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Biosensors for Environmental Monitoring of Aquatic Systems

Bioanalytical and Chemical Methods for Endocrine Disruptors

Volume Editors: Damià Barceló · Peter-Diedrich Hansen

With contributions by

J. Adrián · D. Barceló · S. Belkin · A. Biran · S. Boronat
R. Brix · S. Buchinger · M. Casado · V. Dethlefsen
H. Dizer · M. Farré · F. Fernández · T. Grummt
P.-D. Hansen · M. Kuster · M. López de Alda
M. J. López de Alda · M.-P. Marco · A. Muriano
R. Obregon · A. Olivares · R. Pedahzur · B. Piña
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Preface

This book on "Biosensors for the Environmental Monitoring of Aquatic Systems" is based on the scientific developments and results achieved within a group of European Union (EU) funded projects that will be briefly discussed below. Indeed, everything started with the development of biosensors for environmental measurements in 1991 as part of the EC Environment Programme (1991–1994) under Area II: Technologies and engineering for the environment, section 1: Assessment of environmental quality and monitoring. In the context of this Programme, a biosensor was defined as a compact analytical device containing a biological or biologically derived sensing element (e.g., enzymes antibodies, microorganisms, or DNA) either integrated with or in intimate contact with a physicochemical transducer (e.g., electrochemical, optical, thermometric, or piezoelectric). The first European Workshop on Biosensors for Environmental Monitoring took place at the Technische Universität Berlin (BIT - Berlin Institute of Technology) in February 1993, organized by Prof. P.D. Hansen and Dr. J. Büsing. This was the first focused workshop of a series of workshops in London (1994) organized by P. Bennetto and J. Büsing, Florence (1995), Barcelona (1996), Freising (1997), Kinsale (1998), Paris (1999), Cascais (2000), and Alcala de Henares (2001). All these workshops covered a large variety of technical and scientific topics related to biosensors for environmental monitoring with additional presentations of the progress reports of the specific EU projects. The workshop in Florence had already presented ten biosensor projects supported under the EC Environment Programme, dealing with pesticide detection, microbiological contamination, and xenobiotics in water and air. This workshop involved some 100 scientists from 15 European countries and from Japan. The objective of the workshop in Florence (1995) was to present the state of European research activities supported under the EC Environment and Climate Programme (1995–1998) in the field of biosensor development and to identify priorities for future Research & Development.

During that period the European Commission's Environment and Climate Research Programme supported a three-year co-ordination project to guide technological developments in Biosensors for Environmental Technology (BIOSET). The aim of BIOSET was to enhance the development of biosensors for practical applications in monitoring pollutants in the environment. BIOSET maintained close contact with European industry and explored possibilities for collaboration with the commercial sector. This ensured eventual transfer of the technology demonstrated at the workshops and Technical Meetings to marketable devices. BIOSET provided assistance for researchers from European laboratories to meet to exchange ideas, use equipment, and establish a basis for new joint projects. The secretariat of the Concerted Action BIOSET supported the Technical Meetings.

There were three Technical Meetings held, two in Berlin in 1997 and 1998, and the third in Barcelona, in April 2000. The goal of these technical meetings was to join different research and industrial teams to evaluate the performance of their biosensor technology in field conditions with common and standardized surface and waste waters.

