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EDITORIAL

Toward inline multiplex biodetection of metals, bacteria, and toxins in water networks: the COMBITOX project

Mireille Ansaldi[1] · **Ingrid Bazin**[2] · **Pierre Cholat**[3] · **Agnès Rodrigue**[4] · **David Pignol**[5,6,7]

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This special issue of *Environmental Science and Pollution Research* highlights selected papers whose results have been obtained in the course of the COMBITOX project. COMBITIOX is an interdisciplinary research project funded by the French National Research Agency (ANR) aiming at conceiving an inline multiparametric device for the surveillance of water networks using biosensors. This device is not intended to fully replace chemical methods, but when compared to analytical chromatographic methodologies, biological sensors can offer rapid and on-site monitoring of even trace levels of targeted compounds (Sun et al. 2015) and can quickly raise the alarm in the event of an accidental or intentional pollution. Numerous developments have been published to improve the sensitivity, specificity, and time response of various biosensors in laboratory conditions (Xiong et al. 2012) (der Meer et al. 2010), but their actual transfer into technological devices for the surveillance of water networks remains at a conceptual level. Thus, the challenge here is to go a step beyond and validate biosensors under real-life field conditions by incorporating them in a single inline detector. During the course of COMBITOX, we could define the interface between the biosensors and a common light detector as well as the physical conditioning of the bioreagents and usage protocol. Our resulting prototype allow the detection of bioavailable toxic compounds as well as microorganisms, impacting human health through the drinking water network or interfering with the biological process of modern wastewater treatment plants. We also plan to propose this system to meet the emerging threats such as bioterrorism.

COMBITOX focuses on three families of "objects" to detect: metals (cadmium, mercury, arsenic, nickel, etc.), environmental and/or food toxins, and pathogenic microorganisms. Whole-cell biosensors based on reporter gene under the control of an inducible promoter are used to detect various metals (Hynninen and Virta 2010), the antibody/antigen interaction for toxins (Makaraviciute and Ramanaviciene 2013), and the specific infection of bacteria by bacteriophages for pathogenic microorganisms (Smartt et al. 2012) (Vinay et al. 2015). In all cases, the signal measured is photochemical (fluorescence, bioluminescence, or chemo-luminescence): such a method to transduce the biological recognition is very sensitive and a single photodetector can be used for all biosensors included in the device. The challenge here rather lies in the design and the optimization of the different biological compounds for their use in the field while maintaining a high sensibility and robustness. As a consequence, the different articles presented in this special issue focus on original strategies for the optimization and the adaptation of the three types of biosensors for their use in a semi-autonomous inline water analyzer. In the case of whole-cell biosensors, improvement of the dose-responses

Responsible editor: Philippe Garrigues

✉ David Pignol
david.pignol@cea.fr

[1] Laboratoire de Chimie Bactérienne, UMR7283, Centre National de la Recherche Scientifique, Aix-Marseille Université, Marseille, France

[2] École des Mines d'Alès, Laboratoire de Génie de L'Environnement industriel, 6 Avenue de Clavières, F-30319 Alès CEDEX, France

[3] AP2E, 240, rue Louis de Broglie, Les Méridiens Bâtiment A, CS90537, F-13593 Aix-en-Provence, France

[4] Université de Lyon, Lyon, INSA de Lyon, CNRS, UMR5240, Microbiologie, Adaptation et Pathogénie, Villeurbanne F-69622, France

[5] CEA, DSV, IBEB, Lab Bioenerget Cellulaire, Saint-Paul-lez-Durance F-13108, France

[6] CNRS, UMR Biol Veget & Microbiol Environ, Saint-Paul-lez-Durance F-13108, France

[7] Aix-Marseille Université, Saint-Paul-lez-Durance F-13108, France

and the specificity by genetic modifications of the regulators is exemplified for a nickel bioluminescent biosensor (Cayron et al. in this special issue). The key-issue of the biosensor conservation over a period of time compatible with the autonomy of the device requested by the end-user while maintaining a satisfactory sensitivity, specificity, and time response is also addressed (Prévéral et al. in this special issue). Moreover, Brutesco et al. characterized an original near-infrared fluorescent reporter candidate that represents an interesting alternative to bacterial luciferase to perform biodetection in turbid and complex water samples. The use of a robust environmental bacterial species (*Deinococcus deserti*) as cellular chassis is also shown to correspond to an interesting alternative to laboratory *Escherichia coli* stains for optimal biodetection out of the laboratory benches while offering desiccation as a cost-effective solution to the problem of biosensors long-term storage. Last but not least, transferring biosensors into *Deinococcus* species open the field of metallic radioisotopes (Brutesco et al. in this special issue). In addition, the strategy used and validated for the implementation of immunodetectors in our automatous inline analyzer is based on the development of chitosan support for oriented immobilization of functionally intact polyclonal antibodies (Demey et al. in this special issue). Phage-based biodetectors using a luminescent and substrate independent output to detect different *E. coli* strains have also been developed and tailored to our analyzer (Franche et al. in this special issue). Taken together, these different studies contribute to reach our major objective of using various types of biosensor in an autonomous inline prototype, whose elaboration of the light detector/incubation chamber as well as the procedures to bring the three types of biosensors into play are also described in this special issue (Descamps et al.). The COMBITOX project provides the proof of concept that biosensors can be used in the field to detect different targets (As, Hg, Ni as metals, microcystin as a toxin model, and *E. coli* for bacteria detection). This list could easily be extended in the future to respond to requests of putative clients and meet the societal challenges of environmental survey.

