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Miniaturized and integrated whole cell living bacterial sensors in field applicable autonomous devices

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Live-cell based bioreporters are increasingly being deployed in microstructures, which facilitates their handling and permits the development of instruments that could perform autonomous environmental monitoring. Here we review recent developments of on-chip integration of live-cell bioreporters, the coupling of their reporter signal to the devices, their longer term preservation and multi-analyte capacity. We show examples of instruments that have attempted to fully integrate bioreporters as their sensing elements.

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Introduction

Increasing industrialization in human societies leads to more and more chemical compounds being released into and finishing in the environment. As many of those compounds are toxic at low concentrations, their increased presence in all environmental compartments increases the likelihood of harm caused to human health, plant and animal life. Monitoring of chemicals in the environment is thus critical in order to identify regions of elevated concentrations and to unveil concealed pollution sources. Monitoring results can subsequently be used as basis for appropriate pollution mitigation, treatment or removal strategies [1,2]. The most common method for environmental monitoring is to have fixed stations sampling at regular intervals or to conduct periodical field sampling campaigns. Samples of different matrices (aqueous, soil, air or other) are then transported to the laboratory to analyze their chemical content with advanced methods and instruments (e.g., solid-phase micro-extraction, gas chromatography-coupled to mass spectrometry). These methods give excellent results, but are expensive and time-consuming, and their sampling strategies do not easily capture temporal and spatial dynamics of the contamination. Increasingly, therefore, fixed or manual sampling is replaced by mobile samplers (e.g., gliders, drones, buses or ships) equipped with miniaturized sensors focusing on a single or small, defined group of target chemicals, in order to, ultimately, allow real-time assessment of recurring pollutants [3,4,5•].

The development of sensors that are sufficiently small, target-specific and precise, and which can fit miniaturized mobile samplers, is an active field of research that requires integration and collaboration between various disciplines (e.g., see other contributions in this issue). One of the possible types of sensors that may be integrated in mobile and autonomous samplers are those based on living cells or live microorganisms [6]. The major incentive for deploying live cells in sensors is that they reproduce to some extent the biological effects expected to occur in living organisms exposed to or in the (contaminated) environment [7,8]. Whereas biological effects can be manifold and difficult to discern, in particular the design of synthetic implanted genetic circuits inside live cells (microorganisms or other) has enabled to target specific compounds, compound groups, as well as general distress pathways, with the concomitant output of an easily detectable signal [9,10,11,12]. There is more than two decades experience with bioassays based on live microorganisms or *bioreporters* (as they are more commonly referred to), which therefore appear to be an interesting option for miniaturized sensor development. Bioreporters based on bacteria or yeast are, in principle, extremely easy to reproduce and cheap compared to conventional analytical machines. Because of their small size (1–20 μm) and low population numbers needed for signal detection (10^4–10^6 cells), the integration of bioreporters into field applicable miniaturized and autonomous devices is a realistic option. Nonetheless, important technical and biological challenges remain before such mobile and autonomous live-biosensor devices can be routinely deployed.

In this review, we describe recent attempts to integrate bioreporters into miniaturized devices while focusing on the specific obstacles challenging their field deployment. We will first discuss the different signal outputs of bioreporters and their connection to small integrated detectors. Then we will briefly explore recent studies demonstrating survival, long-term maintenance and

