Bioluminescence microscopy: New chemiluminescent probes for imaging physiology at single cell resolution

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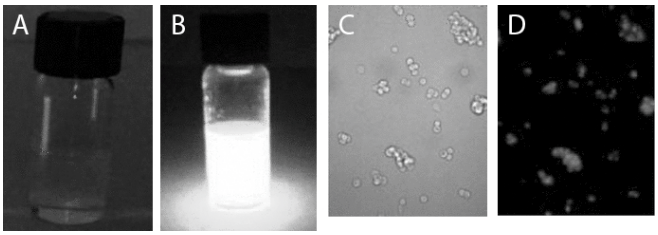
Bioluminescence and chemiluminescent imaging results are highly specific and quantifiable: In distinction to fluorescence, where autofluorescence and light scattering contribute to signal intensities, chemiluminescence is in direct correlation to molecular events.
Here we describe the development of a new class of small-molecule probes that can produce chemiluminescence signal of high enough intensity to allow microscopical imaging at single cell resolution using an Olympus LV200 bioluminescence microscope.
The new turn-ON chemiluminescence dioxetane probes can be used under physiological (aqueous) conditions. They are based on incorporation of a substituent on the benzoate species obtained during the chemiexcitation pathway of Schaap's adamantylidene-dioxetane probe (1). Striking improvement of the chemiluminescence efficiency was obtained when the electron-withdrawing group methyl-acrylate was installed leading to high-quality chemiluminescent images of β-galactosidase in a stable transfected cell line (2). Finally, we developed a probe for cathepsin B. This was of additional interest as this permiited imaging of a natively expressed endogenous enzyme in cancerous leukemia cells (3,4).
We anticipate that the strategy presented here will lead to development of efficient chemiluminescence probes allowing to observe and measure cell physiological events in living cells and organisms.



Chemiluminescent probes (B) are 3000 times brighter than luminol (A), allowing for the first time non-enzymatic chemiluminescent microcopy (D, bright field image C). Reproduced from ref 2. Copyright 2017 American Chemical Society

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