As a result of these field experiments, the additional information that biosensors can offer to environmental monitoring was also evaluated. Thus, these three Technical Meetings were useful accompanying measures and practical additions to the currently organized yearly workshops. The concerted action BIOSET was followed by the SENSPOL network. The 1st SENSPOL Workshop was held on the 9–11 May 2001 on Sensing Technologies for Contaminated Sites and Groundwater at the University of Alcala. There was one special Workshop on "Genotoxicity Biosensing (TECHNOTOX)" supported by the European Commission DG XII D-1 and BIOSET in the year 2000. The TECHNOTOX meeting at the Flemish Institute for Technological Research (VITO) in Mol was organized by Phillippe Corbisier (VITO), Peter-D. Hansen (TU Berlin) and Damia Barcelo (CSIC Barcelona). Thirty scientists participated in this meeting and 14 genotoxicity tests were performed on-site simultaneously on a maximum of 11 samples (chemical compounds and environmental samples). The AMES Test (ISO 16240) and the well-accepted umuC Test (ISO 13829) were performed as reference genotoxicity tests. The panel of genotoxicity tests included six prokaryotic tests, one eukaryotic test, four mammalian tests, one DNA test, and one bacterial test. Four assays were commercially available. It was demonstrated that no single test is capable of detecting all relevant genotoxins. Therefore, a Test Battery for genotoxicity is recommended (see the chapter by T. Grummt et al., this volume). The concept was to transform the standardized and harmonized microplate assay into an automated on-line assay and/or finally into a biosensor. The EuCyanobacteria Electrode was one of the first biosensors developed under the EC Environment Programme (1991–1994) initiated by Jürgen Büsing. The EuCyanobacteria Electrode was developed and optimized as an on-stream biosensor for pesticide detection (EV5V-CT92-0104). The EuCyanobacteria Electrode, in principle a so-called Rawson sensor, was transformed into a single rod electrode. The EuCyanobacteria Electrode with detection limits for atrazine <10 microgram per liter and diuron <2.5 microgram per liter was implemented in the monitoring programme on the river Rhine after the SANDOZ disaster in 1986 and optimized in several research projects in the following years. Special catch system devices were coupled with secondary electrochemical methods of signal transduction based on enzyme or whole-cell reactions. With the aid of mass spectrometry it was possible to evaluate the bio-recognition and detection components.

The Technical Meetings showed that biosensors are useful tools for controlling the degree of pollution of surface waters and of WWTP (wastewater treatment plants).

All biosensors evaluated showed much higher "alarm" values for the influent waste waters as compared to the final treated WWTP effluent. This is already remarkable, since the main advantage of the different biosensors evaluated, like BOD, ToxAlert, or phenols is that the measurements are being carried out very rapidly—a maximum of 20 minutes—so the information is available immediately. This is certainly an advantage over conventional methods that require several days, either for the BOD-5 or for conventional chromatographic/mass spectrometric methods.

The contributions in this book intend to summarize some of the practical achievements in the field of biosensors for environmental monitoring in Europe focusing, mainly, on examples of chemical groups of analytes (or effects) in different water matrices and more specific aspects of biosensor technology. The target objective of this book is to provide an overview of biosensors as a practical alternative and complementary or additional measurement methodology to traditional chromatographic techniques. Emphasis will also be given to the validation of the applied technology and its application to real-world environmental samples. The various chapters cover examples in different areas that are listed below:

General parameters of water quality. Biochemical Oxygen Demand (BOD) which is usually determined by measuring the oxygen consumption of a mixed culture over 5 days (BOD5). In the Third Technical Meeting held in Barcelona, the commercial firm Biosensores presented an on-line biosensor technique for the determination of BOD. This on-line biosensor device was installed in the la Llagosta wastewater treatment plant and after the Technical meeting, was installed in two more WWTP for a period of one month. This is an example of the implementation of biosensors into real-world situations, since the authorities are already using two of their devices for routine control.

Trace organics in surface water. The RIver ANAlyzer (RIANA), an optical immunosensor coupled to a FIA system based on a competitive solid-phase fluorescence immunoassay with immobilized analyte derivative and free fluorescence-labeled anti-atrazine or anti-simazine antibodies has been developed and applied for the measurement of chlorotriazine pesticides in river water samples. A pilot monitoring survey in the Ebre area (Tarragona, Spain) was carried out over a three-month period. Simazine and atrazine were detected at levels ranging from 0.5 to 1 microgram per liter and in soil samples from 19 to 40 g kg^{-1}. Phenylurea herbicides and paraquat were also determined by this immunosensor approach. The validation of the RIANA biosensor for the determination of phenylurea herbicides was carried out by LC-MS whereas in the case of paraquat capillary electrophoresis(CE)-UV detection was used. Enzymatic biosensors based on catalytic inhibition have been widely used and applied for the analysis of organophosphorus and carbamate pesticides in water samples. A cholinesterase biosensor using acethylthiocholine chloride as substrate was able to distinguish between different batches of water containing 3–10 ppb levels of pesticides.

