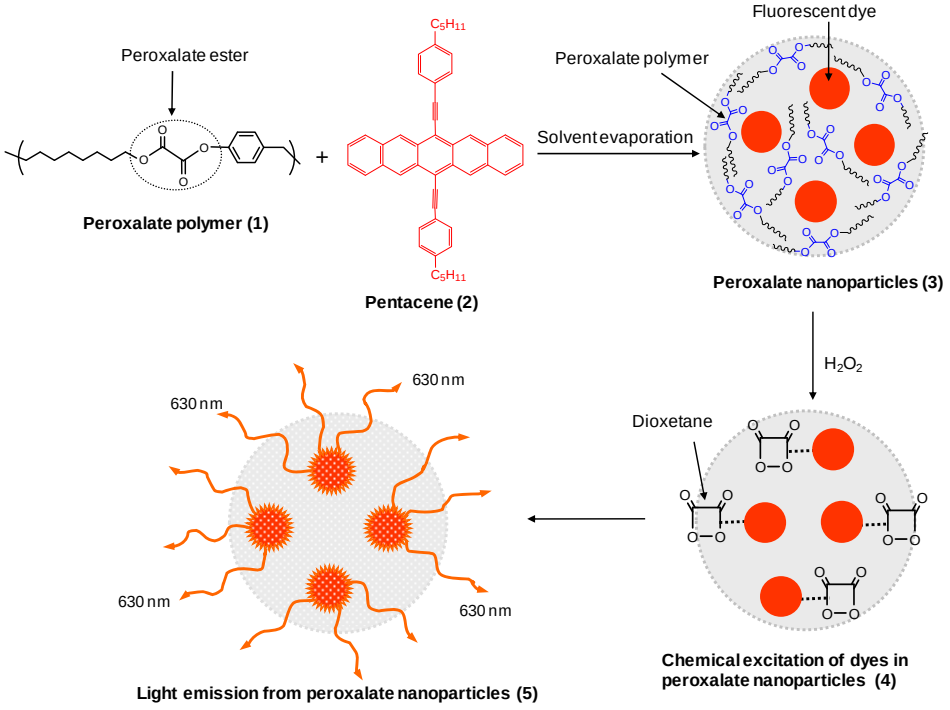
In Vivo Imaging of Hydrogen Peroxide with Chemiluminescent Nanoparticles

Hydrogen peroxide is an important molecule involved in physiological signaling processes, but in a wide range of diseases overproduction of hydrogen peroxide occurs, contributing to inflammation and tissue damage. In a study funded by in part by the NHLBI Programs of Excellence in Nanotechnology, Murthy and colleagues have developed a method for imaging hydrogen peroxide in vivo using nanoparticles that incorporate fluorescent dyes. In a three component reaction, hydrogen peroxide in the body reacts with the peroxolate esters that the nanoparticles are made from, and with the fluorescent dyes. This results in the fluorescent dyes giving off light, a process known as chemiluminescence. By choosing the appropriate dyes, the chemiluminescent light emission can be placed in the near infrared range (> 600 nm), which minimizes the absorbance by tissue and blood. This allows the signal to be detected deep into tissue, more than 1cm from the surface. *In vitro* experiments also demonstrated that the signal generation is highly specific for hydrogen peroxide relative to other reactive oxygen species. The nanoparticles were able to detect hydrogen peroxide at concentrations as low as 250nm.



*Peroxalate nanoparticles (**3**) are formulated from polymer **1** and a fluorescent dye (**2**), in this case pentacene. Hydrogen peroxide reacts with the peroxalate ester of **3** to produce a high energy dioxetane intermediate, within the nanoparticles (**4**). Dioxetane excites the encapsulated dye, leading to light emission from the nanoparticles and the imaging of hydrogen peroxide (**5**).*

To assess the potential of the nanoparticles for *in vivo* imaging, they were tested in a mouse model of acute inflammation, intraperitoneal administration of bacterial lipopolysaccharide. When the peroxalate nanoparticles were injected four hours later, the chemiluminescent signal detected with an external imaging system was almost twice as great as that seen in control mice injected with saline. These findings provide proof-of-principle for the use of the peroxolate- dye nanoparticles for *in vivo* detection of hydrogen peroxide in disease.

Lee D, Khaja S, Velasquez-Castano JC, Dasari M, Sun C, Petros J, Taylor WR, Murthy N. *In vivo* imaging of hydrogen peroxide with chemiluminescent nanoparticles. *Nature Materials* 6:765-769, October 2007.

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