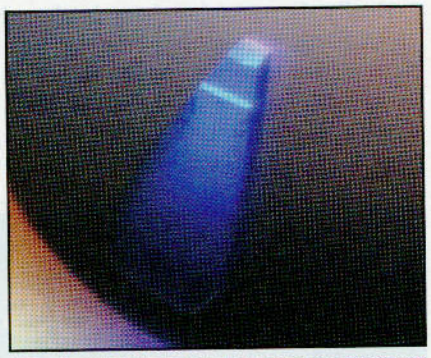
Uncovering why a blue fluorescent antibody is so bright



The antibody EP2-19G2-stilbene complex, shown here in crystal form, emits bright blue at 410 nm when excited by UV light. Courtesy of Erik W. Debler.

Researchers at Scripps Research Institute in La Jolla, Calif., have teased out why one of a group of fluorescent antibodies fluoresces much more brightly and for a much longer time than others of its kind. The work ultimately could lead to improved sensors for a wide variety of applications, including DNA hybridization assays and mercury sensing.

In 2000, the researchers described the group of fluorescent antibodies, which contained about 10 similar antibodies designed to bind stilbene, a gain medium found in dye lasers. Once complexed with stilbene, the antibodies fluoresce to some degree when excited with UV light between 310 and 355 nm. Their emission spectra range from 362 to 442 nm.

Within this group, the stilbene complex of antibody EP2-19G2 stands out for its unusually bright fluorescence and long fluorescence lifetime. Its fluorescence is more than an order of magnitude stronger than the other complexes, said Erik W. Debler, lead author of recently published research unraveling why the fluorescence is stronger.

Since its discovery, EP2-19G2 already has had numerous applications. It is used for chiral sensing in high-throughput synthesis systems, to detect mercury, in DNA hybridization assays and to analyze the residues on viral surfaces. Debler said that better characterizing the molecule could expand its uses as a biosensor.

As published in the Feb. 29 issue of *Science*, the researchers found that the key to the enhanced fluorescence lies in the antibody's molecular structure. When it binds to stilbene, it organizes its structure to "stack" the stilbene molecule so that it has access to a tryptophan residue at the site of the fluorescence activity. Antibody EP2-19G2 is the only one that has this stacking activity. The result is that this allows stilbene and the tryptophan residue to transfer electrons deep within the molecular complex.

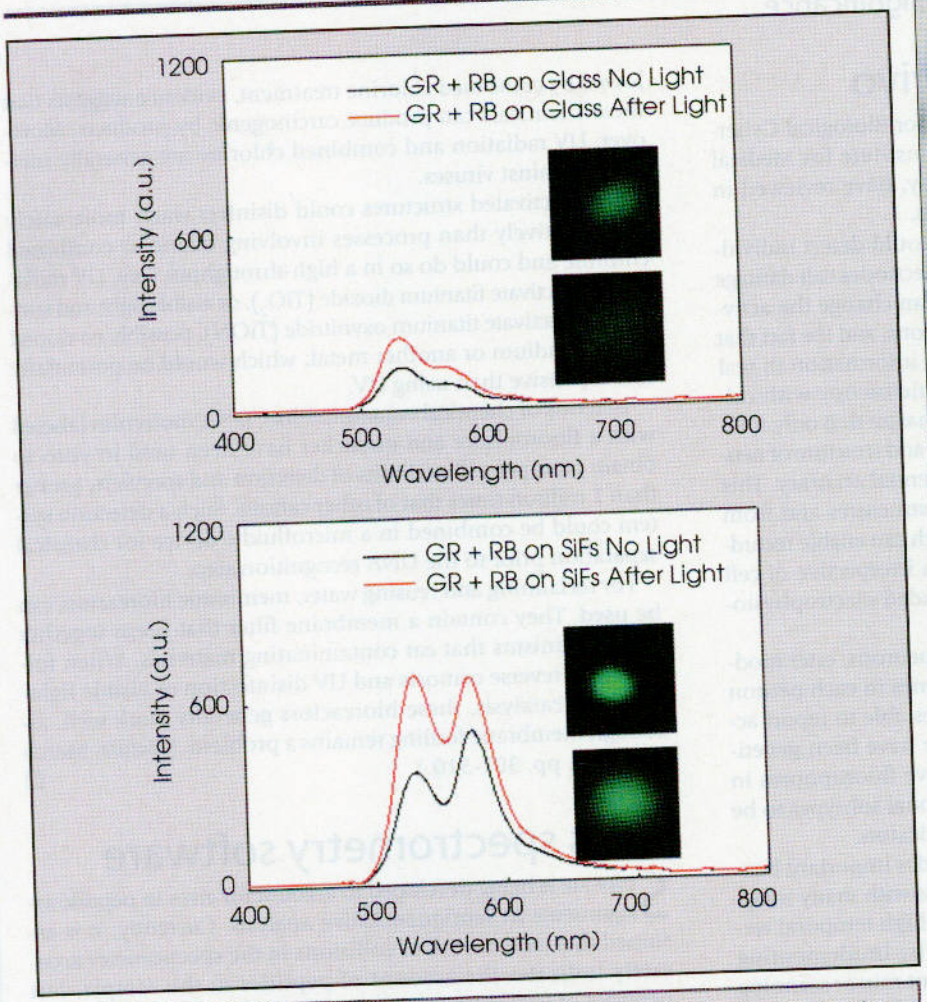
From here Debler said that they plan to continue work on developing additional antibody-chromophore complexes that have charge recombination-induced luminescence phenomena similar to those of EP2-19G2. They also plan to work on further biosensor applications with the existing antibodies. □