Toxic and endocrine-disrupting effects. There are different methods of measuring toxicity. Two of the most commonly used methods are Microtox® and the recently developed ToxAlert@ system. Although they are not strictly biosensors, they are a kind of bioprobe used in a standardized way to measure toxicity of the effluents. They are widely accepted and internationally recognized. During the Third Field Experiment held in Barcelona, Spain more data was generated and a variety of cytotoxic compounds were identified in the wastewater treatment plant during this field experiment. The efficiency of the WWTP was evaluated by collecting and analyzing samples at various stages of the water treatment process. The chemical compounds identified could explain the toxicity observed at the different steps of the WWTP. Endocrine-disrupting effects are today widely studied. One of the most current measurements is the so-called vitellogenin (VTG) level in male fish. If VTG levels are high as compared to control values, exposure to endocrine-disrupting chemicals can be concluded. This approach has been used for the development of a biosensor called VITELLO within the EU projects PRENDISENSOR and SANDRINE. Within the EU project SANDRINE different biosensors, bioassays, and receptor assays are being developed for detecting endocrine-disrupting compounds in WWTP. In this respect, MCF-7 and vitellogenin by the ELRA and Vitello sensor of the SANDRINE project are a few of the different assays in use. A practical example was the determination of the presence and the effects of endocrine-disrupting compounds in two tributaries of the Llobregat river (NE Spain). Water samples and carp, *Cyprinus carpio*, were collected from selected sites along a transect, for chemical and biological determination, respectively. Western blot analysis of male carp plasma using a polyclonal antibody raised in the cyprinid Koi carp, detected this protein in all samples, the VTG increase being more evident at the vicinity of the treatment plants. A certain correlation was also found between NP in water and VTG induction in fish ($r = 0.75$). Within the EU project ALGAETOX a bio-analytical system was developed with the immunotoxic relevant endpoint. Phagocytosis was measured by oxidative burst (ROS) and luminescence. There is now a recent application of this system in the COLUMBUS/BIOLAB programme. The bio-analytical system contributes to risk assessment concerning immunotoxicity under space-flight conditions. A schematic work flow demonstrates the close connection between environmental chemistry and the development and validation of a biosensor.

All these promising results demonstrate some of the European efforts to implement biosensor technology and other assays, including toxicological relevant assays and ELISA, in environmental analysis. The developments achieved during the last years by the different EU Environment Programs have permitted us to show some key examples of the application of biosensors for environmental monitoring, demonstrating not only cost-effective alternatives for screening but also effect-related readouts where new and unpredictable approaches are expected. Biosensor technology has reached maturity for BOD, pesticides, and phenols incorporating whole cells, affinity or catalytic events with electrochemical or optical transduction. Complex and difficult analytes (e.g., surfactants) are progressively being approached by biosensors. The large variety of analytes, the complexity of environmental matrices, the low limits of detection, and the legislative compliance should not shadow the progress achieved in this area. On the contrary, biosensor research is offering new strategies for the evaluation of biological effects where cocktails of compounds may show synergistic, additive, or antagonistic effects that will remain unsolved by chromatographic or spectroscopic techniques. The challenge for environmental biosensors remains open and further research in this area will only prove that new parameters are needed for safer and more complete environmental risk assessment. It is expected that collaborative work, focused research, and interdisciplinary approaches may lead to biosensor indexes that will certainly contribute to a better knowledge and real-time characterization of environmental samples. The book is organized to provide information on relevant principles, novel biosensor techniques and their applications. It is hoped that it will stimulate much discussion and bring new ideas to one of the most exciting and environmentally beneficial areas of environmental chemistry and eco-toxicology. It is hoped that the book and its methods contribute to the broader goal of conserving and protecting our aquatic ecosystems and their biota for future generations.

The book will be of interest to a broad audience of analytical chemists, biologists, environmental chemists, water management operators, and technologists working in the field of wastewater treatment, or newcomers who want to learn more about the topic using new measurement devices such as biosensors. Finally, we would like to thank all the contributing authors of this book for their time and effort in preparing this comprehensive compilation of research papers. Special thanks to Dr. Juergen Büsing for "pushing" this topic since 1991 at the EU level thus allowing excellent scientific and technological developments. The book that you have in your hands is part of such achievements.

February 2009

Damiá Barcelò
Peter-Diedrich Hansen

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Biosensors for Environmental Monitoring at Global Scale and the EU Level

Marinella Farré, Sara Rodriguez-Mozaz, Miren López de Alda, Damià Barceló, and Peter-Diedrich Hansen

Abstract Biosensor technology is based on a specific biological recognition element in combination with a transducer for signal processing. Since their inception, biosensors have been expected to play a significant analytical role in environmental monitoring. The use of biological data to complement chemical analysis, as well as the development of biosensors and other biological approaches, has grown steadily in recent years. However, the commercialization of biosensor technology has significantly lagged behind their search output as reflected by a plethora of publications and patenting activities. The rationale behind the slow and limited technology transfer could be attributed to cost considerations and some key technical barriers.