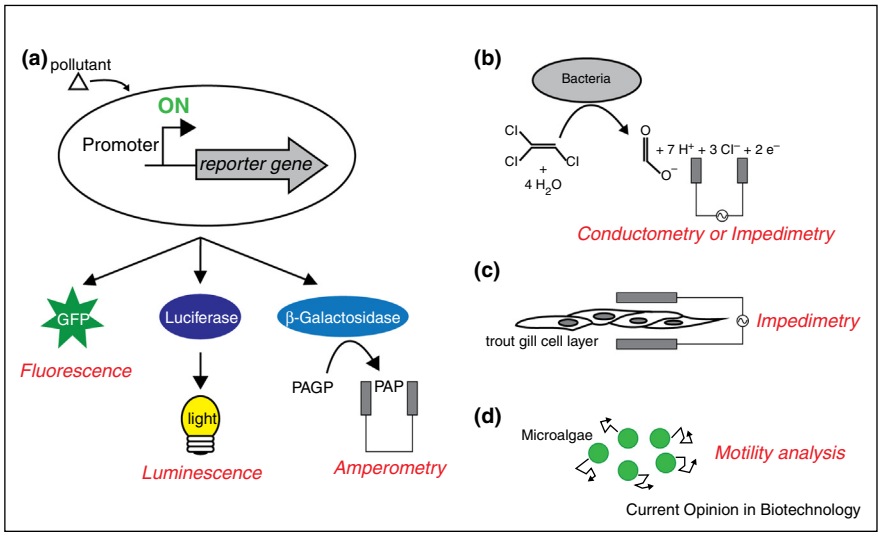
activity of bioreporter cells, which may be used to obtain longer biosensor instrument shelf-lives. Finally, we will present some examples of integrated bioreporter instruments and critically review potential field deployment of automated biosensors.

Generating the signal: transmission between live bioreporters and detectors

The principle of a bioreporter is to produce an easy detectable signal that changes in presence of or upon exposure to the target compound(s). Bioreporter cells are frequently equipped with purposely designed genetic circuits allowing the production of a cellular sensor (protein or aptamer) that can influence expression from the reporter gene [9,13]. To achieve this, the reporter gene itself is fused directly or via some relay to a specific promoter that is controlled by the cellular sensor (for a very simplified conceptual idea, see Figure 1a). Details of synthetic circuitry design have been reviewed elsewhere and will not be repeated here [14,15]. The outcome of the reaction of an exposed bioreporter cell is a proportional production of the reporter protein as a function of the target compound availability (concentration or flux) in the sample (Figure 2a) [16,17].

Reporter proteins come in a wide variety of flavors and their activity or spectroscopic properties (e.g., color, fluorescence, bioluminescence) is routinely detected with macro-size instruments. Miniaturization of bioreporter assays into devices, however, leads to lower overall signal intensity from the cells, and requires both improved signal transmission to the detector and detector sensitivity. The type of coupling depends to a large extent on the type of reporter protein or biological signal [6,18]. Frequently, fluorescence is used as output for bioreporters, being produced from genes for autofluorescent proteins that are non-native to the cell. This results in their specific fluorescence being detected at high sensitivity without too much interference from other fluorescing molecules in the cell. Specific genetic design, however, may be needed to reduce background reporter gene expression in the circuit in absence of the target, in order to obtain the highest signal-to-noise ratio in the assay (see, for example, Refs. [19,20]). In most bioreporter designs, exposure to the target compound induces an increase in the expression of the fluorescent protein. The increase is to some extent dose-dependent and allows the establishment of a calibration curve, from which the target's presence in the environmental sample can be deduced (Figure 2a). Importantly, because of the inherent flexibility and cross-reactivity of the cellular sensors on which bioreporters are based, the cells inevitably react to compound classes rather than a single individual chemical. Consequently, bioreporter reactions to unknown samples can only be interpreted from calibration curves as concentrations *equivalent* to the effect provoked by known target chemicals, when incubated with the bioreporter cells under the same conditions and assay duration. For illustration, the well-known bacterial bioreporters to mercury or arsenic are relatively compound-specific, but respond slightly differently depending on Hg or As chemical speciation [21,22]. An extreme

Figure 1



Concept of live-cell based assays and their output. **(a)** Bioreporter cells are genetically engineered to carry a synthetic DNA construct permitting to sense a polluting chemical (or condition) and turn on expression of a reporter gene. The output of the reporter protein can be measured by, for example, fluorescence, luminescence or by electrochemistry. **(b)** Electron release from native enzymes, such as toluene dioxygenase, can be measured by conductivity or impedance, and can be used to measure the presence of trichloroethylene [35,36]. **(c)** Release of tight cell-cell junctions as a measure for toxicity response can be detected by impedance [37,38••]. **(d)** Changes in random cell motility can be used for detecting the presence of heavy metals [63].