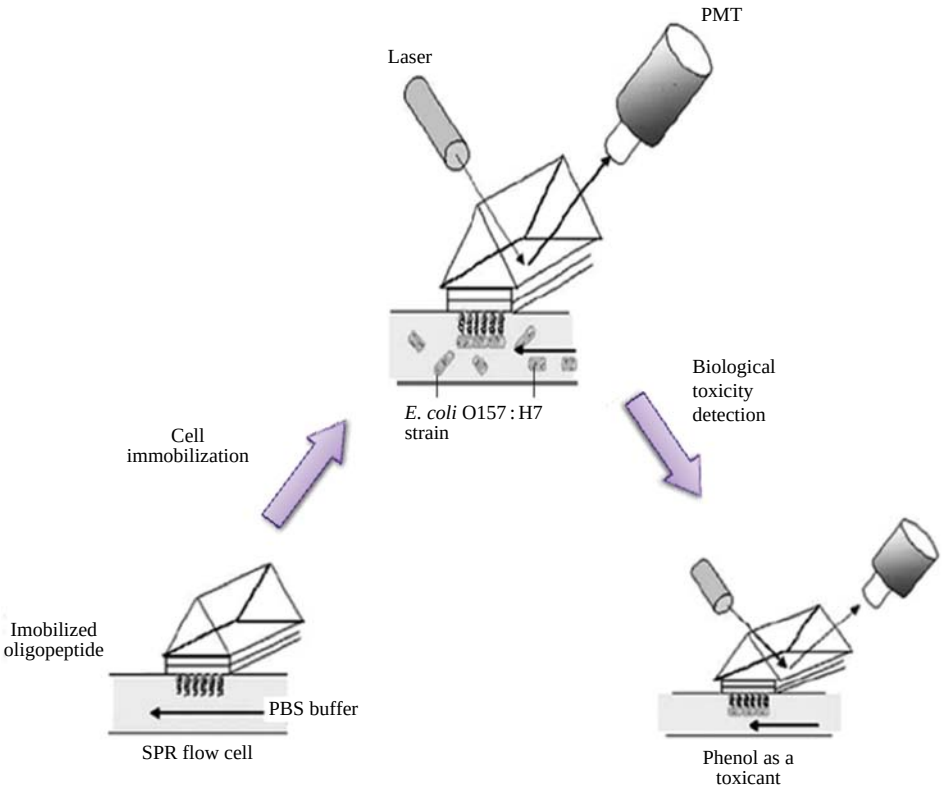


Figure 3. Schematic description of cell immobilization for cell chip and biological toxicity detection of phenol as a pollutant.

Waleed *et al*. have recently been reported that the feasibility of detection of the anticancer drugs effect using immobilized HeLa cells, and the applicability of this approach to a cell chip platform using cyclic voltammetry and potentiometric stripping analysis for use as direct electrochemical detection techniques to monitor cell growth and viability and the effect of anticancer drugs on cell viability[52,53]. Oh *et al*. have also been described an immunosensor based on SPR for the detection of *S. typhimurium* with high sensitivity by controlling the orientation of antibody molecules immobilized on SPR surface using self-assembled protein G[54]. Lee *et al*. fabricated the protein array based on the sol-gel-derived surface for the detection of *E. coli* O157 : H7 as shown in Figure 4. They have found detection limit 10^2 CFU/mL and speculated that the technique involving the preparation of sol-gel derived bioactive platform can be applied to the protein chip based detection such as diagnosis, biochemical research, and so on. These bioactive inorganic platforms will offer many advantages for commercialization and popular use[55]. Jyoung *et al*. have also been developed an immunosensor based on SPR for the detection of *V. cholerae* O1 using immobilization of the antibody, a protein G layer on the mixed-SAM and found detection range of between 10^5 and 10^9 cells/mL. The proposed fabrication technique using protein G and SAM has the potential to be utilized for the development of a versatile immunosensor[56]. A portable fiber-optic biosensor and an impedance-based immunosensor have been reported to determine staphylococcal enterotoxin B[57]. A rapid and sensitive immunosensor developed for the detection of the *Clostridium botulinum* toxin A enabled the detection of the toxin within 1 min at concentrations as low as 5 ng/mL[58]. Recombinant luminescent bacterial sensors were used for the determination of the bioavailable fractions of cadmium, zinc, mercury and chromium in soil and heavy metals in sediments and soil[59]. Urban wastewater treatment regulations aim at reducing pollution, including nitrate/nitrite pollution, from sewage treatment works and industry. A whole-cell fluorescence biosensor based on recombinant *Escherichia coli* allowed the determination of nitrate without the interference of phosphate, chloride and nitrite[60]. The commercial environmental biosensors are substantially less for the measurement of pollutants and other environmental hazards due to their practical stability but SPR biosensors (BIACORE AB, Uppsala, Sweden), REMEDIOS, a whole-cell-based biosensor, employed for diagnosis of contaminated land or soil to detects the levels of any toxic substance that affects the metabolic activity of the biosensor organisms. Unisense (Aarhus, Denmark)