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Dr. Mireille Ansaldi is a CNRS research director at the Laboratoire de Chimie Bactérienne (UMR7283), Centre National de la Recherche Scientifique & Aix-Marseille Université, in Marseille. She is a former member of the CNRS Institute of Biological Sciences (INSB) scientific council (2008–2013), and the scientific organizer of the Molecular Microbiology School (2010, 2014). She obtained a Ph.D. in Microbiology in Marseille in 2000 on the regulation of the *tor* operon encoding an alternative respiratory pathway in *Escherichia coli*. She then moved to the Public Health Research Institute (UMDNJ, Newark, NJ) were she studied the regulation of natural competence in *Bacillus subtilis*. She was hired as a CNRS researcher by the end of 2002 and started her on group entitled "Phage cycle and bacterial metabolism" in 2007. She has renowned expertise in bacterial and prokaryotic viruses genetics. Her group has worked on the mechanistic aspects of site-specific recombination and on the involvement of host factors in this process. Recently, her group unraveled a particular regulation of excessive recombination, involving phage and host-encoded regulatory factors. Her research now focuses on the interplay between host and phages regulatory networks. The primary research interests of her group relate to the evolution of microbial genomes through the acquisition of prophage genes, with a particular interest in dissecting the interconnections between prophages and bacterial genomes from an evolutionary as well as from a mechanistic point of view. Another aspect of her work is the development of bacteriophages as biotechnology tools. Her group recently developed phage-based biosensors to monitor the bacterial load in environmental waters. She is also interested in applications of bacteriophages dealing with therapy, food, and environment preservation.

Dr. Ingrid Bazin is an Associate Professor at Ecole des Mine d'Ales (Ales, France). She began her career in Paris in the biomedical R&D field where she works on the characterization of new drugs designed against cancer. She then moved to the Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA) in Cadarache where she obtained her doctorate in molecular biology and plant physiology in 2002. After a post-doctorate at the Institute of Science and Technology of the drug in Toulouse (ISTMT), she participated in the creation of Smartox (Grenoble, France), a private company specialized in the synthesis of therapeutic peptides. Her group now focuses on the development of biosensors for diagnostics and for detection of environmental pollutants and toxicity, with the characterization of original low-cost biopolymers used for antibodies immobilization and the design of new recognition elements like peptides adapted to ELISA systems.

Dr. Agnès Rodrigue is an Associate Professor at the National Institute of Applied Sciences in Lyon (INSA Lyon). She studied biochemistry at University Lyon 1. Dr Rodrigue obtained her PhD in 1997 at INSA Lyon. Her Ph.D. with Long-Fei Wu sought to understand the biosynthesis of bacterial hydrogenase in *E. coli*. She then moved to the Laboratoire de Chimie Bactérienne at Centre National de la Recherche Scientifique (CNRS) in Marseille to study the translocation of hydrogenase sub-units across the cytoplasmic membrane by a Tat-dependent, hitch-hiking process. Still at CNRS in Marseille, she joined the group of Andrée Lazdunski to study two-component systems in *Pseudomonas aeruginosa*. In 1999, she was appointed as Assistant Professor at INSA Lyon. Her research is focusing on the interactions between bacteria and metals at the mechanistic and adaptive levels. She also developed tools for the bioremediation and biosensing of metals.

Pierre Cholat is an AP2E chief executive officer. This private company (29 persons) is located close to Aix-en-Provence in France. AP2E creates, builds up, and distributes innovative measurement systems for process and environmental issues, and collaborates with both industrial and public research organizations. Graduated from ESIM (Ecole Supérieure des Ingénieur de Marseille), Pierre Cholat entered the industrial word in R&D departments and rose up rapidly to manager positions. In 2006, he decided to create AP2E with collaborators, with the same R&D spirit but with new kind of technologies aiming at proposing original measurement systems. After only 4 years of effort, AP2E starts gathering innovative prices (as CREA13, from the French publication "Mesure," and from the American R&D100). Since 2014, he undertakes the CEO responsibility, but keeps a close contact to the "field" which he considers as a major part of his engagement.

Dr. David Pignol is a research director at the health science division (DSV) of the Commissariat à l'Energie Atomique et aux Energy alternatives (CEA). He is the head of the Bioenergetics Laboratory (LBC), a molecular microbiology laboratory located in Cadarache (Institute of Environmental Biology and Biotechnology). His group has internationally renowned expertise in the characterization of mechanisms responsible for the adaptation of different bacterial species (photosynthetic, radiotolerant, and magnetotactic bacteria) to their environments (web site of the LBC: http://ibeb.cea.fr/dsv/ibeb/english/Pages/laboratories/lbc.aspx). Since his Ph.D. defense in 1994 (Institute of Structural Biology, Grenoble), he published over 75 peer-reviewed papers in molecular microbiology, focusing on the mechanisms that govern bacterial resistance, acquisition, sequestration, and mineralisation of physiological and toxic heavy metals. Using multidisciplinary approaches, he tackled more recently with his group the field of metal biomineralization and was able to identify and characterize key proteins from magnetotactic bacteria, unique microorganisms able to produce intracellular magnetite nanomagnets, becoming one pioneering lab in the field. The fundamental studies are always developed in his laboratory keeping in mind the potential biotechnological applications (three active patents in the field of heavy metal depollution). In the last 4 years, he was the coordinator of the COMBITOX project, an interdisciplinary research project funded by the French National Research Agency (ANR) aiming at using biosensors for the surveillance in the field of water networks.