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CHEMICAL & ENGINEERING NEWS

Volume 95 Issue 12
Issue Date: March 20, 2017 | Web Date: March 15, 2017

Light-generating reporter molecules eas cell monitoring

Chemiluminescent reagents help track activity in cells

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A reaction caused by an enzyme or reactive compound removes the protecting group (PG) from a Schaap's reagent bearing an electron-withdrawing group (EWG), producing an unstable intermediate that chemiluminesces brightly in aqueous solution and in cells.

Credit: *ACS Central Sci*

To detect the goings-on inside cells without the need for an external light source, scientists genetically engineer cells to produce chemiluminescent reporter molecules. A new class of molecules, however, can penetrate cells and monitor their biological processes by chemiluminescence, avoiding genetic modification.

Chemiluminescence, the process that lights up glow sticks, occurs when a chemical reacti generates light. In the lab, researchers use it to monitor reactive oxygen species, diagnose pathogenic infections, and detect the results of chromatography, electrophoresis, immunoassays, nucleic acid assays, and blotting experiments.

The new reagents are modified versions of a set of widely used chemiluminescent Schaap' adamantylidene-dioxetanes, each of which has a characteristic protecting group that react when a specific enzyme or reactive compound is present. For example, the protecting grou may be substrates for a particular enzyme. When that enzyme is present, it cleaves the protecting group from the Schaap's reagent, yielding an unstable phenolate-dioxetane that chemiluminesces.

Schaap's reagents work well in organic solvents but emit light only weakly in water. Three-component systems—each a mixture of a Schaap's reagent, a surfactant, and an excitable fluorescent dye—shine about 100 times as brightly in water as Schaap's reagents by thems but the mixtures aren't used in cells because they are toxic. Scientists can also use the fire substrate-enzyme pair luciferin and luciferase to monitor gene expression and other proces inside cells, but they must first engineer the cells to produce luciferase.

Doron Shabat of Tel Aviv University and coworkers at the University of Geneva have now brightened up Schaap's reagents in a way that permits their use in cells (*ACS Cent. Sci.* 20 DOI: **10.1021/acscentsci.7b00058 <http://cgi.cen.acs.org/cgi-bin/cen/trustedprox redirect=http://pubs.acs.org/doi/abs/10.1021/acscentsci.7b00058?source=cen>**

The team adds electron-withdrawing substituents to conjugated positions on the reagents' phenolate group, creating long π-electron systems that emit more light in water-based me One modified reagent, with added acrylonitrile and chlorine groups, emits in aqueous solut 1,000 times as much light as a conventional Schaap's reagent and 10 times as much as a component system. It is nearly as bright as luciferin-luciferase, it can simply diffuse into ce it doesn't require genetic engineering.

"This is a game changer for chemiluminescent diagnostics,"says Phil S. Baran of Scripps Research Institute California, who has collaborated with Shabat.

By adding different protecting groups as triggering substrates for various enzymes or react compounds, Shabat and coworkers used the new reagents to image β-galactosidase activi single cells and to detect alkaline phosphatase, glutathione, and hydrogen peroxide in aqu solution.

"This simple and elegant molecular design provides a dramatic enhancement," comments

Alexander R. Lippert of Southern Methodist University. “No excitation light source is neede eliminating several problems associated with fluorescence-based cell analysis, including si fading, toxicity, and background interference, he says.

Shabat and coworkers have applied for a patent on the new reagents. The researchers hop extend the molecules’ light emission range from the visible to the near infrared to improve ability to penetrate tissue deeply for possible in vivo use.

This article has been **translated into Spanish <http://cenm.ag/quimiluminece>** by **Divulgame.org <http://divulgame.org/>** and can be found **here. <http://cenm.ag/quimiluminece>**

Chemical & Engineering News
ISSN 0009-2347