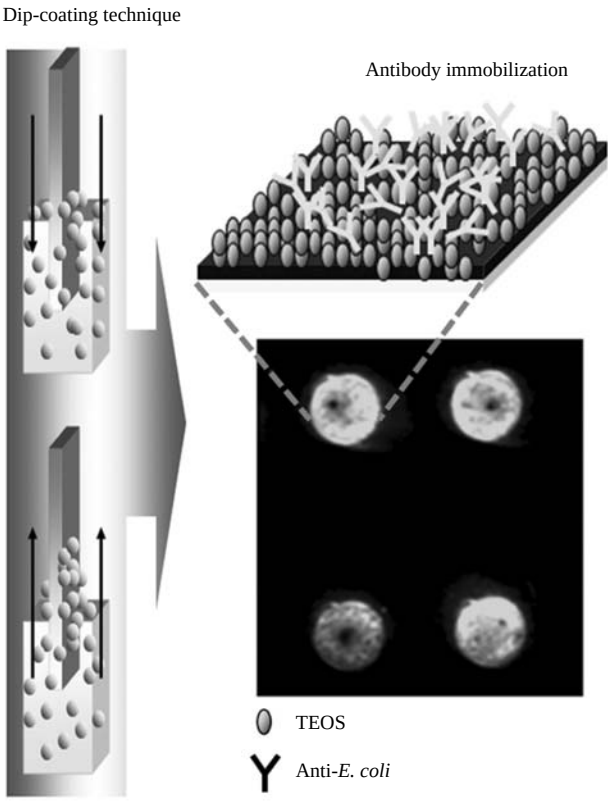


Figure 4. Schematic description of protein array fabrication based on sol-gel bioactive platform. During the hydrolysis of tetraethoxysilane (TEOS), the bioactive thin film was prepared through sol-gel dip coating.

and NECi's Nitrate Biosensor (Nitrate Elimination, MI, USA) launched microbial and enzymatic biosensors, respectively, for nitrate determination[61,62]. Multi-analyte determinations of several analytes simultaneously allow a reduction in time and sample volume and reagents required are a valuable tool for environmental monitoring. A portable SPR immunosensor designed for on-site analysis, which was applied to the simultaneous determination of benzopyrene and 2-hydroxybiphenyl and a planar array immunosensor, equipped with a charge-coupled device (CCD) as a detector and a diode laser as light source, has been also developed and applied to either the determination of multiple compounds, such as viruses, toxins and bacterial spores, in a single sample analysis or a single analyte in multiple samples simultaneously[63]. Mastichiadis *et al*. have recently been applied an optical capillary immunosensor to the simultaneous determination of the pesticides mesotrione, hexaconazole, paraquat and diquat and recently, quantum dots are also being applied for multi-analyte determinations[64].

Nanotechnology is playing an increasingly important role in the development of biosensors by using nanomaterials for biosensor construction, the sensitivity and performance is improved. The quantum dot or nanocrystal display extraordinary optical properties, tunable by regulating their size[65]. The microelectronics and microfluidics are miniaturisation of analytical systems using low volume samples and reduce reagent consumption and waste generation, and increasing sample throughput by decreasing analysis time, increasing reliability and sensitivity through automation and integrating several processes in a single device[66]. New sensing elements are those which improve the affinity, specificity and mass production of the molecular recognition components in future biosensor development by using biotechnology or gene engineering. Modified cell biosensors are being constructed by fusing a reporter gene to a promoter element that is induced by the presence of a target compound[67].

While in days to come antibodies are likely to remain the recognition molecules, a number of alternatives are being investigated. One type of recognition molecule that has received significant attention in recent years is peptide-nucleic acids (PNAs). PNAs have been used as a platform for attachment of an analyte derivative or capture antibody. Specific nucleic acids, aptamers, have been shown to bind small molecules with high affinity and can thus be considered as a valid alternative to antibodies or other biomimetic receptors for the development of biosensors[68].

NA Biosensors for Environmental Applications

Various signal transduction mechanisms are used in NA biosensing applications including optical, electrochemical, mass, magnetic, and micromechanical. Genosensors are the integration of a sequence-specific probe (usually a short synthetic oligonucleotide) and a signal transducer. Environmental applications of genosensors are extensively exploited in the detection of pathogenic microorganisms relevant to food, biodefense and environmental contamination applications[69]. Recently, probes produced by chemical changes to the backbone of naturally-occurring DNA or RNA are used more and more in NA sensing techniques. Among these, locked nucleic acid (LNA) and peptide nucleic acid (PNA) are the most used and their structure shown in Figure 5. LNA is a bicyclic nucleic acid where a ribonucleoside is linked between the 2′-oxygen and the 4′-carbon atoms with a methy-

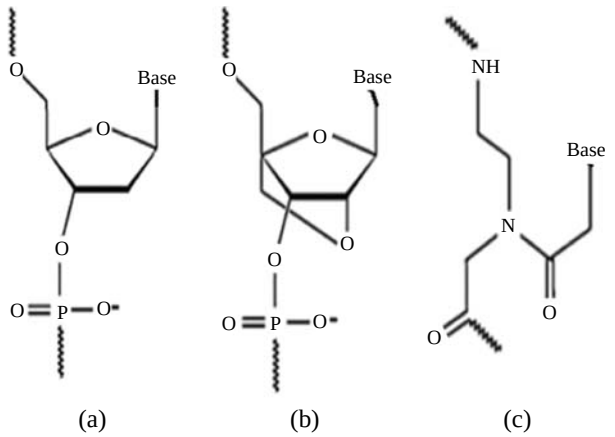


Figure 5. Structure of DNA (a), LNA (b) and PNA (c) monomers.

