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# WHOLE CELL BIOSENSORS *vs* CELL-FREE BIOSENSORS FOR BIOANALYTICAL APPLICATIONS: A SIDE BY SIDE COMPARISON

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Whole-cell biosensors have been widely used for several applications as they provide useful information about the bioavailability, general toxicity, and bioactivity of a target analyte or a sample. During the last decade, thanks to the implementation of smartphones and other user-friendly light detectors, they have been also integrated into compact low-cost analytical devices. However, the scarce robustness of living cells still represents an issue and several immobilization methods have been developed for improving cell's shelf-life and obtain ready-to-use biosensors. More recently, cell-free transcription-translation (TX-TL) systems have been also proposed as a valid alternative. Conversely to whole-cell biosensors, TX-TL systems do not rely on living cells but rather include the biological machinery and energy source to express a reporter protein as consequence of target activation. Whole-cell and cell-free biosensors have become preferential alternatives to conventional analytical methods for rapid detection of analytes of environmental interest as they are cost effective and easy to implement into portable devices. The choice of reporter genes in biosensors, is also a key factor especially for on-site monitoring. A reporter gene is a gene which can be easily and quantitatively distinguished over a background of endogenous proteins. Several reporter genes have been widely employed to monitor cellular events associated to signal transduction, including the application in biosensors.
Here we compared three optical reporter categories, e.g., fluorescent, colorimetric and bioluminescent reporters in both whole cell biosensors and cell-free transcriptional and translational system for heavy metal and bacterial contamination in water. Particularly, green fluorescent reporters (GFP and deGFP), red fluorescent reporters (mCherry and mScarlet-I), colorimetric reporter (LacZ) and bioluminescent reporters (NanoLuc luciferase and lux operons from Aliivibrio fischeri and Photorhabdus luminescens) have been analysed. A comprehensive profile of the analytical performance, in terms of limit of detection (LOD), sensitivity, input/output dynamic ranges and response time, obtained with diverse optical reporters is reported (Fig.1).

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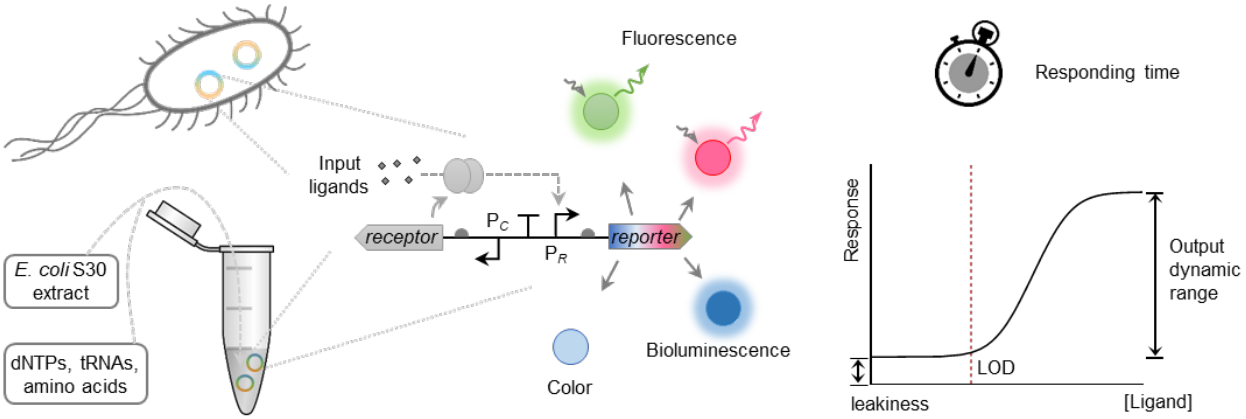


Figure 1. Schematic representation of the comparison of different reporter genes in whole cell and cell-free system

According to our results, enzymatic reporters are the best candidates. Especially NanoLuc luciferase showed the lowest LOD (50.0 fM of $HgCl_2$) within the shortest response time (30 min), proving its eligibility as reporter gene for rapid and sensitive on field monitoring.
Despite this, the selection of reporters needs to be a balanced compromise with other important factors, such as the background signals (higher in fluorescent gene), cost of the assays and need for substrate addition, especially for point of care and point of need applications.