During the last 15 years a relevant support has been provided by public institutions for biosensor research in the USA, Japan and, especially, in Europe. In addition, biomonitoring is an essential tool for rapid and cost-effective environmental monitoring. For this reason, biosensor technology has been considered as a key tool for the complete implementation of the new European Union (EU) directives, such as the Water Framework Directive (WFD), and other related directives such as the recent Marine Strategy Framework Directive.

Analytical chemistry has changed considerably, driven by automation, miniaturization and system integration with high throughput for multiple tasks. Such requirements pose a great challenge in biosensor technology where the biosensor is

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M. Farré, M.L. de Alda, and D. Barceló (✉)
Department of Environmental Chemistry, IDAEA-CSIC,
c/Jordi Girona, 18-26, 08034, Barcelona, Spain
e-mail: dbcqam@iiqab.csic.es

S. Rodriguez-Mozaz and D. Barceló
Catalan Institute of Water Research, ICRA, C/Pic de Peguera, 15, 17003,
Girona, Spain

P.-D. Hansen
Department of Ecotoxicology, Technische Universitaet Berlin,
Franklinstrasse 29, 10587, Berlin, Germany

often designed to detect a single or a few target analytes. Successful biosensors must be versatile to support interchangeable biorecognition elements, and, in addition, miniaturization must be feasible to allow automation for parallel sensing with ease of operation at a competitive cost. A significant up front investment in research and development is a prerequisite in the commercialization of biosensors. The progress in such endeavours is incremented with limited success, thus, the market entry for a new venture is very difficult unless a niche product can be developed with a considerable market volume.

Recent technological developments in the miniaturization of electronics and wireless communication technology have led to the emergence of Environmental Sensor Networks (ESN). These will greatly enhance monitoring of the natural environment and in some cases open up new techniques for taking measurements or allow previously impossible deployments of sensors.

Herein we present the principles, advantages and limitations of biosensor technology for environmental diagnosis, with special emphasis on those based on nanomaterials and technologies for remote biosensing developed under the support provided by public institutions for research in USA, Japan and Europe.

Keywords Biosensor, Environmental monitoring, Network, Sensor, Water Framework Directive

Contents

1 Introduction

Because of the difficulty of predicting the collective effects of the increasing number of pollutants in receiving ecosystems, there is a need for screening methods in environmental monitoring.

The implementation of safety programs calls for environmental analysis comprising two parts: screening methods based on high-throughput analysis and capable of continuous monitoring on-line at site with low costs, and analysis of the positive samples with confirmatory analytical techniques.

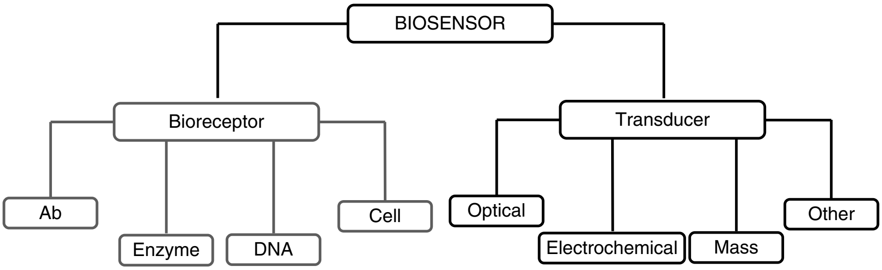

Fig. 1 General scheme of biosensors

Bioresponse-linked instrumental analysis combines two processes, biological recognition initiating a biological effect, and chemical analysis. The biomolecular components are used as targets for active substances. Although it is possible to apply, for example, binding assays that provide effects-linked equivalents, information on the responsible contaminants is only accessible by chemical analysis. Therefore a subsequent step is provided by the chemical analysis of the substances that are bound by the biorecognition components and that are therefore bio-effective.

Biosensors are usually classified either according to the transduction element (e.g. electrochemical, optical, piezoelectrical, or thermal), or according to the biorecognition principle (enzymatic, immunoaffinity recognition, whole-cell sensors, or DNA) (see Fig. 1).