lene unit and used in any hybridization assay that requires high specificity and/or reproducibility. General properties of LNA oligonucleotides include highly stable base pairing with DNA and RNA[70] exceptionally high thermal stability, improved discrimination, compatibility with most enzymes and predictable melting behaviour. PNA is a synthetic nucleic acid reported in the early 1990s that has an achiral neutral polyamide backbone formed by repetitive units of *N*-(2-aminoethyl) glycine linked to N bases. The PNA molecule that mimics DNA is advantageous as a probe molecule, owing to improved chemical and enzymatic stability relative to nucleic acids. The PNA molecule, being resistive to attack by nucleases, provides an extra edge over the use of conventional or naturally existing nucleic acids. Several variables affect the hybridization event at the transducer-solution interface and should be controlled carefully. These are salt concentration, temperature, and the presence of accelerating agents, contact time and length of the probe sequence[71]. Conducting polymer based nucleic acid biosensor is also suitable for environmental toxicant detection. Arora *et al*. have physically been immobilized ds-CT-DNA onto electrochemically prepared polypyrrole-polyvinylsulphonate (PPy-PVS) films and investigated the amperometric response studies of the DNA/PPY-PVS electrodes carried out at 25°C as a function of 2-aminoanthracene (2-AA) concentration (0.01-20 ppm) and o-chlorophenol (OCP, 0.1-30 ppm) reveal that 10 ppm is sufficient to reduce the observed guanine oxidation current and that 25 ppm of OCP reduced the oxidation current to zero[72]. Lermo *et al.* have been described a genomagnetic assay for the electrochemical detection of food pathogens based on *in situ* DNA amplification with magnetic primers[73]. Elsholz *et al.* have been reported a low-density electrical 16S rRNA -specific oligonucleotide microarrays and an automated analysis system for the identification and quantization of pathogens, such as *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *S. aureus*, and *S.* epidermides[74].

Aptasensors for Environmental Analysis

Aptamers are single-stranded DNA or RNA ligands that can be selected for different targets starting from a huge library of molecules containing randomly created sequences. An application of aptamer-based biosensors for the detection of environmental analysis related molecules is very limited[75]. Brockstedt *et al.* reported for the first time the selection of RNA aptamers for the recognition of hydrophobic aromatic carcinogens[76]. Among those aptamers that have actually been applied to the detection of target molecules for environmental analysis, find a 17β-estradiol DNA aptamer i.e. 76-mer DNA aptamer, with a K_d of 0.13 mM selected and used for the development of an electrochemical biosensor for the detection of 17β-estradiol, a well-known endocrine disrupting molecule[77]. The aptamer was immobilized on gold electrodes via the avidin-biotin interaction, and cyclic voltammetry and square wave voltammetry were used to evaluate the target binding to the aptamer as shown in Figure 6 (a) and (b) along with secondary structure of DNA aptamer.

Catalytic Biosensors for Environmental Analysis

The term nucleic acid enzyme is used to identify nucleic acids that have catalytic activity. Ribozymes (made of ribonucleic acid or RNA) are found in nature and mediate phosphodiester bond cleavage and formation and peptide bond formation. Artificial ribozymes have been obtained by means of combinatorial chemistry approaches, such as *in vitro* selection and *in vitro* evolution and have been shown to catalyze quite a broad array of other chemical reactions. DNAzymes are artificial molecules and are not found in nature[78].

DNAzymes for Environmental Analysis

DNAzymes are DNA-based biocatalysts capable of performing chemical transformations. DNAzymes can therefore provide additional control over nucleic acid-based nanodevices. Among the many classes of DNAzymes, RNA-cleaving DNAzymes are the most widely used, mainly because of their simple reaction conditions, fast turnover rates, and significant modifications on their substrate lengths[79]. Liu *et al*. have been used 17E (a variant of the '8-17' deoxyribozy-

