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GET INSPIRED BY NATURE WITH BIOLUMINESCENT BIOSENSORS: "*PRENDERE LUCCIOLE PER LANTERNE*"

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Irrespective of the different molecular mechanisms, all chemical reactions catalysed by bioluminescent enzymes require molecular oxygen. An intriguing hypothesis suggests that bioluminescence arose in the early evolution of life because of its ability to remove oxygen, which was toxic to life when it first appeared on earth. Then, when oxygen became abundant, other more efficient antioxidant mechanisms evolved for oxygen removal, and most luminous species became extinct. Today, still many different bioluminescent species exist in nature, such as fireflies, bacteria, mushrooms, invertebrates, as well as fish.

Thanks to advancements in synthetic biology, organic chemistry and computational models, bioluminescence is widely exploited in several fields, ranging from the detection of microbial contamination to in vivo imaging to track cancer and stem cells, from cell-based assays for drug discovery to optogenetics to understand circuitry in the brain.

Here we report the generation of a portfolio of biosensors based on living cells and cell-free systems. We developed bioluminescence smartphone-based biosensing platforms exploiting highly sensitive luciferases as reporters in bacteria, yeast, and human cells lines. These biosensors, relying on reporter gene technology and split complementation strategies were integrated into 3D-printed cartridges. Smartphone-based cell biosensor were developed and applied to the detection of compounds with estrogenic, androgenic, and pro/anti-inflammatory activities. The comparison of analytical performance of whole-cell biosensors with the corresponding cell-free transcription/translation systems, which include the biological machinery and energy source to express a reporter protein as consequence of target activation, is also discussed.

Cell-free systems relying on "nano-lanterns" were developed to provide a ready-to-use and stable ATP sensing paper that can be easily integrated in miniaturized devices with smartphone detection. The feasibility of origami paper-based enzyme biosensors for detection of neurotoxic compounds is also reported exploiting both bioluminescence and its sibling, chemiluminescence, as detection principle.

Proof-of-concept applications of these biosensors are presented together with main limitations, such as those related to sensitivity and robustness, and current unsolved challenges to turn them into marketable biosensors.