The main advantages of biosensors are short times of analysis, low cost of assays, and possibility of portability, real-time measurements and remote control. These new technologies have been applied equally in the quantitative analysis of target analytes and in ecotoxicological measurements.

At present, biomonitoring is an essential tool for the complete implementation of the EU directives, such as the Water Framework Directive (WFD), and the Marine Strategy Framework directive. Nevertheless, many of these new technologies are still being developed and rely on combined efforts from diverse scientific fields. In Europe, the European Commission (EC) has been the flagship for the development of these new technologies in environmental analysis. Under the 3rd, 4th, 5th and 6th Framework Programs (FP) a great number of funded research projects were dedicated to the development of new analytical tools, and more than 300 projects on sensors, biosensors and biological techniques have been carried out [1].

Several reviews on biosensors, optical sensors for different analytical fields, or biological techniques for environmental analysis have been published during the last years: see Rodriguez-Mozaz [1], González-Martínez [2], Kurosawa [3], Farré [4].

Herein we aim to present the principles, advantages and limitations of biosensor technology for environmental monitoring, and summarize the advances obtained in this field under the support provided by public institutions.

2 Biosensor Research Funding Schemes

Europe, USA and Japan have been trend leaders in biosensor research and manufacture, especially for health, food and environmental analysis.

The European research funding in the area of biosensors for environmental monitoring as a specific topic started in 1992 and the first workshop was held in Berlin in 1993. A selection of projects developed in the past years under different framework programs is listed in Table 1.

During the first EU Framework Programmes, FP4 and FP5, funding was devoted to supporting research projects related to the determination of pesticides in water, in support to the European Union Drinking Water Directive (98/83/EC).

Table 1 Research projects involving biosensors supported by European Union funding

Title, acronym	Contract no.	Duration
Optical biosensing techniques for monitoring organic pollutants in the aquatic environment, BIOPTICAS	EV5V-CT92-0067	1993–1996
Development of new multi-sensing biosensors for the detection of bioavailable heavy metals in solid matrices, BIOMETSENSOR	ENV4-CT95-0141	1996–1999
Integrated immuno extraction sampling and portable biosensor prototype for in field monitoring, INExSPORT	ENV4-CT97-0476	1997–2000
Prediction of the behaviour of potential endocrine disruptors in soils using vitellogenin ELISA as biosensors, PRENDISENSOR	ENV4-CT97-0473	1997–2000
The application of integrated biosensors with antibody and macrocyclic receptor libraries in the measurement of algal cells and toxins in water, ALGAETOX	ENV4-CT98-0784	1999–2002
Development of improved detection systems for monitoring of toxic heavy metals in contaminated groundwaters and soils, DIMDESMOTOM	EVK1-CT-1999-00002	2000–2003
Protection of groundwater resources at industrially contaminated sites, PURE	EVK1-CT-1999-00030	2000–2003
Automated water analyzer computer supported system, AWACSS	EVK1-CT-2000-00045	2001–2004
Artificial recharge demonstration project, ARTDEMO	EVK1-CT-2002-00114	2002–2005
Water catchment areas: tools for management and control of hazardous compounds, WATCH	EVK1-CT-2000-00059	2001–2004
A biological nanoactuator as a molecular switch for biosensing, BIONANO-SWITCH	Specific Targeted Research Project 43288	2006–2009
Tools and technologies for the analysis and synthesis of nanostructures TASNANO	NMP-2003-3.4.1.4-1	2005–2007
DIagnostic NAnotech and MICrotech Sensors, DINAMICS	Integrated Project 26804	2007–2010

A good example of such projects was BIOPTICAS, which was followed up by RIANA, AWACSS and SANDRINE being the most successful projects at the EU level in that area. In the frame of these projects biomolecular receptors were obtained, and different biosensor configurations were developed and applied to real water samples. Under the RIANA and AWACSS projects optical immunosensors were developed and validated against several groups of organic pollutants, for example, for the simultaneous determination of pesticides and estrogens in natural water samples [5]. It is important to highlight BIOSET and SENSPOL concerted actions, which have been organizing various annual workshops and technical meetings on different areas of biosensors for environmental monitoring in order to stimulate research in that topic in Europe and to demonstrate the applicability of biosensors under real-world conditions.