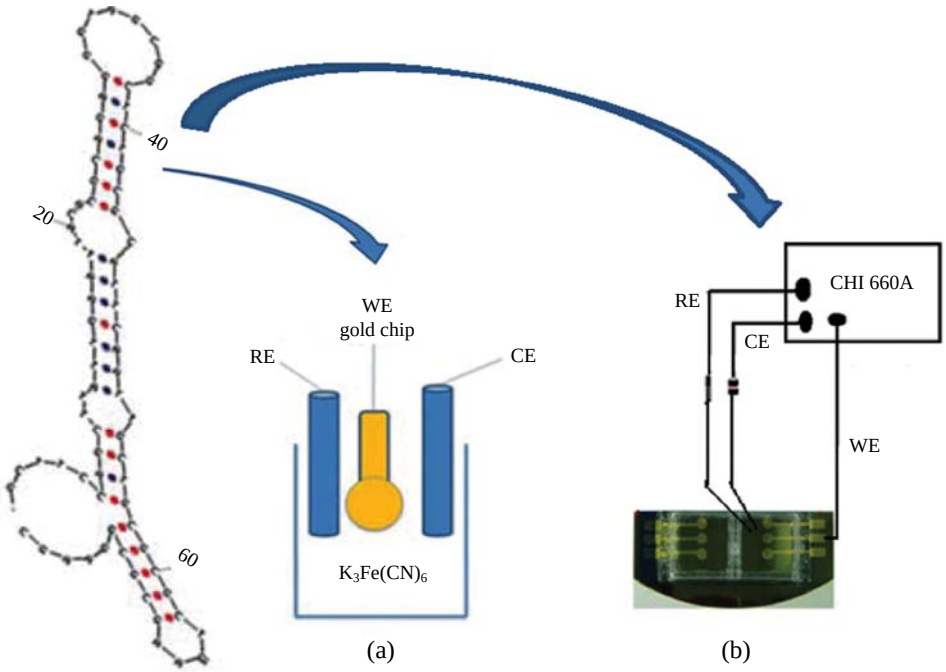


Figure 6. Secondary structure of the selected DNA aptamer and (a) & (b) electrochemical detection system and the mode for electrochemical signal production. The DNA aptamer-immobilized gold electrode chip as a working electrode. The sample solution was tested by exposing the DNA aptamerimmobilized gold electrode to it and then inserting the chip into $K_3Fe(CN)_6$ containing buffer and then signal measured by CV or SWV or DPV by electrochemical analyser. (b) Shows schematic description of cell chip set-up for detection of anticancerous drugs and biological toxicity.

me) with gold nanoparticles for the design of a colorimetric assay of lead and found detection range between 100 nM and 4 mM[80]. Yim *et al*. have been reported Pb^{2+}-specific DNAzyme, immobilized onto carbon nanotubes[81]. Along the same lines of surface immobilization, the DNAzyme was covalently attached to gold surfaces[82] and gold-coated nanocapillary membranes for the analysis of environmental lead pollution in groundwater or drinking water[83]. Garibotti *et al.* have recently been incorporated a Cu^{2+}-dependent DNAzyme with optimized cleavage efficiency into a two-dimensional array based on the DNA double crossover motif (a rigid DNA building block frequently used in DNA nanotechnology)[84].

Aptazymes for Environmental Pollution Analysis

DNAzymes can perform chemical modifications on nucleic acids, while aptamers can bind a broad range of molecules. A combination of the two has generated a new class of functional nucleic acids known as allosteric DNAzymes or aptazymes. Liu and Lu have been used an adenosine-dependent aptazyme built on the basis of the Pb^{2+}-specific DNAzyme, to assemble gold nanoparticles. In the presence of adenosine, the substrate was cleaved and the assembly inhibited. Several aptazymes (activated RNAzymes) have been shown to have metal-ion-dependent activities[85]. In conclusion, the potentialities of aptazymes have been reported after their selection, but no direct application in environmental monitoring has been published so far.

DNA Biosensors for the Detection of Chemically-induced DNA Damage

The environmentally toxic compounds chemically-induced damage to DNA is an important challenging issue in environmental pollutants detection. A short-term test for genotoxicity/mutagenicity are avialable to determine the extent of environmental hazards in polluted water and sediments but these tests are expensive to run, require sophisticated technical expertise, and are not well suited to be adapted to screening applications. In recent years, use of nucleic acids as a tool in the recognition or monitoring of chemical compounds of environmental interest by electrochemical DNA biosensors for DNA strand breaks and base damage as well as electroactive substances that specifically interact with DNA. The guanines are available to react rapidly with the mediator, providing an increased current in the square wave oxidation peak which correlates sensor for toxicity screening based on the detection of DNA damage for the various toxic aromatic amines, benzo[*a*]pyrene, and hydrazine etc. Some heavy metals are known to have great affinity for DNA and to cause mutagenesis and carcinogenesis[86]. Babkina and Ulakhovich, have been proposed a method for the determination of heavy metals based on the biospecific pre-concentration of metal ions on an electrochemical DNA biosensor followed by the destruction of DNA-metal complexes with EDTA and voltammogram recording. DNA da-

mage has also been measured using fluorescence-based biosensors[87]. The changes in melting-annealing behaviour that were observed in real time using a double-strand-selective fluorescent indicator dye have also been used to measure DNA damage induced by radiation and chemical mutagens such as styrene oxide, glutaraldehyde, acrolein, allylamine, chloroacetone, acrylonitrile, bromoethane, crotonaldehyde and benzo[*a*]pyrene. This screening assay was sensitive to various forms of DNA damage including strand breaks, cross-links and adducts formation[88,89]. The assay was also demonstrated with respect to the effects of well-characterized genotoxins such as mitomycin C as well as cytotoxic but non-genotoxic compounds such as phenol, cyclohexane and toluene. DNA damage generated by organophosphate pesticides was studied by Hianik *et al.* using the sensitivity of the acoustic shear wave technique to the interaction of DNA strands with damaged DNA containing apurinic or apyrimidinic (AP) sites[90].