Kevin Robinson

Metal-enhanced fluorescence could aid photodynamic therapy

Photodynamic therapy uses light-activated chemicals to treat diseases such as cancer and age-related macular degeneration, but the technique can be difficult to control precisely. The chemicals — drugs called photosensitizers — are injected into a patient and are taken up by diseased cells. The key to treatment is that, when light illuminates the photosensitizer, singlet oxygen is generated. A highly reactive form of oxygen, $^1O_2$ is highly cytotoxic — damaging or destroying the tumor or other cells in close proximity.

Unfortunately, creating too little $^1O_2$ results in ineffective treatment; too much increases the likelihood that healthy cells surrounding a tumor, for example, also will be affected. Adjusting the light intensity can help but is tricky because high intensity can lead to photobleaching of the sensitizer, and low intensities can increase exposure time and the risk of vascular shutdown.



A solution of the photosensitizer Rose Bengal (RB) and a reagent (GR) that senses singlet oxygen exhibits enhanced fluorescence intensity when brought into contact with silver island film (SiF). Reprinted with permission of PNAS.

Now, however, a group of researchers from the University of Maryland Biotechnology Institute in Baltimore has developed a technique that uses metal nanoparticles to provide a higher degree of control over the amount of $^1O_2$ that is created during exposure to light of constant intensity.

Led by Yongxia Zhang of Chris D. Geddes' fluorescence group at the institute, the investigators placed a solution comprising the photosensitizer Rose Bengal and green reagent, a selective sensor for $^1O_2$, on glass substrates dotted with islands made of silver film. The reagent and the photosensitizer have fluorescence peaks of 525 and 588 nm, respectively.

When irradiated with UV light from a 100-W mercury lamp for 2 min, the fluorescence emission intensity of the green reagent that was located over the silver islands increased by about 3.3 times compared with that of the reagent over uncoated glass. The researchers used a fiber optic spectrometer made by Ocean Optics Inc. of Dunedin, Fla., to measure the fluorescence emissions.

As they report in the Feb. 12, 2008, issue of *PNAS*, the increased emission intensity is a function of the increased absorption cross section of the photosensitizer — in essence, the probe absorbed significantly more light. The group has tested the system using various photosensitizers and different thicknesses of silver islands, which has led to consistent findings. The investigators extrapolate that converting the silver islands into silver-coated nanoscale particles will enable them to bring the process to the cellular level, thereby enhancing photodynamic therapy through precise control of $^1O_2$ production. □

Lynn M. Savage