Green and red light for cellular stress: Harnessing a single firefly luciferase for ratiometric biosensing of intracellular pH or heavy metals, and dual reporting

Vadim Viviani*[1,2] and Gabriele Gabriel[2]

[1]Graduate School of Biotechnology and Environmental Monitoring (UFSCar), Sorocaba, SP, Brazil (UFSCar) – Brazil

[2]Graduate School of Evolutive Genetics and Molecular Biology (UFSCar), São Carlos, SP, Brazil (UFSCar) – Brazil

Abstract

Firefly luciferases have been extensively used for bioanalytical purposes during the last 5 decades. Although they catalyze the production of yellow-green light under normal physiological conditions, they produce red light under acidic conditions, high temperature and presence of heavy metals, a property that has been called pH-sensitivity or pH-dependency. Because acidic pH, heavy metals and temperature fluctuations reflect cellular stress, we thought to harness firefly luciferase spectral sensitivity to ratiometrically estimate these factors inside the cells. Using the Brazilian *Macrolampis* sp2 firefly luciferase, we showed, for the first time, that pH-sensitivity could be harnessed to ratiometrically estimate intracellular pH in bacteria (Gabriel & Viviani 2014) and more recently in mammalian cells. We also showed that firefly luciferases can be potentially harnessed to estimate concentration of heavy metals such as Zn2+, Ni2+, Hg2+. We have engineered the metal binding site, increasing the spectral sensitivity to some heavy metals such as Hg2+ and Cd2+, allowing to create novel metal biosensing luciferases which could be potentially useful for estimating intoxication and bioavailability of these heavy metals. Whereas the ratiometric analysis can be successfully used to estimate intracellular pH, the lower specificity and sensitivity still limits such application in the case of heavy metals detection. Nevertheless, this methodology allows, for the first time, to use a single luciferase gene for dual reporting: (1) the bioluminescence intensity to report luciferase expression or ATP concentration, and (2) the spectral ratiometric analysis to report pH and metal fluctuations. This technology may bring a new dimension in real-time imaging of cellular stress processes such as apoptosis, inflammation and necrosis, and of heavy metal intoxication and bioavailability. (FAPESP 2010/05426-8; CNPq 401867/2016-1)

Keywords: firefly luciferase, bioluminescence, pH, sensitivity, biosensors, dual reporter, heavy metal

*Speaker