New Trends, Future Perspectives and Conclusions

The research activity on the development of biosensors based on enzyme and their analytical applications are limited because these are not usually able to discriminate various toxic compounds in the same sample. The simultaneous presence of heavy metals and pesticides in contaminated samples are a challenging concern which suggest that these biosensors can be used as alarm systems and they would provide either quantification of one contaminant when this analyte is present alone or an indication of total contamination of particular samples. In few cases, the biosensor based on inhibitory effects of pesticides or toxicants/contaminants show high sensitivity and low specificity with rapid and cost-effective. Current research studies enhancing the analytical performance of the biosensing systems to monitor a wide range of pollutants in environmental samples (eg genetically modified enzymes based biosensors) using microporous-activated carbon technology; improved the efficiency of enzyme-based biosensors for environmental monitoring. This will be the future perspectives to targetd engineered variants of enzymes for the biosensor design for the discrimination and detection of numerous enzyme-inhibiting toxicants/contaminants. Besides this the use of transducers fabricated from nano-structured material may improve the sensitivity and specificity of biosensors along with the simultaneous monitoring of several toxicants/contaminants in a multicomponent sample which will enable into commercialization of biosensing devices for large scale environmental monitoring applications.

NA biosensing of environmental pollution is a faster, simpler and cheaper when compared to the enzymatic biosenscing assays. The functional DNA molecules such as aptamers, DNAzymes and aptazymes have been found application in almost every aspect of DNA nanotechnology, and the resulting new materials and devices enter into many other domains especially medical and environmental pollution monitoring. In the sake of this, nanomaterials including CNT, metal NPs, or metal oxide nanostructures are targeted for the development of ultrasensitive NA-based biosensors (Nanobiosensors) for environmental pollution monitoring to check the proliferation of cancer, cardiovascular diseases, diabetes and infectious organisms. In the future, Micro Total Analysis Systems (μTAS) or Lab-on-a-chip (LOC) kind of devices to be come into the existence to measure extremely small fluid volumes down to less than picolitres of desire samples. Thus, integration of nanotechnology, microfluidics, and bioanalytical systems clearly represent one of the future directions of all biosensor research. In conclusion, in the near future, NA sensing and biosensor technology itself will undoubtedly benefit from nanotechnology and μTAS technology.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2004-005-D00003) and by the Ministry of Environment of the Republic of Korea as “The Eco-technopia 21 project”.

References

1. Amine, A., Mohammadi, H., Bourais, I. & Galleschi, G. Enzyme inhibition-based biosensors for food safety and environmental monitoring. *Biosen. Bioelectron*. **21**, 1405-1423 (2006).
2. Rogers, K.R. Recent advances in biosensor techniques for environmental monitoring. *Anal. Chim. Acta* **568**, 222-231 (2006).
3. Lee, W. *et al*. Fabrication of self-assembled protein A monolayer and its application as an immunosensor. *Biosen. Bioelectron*. **19**, 185-192 (2003).
4. Castillo, J. *et al*. Biosensors for life quality Design, development and applications. *Sens. Actuator B-Chem.* **102**, 179-194 G (2004).
5. Wang, J. *et al*. DNA electrochemical biosensors for environmental monitoring. A review. *Anal. Chim. Acta* **347**, 1-8 (1997).