At the same time, in the USA, "biodefence" and research with potential contribution to national security was an issue of particular concern, and biosensors for biohazards detection were developed [6, 7]. In addition, important initiatives were carried out under the auspices of the National Science Foundation (NSF), which funded biosensing network proposals, and "The National Centre For Environmental Research" (NCER), which funds research grants and graduate fellowships in numerous environmental science and engineering disciplines. The Office of Naval Research also promoted the science and technology programs through government laboratories, such as the "Naval Research Laboratory", which is one of the leading groups in biosensors research in the USA. They have developed several single and multi-analyte biosensors for toxins, bacteria and virus detection in clinical and environmental samples. Another remarkable research group, the biosensor group at UC Davis undertakes research into the development of miniaturized, fast and sensitive biosensors for use in environmental research and monitoring.

Regarding biosensor research in Japan, it is important to mention that biocomputing and nanotechnology are areas of increasing interest. One of the main research groups devoted to biosensor development is Karube's group in the "Research Centre for Advanced Science and Technology", at the University of Tokyo. Japan Fund for Global Environment (JFGE) provides grants for environmental conservation activities. For example, support is provided for activities, such as conserving air and water quality, and organizing symposiums, seminars and workshops. For example, Biosensors 2002, the Seventh World Congress on Biosensors, was held in Kyoto, Japan.

More recently, in Europe under FP6 less funding was directly devoted to biosensors for environmental monitoring; however, other important fields related to the development of environmental biosensing were boosted, such as nanotechnology. Some examples of research funded projects in this period are BIONANO-SWITCH, BIOMED-nano, OPTANANOGEN, TASNANO, DINAMICS and ISAMCO, most of them related to nanotechnology and material science advancements. For example, BIONANO-SWITCH is still on-going, and the main aim is the development of a nanoactuator/biosensor system.

The BIOMED-nano STREP-project aimed at improving enzymatic electron transfer reactions for application towards integrated bio-powered biosensing systems for diagnosis and healthcare. Such improvements were based on novel enzymes; modification of enzymes; design of novel nano-structured scaffolds for enzyme immobilization, in order to obtain biosensing devices with improved stability and electron transfer efficiency. Another example was OPTONANOGEN: Integrated opto-nanomechanical biosensor for functional genomic analysis. The aim of this project was to develop a portable genotoxicity biosensor microsystem able to detect hybridization of nucleic acids with sensitivity to distinguish single base substitutions, deletions and insertions.

In addition to initiatives for biosensing development, an important restriction is the successful marketing of biosensors for environmental applications. In order to overcome implementation barriers and bridge the gap between innovative and accepted techniques for environmental monitoring in Europe an Environmental Technology Verification (ETV) system has been developed following the model of the US and Canada. Therefore, Europe is now heading towards an Environmental Technology Verification System. Under the 6th FP several projects have been devoted to the development of this verification system including PROMOTE-ETV, Eurodemo, Testnet, AirETV and Tritech-ETV. A proposal for an EU framework on ETV is planned to be published by the European Commission in autumn 2008.

Research based on nanotechnology has been extremely important worldwide, and has been a new motor for biosensors development. In addition to the use of nanomaterials to improve analytical properties of new biosensors, the development of nanobiosensing devices is one of the main promising perspectives for future developments.

In this sense, during the last years in the US the National Science Foundation (NSF), through the Directorate for Engineering and the Directorate for Computer and Information Science and Engineering, carried out a broad interdisciplinary program of research and education in the area of advanced sensor and biosensors development. An example of successful achievements has been the creation of the world's first all-integrated sensor circuit based on nanowire arrays, combining light sensors and electronics made of different crystalline materials, by Scientists at the U.S. Department of Energy's Lawrence Berkeley National Laboratory and the University of California at Berkeley [8, 9]. Several projects are under development such as MEMS-Based Magnetic Probe Microscopy Project, in which a scanning-probe magnetic microscope based on a high-resolution magnetic tunnel junction (MTJ) sensor is under construction. The completed microscope will be used to characterize the magnetic properties of ferromagnetic metal samples.

In the same way the Japan Science and Technological Agency has been promoting a number of initiatives focused on nanobiosensors.

In Europe the 7th FP presents a thematic area on Nanosciences, nanotechnologies, materials and new production technologies. Also under the 7th FP, the first call has included funding for projects to develop sensors and biosensors platforms for remote environmental monitoring. The last tendency in Europe is research in new autonomous platforms able to operate on-line in an autonomous manner.