Vadim R Viviani, J Biotechnol Biomater 2016, 6:5(Suppl)
http://dx.doi.org/10.4172/2155-952X.C1.058

Brazilian beetle luciferases: Developing a bright future for cell toxicity assays, bioimaging and environmental analysis

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Firefly luciferases catalyze the ATP-dependent oxidation of D-luciferin, leading to the production of bioluminescence in the yellow-green region of the spectrum with high quantum yield (41-61%). Thus, they have been extensively used for decades as bioanalytical reagents to measure ATP content, biomass estimation and then as bioluminescent reporter genes to investigate cellular events and bioimaging and biosensors. However, until the nineties, only a few firefly luciferases which produced yellow-green light and were pH-sensitive were used for such bioanalytical purposes. In the past 15 years, we have cloned and characterized 10 new luciferases from Brazilian bioluminescent beetles, which elicit production of different bioluminescence colors, kinetics and pH-sensitivities. Among them *Phrixotrix hirtus* railroad worm luciferase is the only naturally red emitting luciferase (623 nm), *Pyrearinus termitilluminans* larval click beetle luciferase is the most blue-shifted (534 nm) and most efficient one (61%), and *Macrolampis* sp2 firefly luciferase displays a pH-sensitive bimodal spectrum (569/610 nm). With these enzymes, we have investigated the structural determinants of bioluminescence colors, pH-sensitivity and luminescent activity. Based on the acquired knowledge, we have engineered new luciferases with different bioluminescence colors from green to red (534, 550, 564, 575, 590, 605, 615, 628 nm), kinetics and pH-sensitivities, suited for specific biotechnological and environmental purposes. The red emitting luciferase of *Phrixotrix* and *Pyrearinus termitilluminans* green-emitting luciferase are currently used as multicolor reporter gene for mammalian cells assays and cell bioimaging. The luciferases of *Macrolampis* and *Pyrearinus termittilluminans* were shown to be suitable for general toxicity light off whole cell biosensors. Very recently, based on the spectral sensitivity of firefly luciferases, we have developed the first ratiometric intracellular pH and heavy metal-biosensors, being the first dual reporter system using a single luciferase gene to simultaneously monitor intracellular ATP or gene expression based on luminescent intensity (I) and intracellular pH or heavy metals based on the ratio of intensities at different wavelengths (I_{550}/I_{614} nm). Finally, we have developed for the first time a totally new orange emitting luciferase departing from a non-luminescent CoA-ligase, which has potential applicability as carboxylic xenobiotics biosensor for environmental and drug toxicity assays. These luciferases and their modified genes generated patents and products, expanding the range of bioluminescence applications in cell assays and environmental analysis.

Biography

Vadim R Viviani has been completed his degree in Biological Sciences from the Catholic University of Campinas (1990), doctorate in biochemistry from the Institute of Chemistry, University of São Paulo (1996), postdocs in Shizuoka- Japan University (1997-1999) and Harvard University (1999-2002) and professor (2014) by the Institute of Chemistry, University of São Paulo. He is an Associate Professor of Biochemistry at the Federal University of São Carlos, leads the research group "Bioluminescence and Biophotonics" (CNPq), and guest researcher at the Nat. Inst. of Advanced Industrial Science and Technology (Tsukuba, Japan) and Univ. Vanderbilt (Nashville, TN, USA), and president of the International Society for Bioluminescence and Chemiluminescence . Investigates bioluminescence enzymes luciferases and biotechnological and environmental use.

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