6. Bagni, D., Osella, D., Sturchio, E. & Mascini, M. Deoxyribonucleic acid (DNA) biosensors for environmental risk assessment and drug studies. *Anal. Chim. Acta* **573**, 81-89 (2006).
7. Marrazza, G., Chianella, I. & Mascini, M. Disposable DNA electrochemical sensor for hybridization detection. *Biosen. Bioelectron.* **14**, 43-51 (1999).
8. Ellington, A.D. & Szostak, J.W. In vitro selection of RNA molecules that bind specific ligands. *Nature* **346**, 818-822 (1990).
9. Tuerk, C. & Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249**, 505-510 (1990).
10. Wanekaya, A.K., Chen, W. & Mulchandani, A. Recent biosensing developments in environmental security. *J. Environ. Monit.* **10**, 703-712 (2008).
11. Guilbault, G.G., Pravda, M. & Kreuzer, M. Biosensors-42 years and counting. *Anal. Lett.* **37**, 14481-14496 (2004).
12. Andreescu, S., Noguer, T., Magearu V. & Marty, J.L. Screen-printed electrode based on AChE for the detection of pesticides in presence of organic solvents. *Talanta* **57**, 169-176 (2002).
13. Durrieu, C., Chouteau, C., Barthet, Chovelon, J.M. & Tran-Minh, C. A bi-enzymatic whole-cell algal biosensor for monitoring waster pollutants. *Anal. Lett.* **37**, 1589-1599 (2004).
14. Choi, J.W., Kim, Y.K., Song, S.Y., Lee, I.H. & Lee, W.H. Optical biosensor consisting of glutathione-*S*-transferase for detection of captan. *Biosens. Bioelectron.* **18**, 1461-1466 (2003).
15. Choi, J.W., Kim, Y.K., Oh, B.K., Song, S.Y. & Lee, W.H. Optical biosensor for simultaneous detection of captan and organophosphorus compounds. *Biosens. Bioelectron.* **18**, 591-597 (2003).
16. Ivanov, A. *et al*. Cholinesterase sensors based on screen-printed electrode for detection of organophosphorus and carbamic pesticides. *Anal. Bioanal. Chem.* **377**, 624-631 (2003).
17. Choi, J.W., Kim, Y.K., Lee, I.H., Min, J. & Lee, W.H. Optical organophosphorus biosensor consisting acetyl cholinesterase/viologen hetero Langmuir-Blodjett film. *Biosens. Bioelectron.* **16**, 937-943 (2001).
18. Kuswandi, B. Simple optical fibre biosensor based on immobilized enzyme for monitoring of trace heavy metal ions. *Analytical Bioanalytical Chemistry* **376**, 1104-1110 (2003).
19. Shan, D., Mousty, C. &. Cosnier, S. Subnanomolar cyanide detection at polyphenol oxidase/clay biosensors. *Anal. Chem.* **76**, 178-183 (2004).
20. Mazzei, F. *et al*. Alkaline phosphatase inhibition based electrochemical sensors for the detection of pesticides. *J. Electroanal. Chem.* **574**, 95-100 (2004).
21. Simonian, A.L., Good, T.A., Wang, S.S. & Wild, J.R. Nanoparticle-based optical biosensors for the direct detection of organophosphate chemical warfare agent and pesticides. *Anal. Chim. Act.* **534**, 69-77 (2005).
22. Kuusk, E. & Rinken, T. Transient phase calibration of tyrosinase-based carbaryl biosensor. *Eng. Microb. Technol.* **234**, 657-661 (2004).
23. Alexander, P.W. & Rechnitz, G.A. Enzyme inhibition assays with an amperometric glucose biosensor based on thiolate self-assembled monolayer. *Electroanalysis* **12**, 343-350 (2000).
24. Pirvutoiu, S. *et al*. Application of the enzyme thermistor for determination of mercury and other heavy metals using free and immobilised alcohol oxidase. *Roum. Biotechnol. Lett.* **7**, 975 (2002).
25. Trojanowicz, M. *et al*. Limitations in the analytical use of invertase inhibition for the screening of trace mercury content in environmental samples. *Anal. Sci.* **20**, 899-904 (2004).
26. Mohammadi, H., El Rhazi, M., Amine, A., Brett, A.M.O. & Brett, C.M.A. Determination of mercury (II) by invertase enzyme inhibition coupled with batch injection analysis. *Analyst* **127**, 1088-1093 (2002).
27. Soldatkin, A.P., Volotovsky, V., El'skaya, A.V., Jaffrezic-Renault, N. & Martelet, C. Improvement of urease based biosensor characteristics using additional layers of charged polymers. *Anal. Chim. Acta* **403**, 25-29 (2000).
28. Rodriguez, B.B., Bolbot, J.A. & Tothill, I.E. Development of urease and glutamic dehydrogenase amperometric assay for heavy metals screening in polluted samples. *Biosens. Bioelectron.* **19**, 1157-1167 (2004).
29. Tsai, H.C. & Doong, R.A. Simultaneous determination of pH, urea, acetylcholine and heavy metals using array-based enzymatic optical biosensor. *Biosens. Bioelectron.* **20**, 1796-1804 (2005).
30. De Melo, J.V., Cosnier, S., Mousty, S., Martelet, C. & Jaffrezic-Renault, N. Urea biosensors based on immobilization of urease into two oppositely charged clays (laponite and Zn-Al layered double hydroxides). *Anal. Chem.* **74**, 4037-4043 (2002).
31. Krawczynski vel Krawczyk, T., Mosszczynska, M. & Trojanowicz, M. Inhibitive determination of mercury and other metal ions by potentiolmetric urea biosensor. *Biosens. Bioelectron.* **15**, 681-691 (2000).
32. Schulze, H., Vorlova, S., Villatte, F., Bachmmann, T.T. & Schmid, R.D. Design of acetylcholinesterases for biosensor applications. *Biosens. Bioelectron.* **18**, 201-209 (2003).
33. Sotiropoulou, S. & Chaniotakis, N.A. Lowering the detection limit of the acethylcholinesterase biosensor using a nanoporous carbon matrix. *Anal. Chim. Acta* **530**, 199-204 (2005).
34. Lee, H.S., Kim, Y., Chi, Y. & Lee, T.Y. Oxidation of organophosphorus pesticides for the sensitive detection by cholinesterase-based biosensor. *Chemosphere* **46**, 571-576 (2002).
35. Del Carlo, M. *et al*. Determining pirimiphos-methyl in durum wheat samples using an acethylcholinesterase inhibition essay. *Anal. Bioanal. Chem.* **381**,

1367-1372 (2005).
36. Dzyadevych, S.V. *et al*. Potentiometric biosensors based on ISFETs and immobilized cholinesterases. *Electroanalysis* **16**, 1873-1882 (2004).
37. Jeanty, G., Wojiciechowska, A. & Marty, J.L. Flow-injection amperometric determination of pesticides on the basis of their inhibition of immobilized acetylcholinesterases of different origin. *Anal. Bioanal. Chem.* **373**, 691-695 (2002).
38. Young, S.J., Hart, J.P., Dowman, A.A. & Cowell, D.C. The non-specific inhibition of enzymes by environmental pollutants: a study of model system toward the development of electrochemical biosensor arrays. *Biosens. Bioelectron.* **16**, 887-894 (2001).
39. Nistor, C. *et al*. In-field monitoring of cleaning efficiency in water treatment plants using two phenol sensitive biosensors. *Anal. Chim. Acta* **456**, 3-17 (2002).
40. Casero, E., Losada, J., Pariente, F. & Lorenzo, E. Modified electrode approaches for nitric oxide sensing. *Talanta.* **61**, 61-70 (2003).
41. Kilinc, E., Ozsoz, M. & Sadik, O.A. Electrochemical detection of NO by inhibition on oxidase activity. *Electroanalysis* **12**, 1467-1471 (2000).
42. Campanella, L., Persi, L. & Tomasseti, M. A new tool for superoxide and nitric oxide radicals determination using suitable enzymatic sensors. *Sens. Actuator B-Chem.* **68**, 351-359 (2000).
43. Lee, J.H., Mitchell, M.J., Kim, B.C., Cullen, D.C. & Gu, M.B. A cell array biosensor for environmental toxicity analysis. *Biosens. Bioelectron.* **21**, 500-507 (2005).
44. Giardi, M.T., Koblízek, M. & Masojídek, J. Photosystem II-based biosensors for the detection of pollutants. *Biosens. Bioelectron.* **16**, 1027-1033 (2001).
45. Kasai, A. *et al*. Fast-track DRESSA: a bioassay for fast, sensitive, and selective detection of halogenated and polycyclic aromatic hydrocarbons. *Anal. Biochem.* **337**, 84-88 (2005).
46. Andreescu, S. & Sadik, O.A. Correlation of analyte structures with biosensor responses using the detection of phenolic estrogens as a model. *Anal. Chem.* **76**, 552-560 (2004).
47. Berno, E., Pereira Marcondes, D.F., Ricci Gamalero, S. & Eandi, M. Recombinant Escherichia coli for the biomonitoring of benzene and its derivatives in the air. *Ecotoxicol. Environ. Saf.* **57**, 118-122 (2004).
48. Hansen, L.H. & Sorensen, S.J. Detection and quantification of tetracyclines by whole cell biosensors. *FEMS Microbiol. Lett.* **190**, 273-278 (2000).
49. Oh, B.K. *et al*. The fabrication of protein chip based on surface Plasmon resonance for detection of pathogens. *Biosen. Bioelectron.* **20**, 1847-1850 (2005).
50. Choi, J.W., Park, K.W., Lee, D.B., Lee, W. & Lee, W.H. Cell immobilization using self-assembled synthetic oligopeptide and its application to biological toxicity detection using surface plasmon resonance. *Biosen. Bioelectron.* **20**, 2300-2305 (2005).
51. Oh, B.K., Lee, W., Lee, W.H. & Choi, J.W. Nanosacle probe fabrication using self assembly technique and application to detection of *E. coli* O157 : H7. *Biotech. Bioprocc. Eng.* **8**, 227-232 (2003).
52. El-Said, W.A., Yea, C.H., Kim, H., Oh, B.K. & Choi, J.W. Cell-based chip for the detection of anticancer effect on HeLa cells using cyclic voltammetry. *Biosen. Bioelectron.* (In press, 2008, doi.org/10.1016/j.bios.2008.07.037).
53. Yea, C.H., Min, J. & Choi, J.W. The fabrication of cell chips for use as Bio-sensors. *Biochip J.* **1**, 219-227 (2007).
54. Oh, B.K., Kim, Y.K., Park, K.W., Lee, W.H. & Choi, J.W. Surface plasmon resonance immunosensor for the detection of *Salmonella typhimurium*. *Biosen. Bioelectron.* **19**, 1497-1504 (2004).
55. Lee, W., Park, S.K., Kim, Y.W., Lee, W.H. & Choi, J.W. Protein array consisting of sol-gel bioactive platform for detection of *E. coli* O157 : H7. *Biosen. Bioelectron.* **20**, 2292-2299 (2005).
56. Jyoung, J.Y., Hong, S.H., Lee, W. & Choi, J.W. Immunosensor for the detection of *Vibrio cholerae* O1 using surface plasmon resonance. *Biosen. Bioelectron.* **21**, 2315-2319 (2006).
57. Tempelman, L.A., King, K.D., Anderson, G.P. & Ligler, F.S. Quantitating staphylococcal enterotoxin B in diverse media using a portable fiber-optic biosensor. *Anal. Biochem.* **233**, 50-57 (1996).
58. Ogert, R.A., Brown, J.E., Singh, B.R., Shriver-Lake, L.C. & Ligler, F.S. Detection of Clostridium botulinum toxin A using a fiber optic-based biosensor. *Anal. Biochem.* **205**, 306-312 (1992).
59. Liao, V.H.C., Chien M.T., Tseng, Y.Y. & Ou, K.L. Assessment of heavy metal bioavailability in contaminated sediments and soils using green fluorescent protein-based bacterial biosensors. *Environ. Pollut.* **142**, 17-23 (2006).
60. Taylor, C.J., Bain, L.A., Richardson, D.J., Spiro, S. & Russell, D.A. Construction of a whole-cell gene reporter for the fluorescent bioassay of nitrate. *Anal. Biochem.* **328**, 60-66 (2004).
61. Brecht, A. Multianalyte bioanalytical devices: scientific potential and business requirements. *Anal. Bioanal. Chem.* **381**, 1025-1026 (2005).
62. Glazier, S.A., Campbell, E.R. & Campbell, W.H. Construction and characterization of nitrate reductase-based amperometric electrode and nitrate assay of fertilizers and drinking water. *Anal. Chem.* **70**, 1511-1515 (1998).
63. Rowe-Taitt, C.A. *et al.* Array biosensor for detection of biohazards. *Biosens. Bioelectron.* **14**, 785-794 (2000).
64. Mastichiadis, C. *et al*. Simultaneous determination of pesticides using a four-band disposable optical capillary immunosensor. *Anal. Chem.* **74**, 6064-6072 (2002).
65. Jianrong, C., Yuqing, M., Nongyue, H., Xiaohua, W. & Sijiao, L. Nanotechnology and biosensors. *Biotechnol. Adv.* **22**, 505-518 (2004).
66. Bange, A., Halsall, H.B. & Heineman, W.R. Micro-

fluidic immunosensor systems. *Biosens. Bioelectron.* **20**, 2488-2503 (2005).
67. Keane, A., Phoenix, P., Ghoshal, S. & Lau, P.C.K. Exposing culprit organic pollutants: a review. *J Microbiol. Method.* **49**, 103-119 (2002).
68. Tombelli, S., Minunni, M. & Mascini, M. Analytical applications of aptamers. *Biosens. Bioelectron.* **20**, 2424-2434 (2005).
69. Lucarelli, F., Marrazza, G., Turner, A.P.F. & Mascini, M. Carbon and gold electrodes as electrochemical transducers for DNA hybridisation sensors. *Biosens. Bioelectron.* **19**, 515-530 (2004).
70. Kaur, H., Arora, A., Wengel, J. & Maiti, S. Thermodynamic, counterion, and hydration effects for the incorporation of locked nucleic acid nucleotides into DNA duplexes. *Biochemistry* **45**, 7347-7355 (2006).
71. Nelson, K.E., Levy, M. & Miller, S.L. Peptide nucleic acids rather than RNA may have been the first genetic molecule. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 3868-3871 (2000).
72. Arora, K. *et al*. Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films to DNA biosensor. *Biosens. Bioelectron.* **21**, 1777-1783 (2006).
73. Lermo, A. *et al*. *In situ* DNA amplification with magnetic primers for the electrochemical detection of food pathogens. *Biosens. Bioelectron.* **22**, 2010-2017 (2007).
74. Elsholz, B. *et al*. Automated detection and quantitation of bacterial RNA by using electrical microarrays. *Anal. Chem.* **78**, 4794-4802 (2006).
75. Song, S., Wang, L., Li, J., Fan, C. & Zhao, J. Aptamer-based biosensors. *Trds. Analyt. Chem.* **27**, 108-116 (2008).
76. Brockstedt, U., Uzarowska, A., Montpetit, A., Pfau, W. & Lauda, D. In vitro evolution of RNA aptamers recognizing carcinogenic aromatic amines. *Biochem. Biophys. Res. Commun.* **313**, 1004-1008 (2004).
77. Kim, Y.S. *et al*. Electrochemical detection of 17β-estradiol using DNA aptamer immobilized gold electrode chip. *Biosens. Bioelectron.* **22**, 2525-2531 (2007).
78. Fiammengo, R. & Jaschke, A. Nucleic acid enzymes. *Curr. Opin. Biotechnol.* **16**, 614-621 (2005).
79. Navani, N.K. & Li, Y. Nucleic acid aptamers and enzymes as sensors. *Curr. Opin. Chem. Biol.* **10**, 272-281 (2006).
80. Liu, J. & Lu, Y. A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles. *J. Am. Chem. Soc.* **125**, 6642-6643 (2003).
81. Yim, T.J., Liu, J., Lu, Y., Kane, R.S. & Dordick, J.S. Highly active and stable DNAzyme-carbon nanotube hybrids. *J. Am. Chem. Soc.* **127**, 12200-12201 (2005).
82. Xiao, Y., Rowe, A.A. & Plaxco, K.W. Electrochemical detection of parts-per-billion lead via an electrode-bound DNAzyme assembly. *J. Am. Chem. Soc.* **129**, 262-263 (2007).
83. Wernette, D.P. *et al*. Incorporation of a DNAzyme into Au-coated nanocapillary array membranes with an internal standard for Pb(II) sensing. *Analyst* **131**, 41-47 (2006).
84. Garibotti, A.V., Knudsen, S.M., Ellington, A.D. & Seeman, N.C. Functional DNAzymes organized into two-dimensional arrays. *Nano Lett.* **6**, 1505-1507 (2006).
85. Liu, J. & Lu, Y. Adenosine-dependent assembly of aptazyme-functionalized gold nanoparticles and its application as a colorimetric biosensor. *Anal. Chem.* **76**, 1627-1632 (2004).
86. Lucareli, F., Palchetti, I., Marrazza, G. & Mascini, M. Electrochemical DNA biosensor as a screening tool for the detection of toxicants in water and wastewater samples. *Talanta* **56**, 949-957 (2002).
87. Babkina, S.S. & Ulakhovich, N.A. Complexing of heavy metals with DNA and new bioaffinity method of their determination based on amperometric DNA-based biosensor. *Anal. Chem.* **77**, 5678-5685 (2005).
88. Pumera, M., Samuel, S., Izumi, I. & Tang, J. Electrochemical nanobiosensors. *Sens. Actuator B-Chem.* **123**, 1195-1205 (2007).
89. Erdem, A. Nanomaterial-based electrochemical DNA sensing strategies. *Talanta* **74**, 318-25 (2007).
90. Hianik, T. *et al*. DNA-duplexes containing abasic sites: correlation between thermostability and acoustic wave properties. *Analyst* **131**, 1161-1166 (2006).