Detection scheme takes lesson from plants

Borrowing a trick from photosynthesis, scientists are using light to detect faint traces of biochemicals. Their work suggests a route to developing speedy new tools for sniffing out environmental toxins and telltale markers of disease.

Techniques to detect specific molecules often form the cornerstones of research. Many of the quickest, most sensitive methods employ fluorescent molecules as signals. If a system can turn on just one of these miniature lightbulbs for every molecule in a sample, it's a tough tool to beat. The new findings, however, suggest how single molecules can light up dozens of bulbs at a time.

"It's really a fascinating concept," comments Anne L. Plant, a biochemist at the National Institute of Standards and Technology in Gaithersburg, Md.

The new approach, reported in the Oct. 26 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, makes use of MPS-PPV, a fluorescent polymer—a giant molecular chain of repeating units—belonging to the polyphenylene vinylene family. This polymer resembles a string of Christmas tree lights of one color.

When researchers shine green light on water containing dissolved MPS-PPV, it glows orange. The new discovery, which puts the entire string of lights under a single switch, exploits a quantum mechanical effect known as resonance energy transfer. It enables fluorescence energy to jump from molecule to molecule before being emitted as light.

The energy can hop between similar fluorescent molecules or between two segments of one polymer molecule. Molecules or segments close together swap energy so rapidly that it might move a long way before being emitted. This indeed happens in MPS-PPV, says Duncan W. McBranch, a scientist at Los Alamos (N.M.) National Laboratory, who collaborated in the new research.

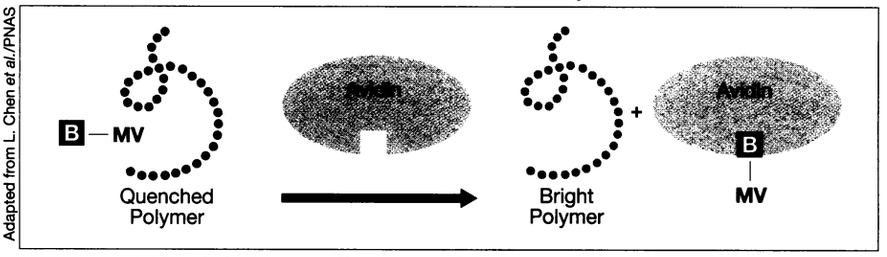
To create a switch for the polymer's fluorescence, McBranch and his coworkers mixed the MPS-PPV with a chemical known as a quencher, which saps energy from the fluorescent molecules with which it collides. In the presence of dilute fluorescent molecules that aren't polymers, a quencher molecule only dims the bulb that it touches. However, a single quencher dimmed all the bulbs on an MPS-PPV molecule, the researchers found.

The only way the quencher could be so potent, says McBranch, is if energy in the distant bulbs can hop to the bulb being quenched.

The scientists knew that nature exploits the same type of energy movement in the light-harvesting enzyme complex within plant chloroplasts. The complex uses long fluorescent molecules to funnel energy from a wide area to its center by a fast succession of quantum handoffs.

In their first application of the method, researchers used the MPS-PPV–quenching scheme to detect a protein—called avidin because it avidly binds the vitamin biotin. The scientists chemically linked the quencher to biotin, thereby creating a handle for avidin to grab onto the quencher. Once avidin binds the quencher, it would no longer get close enough to the polymer to dim flouresecence, they predicted. In the absence of avidin, both the modified and unmodified quenchers made the MPS-PPV solutions go dark. Even small amounts of avidin, however, caused orange fluorescence to reappear in the presence of modified quencher.

To prove the technique useful in medical and environmental applications, researchers must get it to work with other molecules, says McBranch. Preliminary efforts under way make him and his colleagues expect success. Plant, too, is optimistic. "It has real potential as a biosensor," she says. *—O. Baker*



A scheme yielding light from small amounts of the protein avidin: Avidin binds biotin (B), engulfing the quencher methyl viologen (MV) and enabling the polymer to fluoresce.

Collagen transplant replaces rabbit artery

When transplanted into rabbits, blood vessels fashioned from pig and cow tissue can keep blood flowing normally, a new study shows. The work suggests that such vessels might function in people as replacements for blocked arteries in heart-bypass surgery.

Doctors perform more than 360,000 coronary-bypass operations in the United States annually. They usually remove a vein from a patient's leg and use the vessel to circumvent blocked arteries feeding heart muscle. However, some patients, especially those with vascular disease, have veins unsuitable for the task. These people must resort to synthetic arteries, which often clog.

For decades, researchers have explored the concept of making substitute vessels from natural tissue. One candidate, a protein called collagen, is a basic component of tendons, ligaments, cartilage, and skin. Collagen-based and synthetic materials show promise but can attract unwanted attention from the immune system, causing rejection or scarring. They also can weaken or clog.

In the new study, a team of U.S. researchers cleansed durable sheets of pig intestine by removing stray proteins and cells and formed the collagen into a cylinder. Next, they coated the inside of this 4-millimeter tube with malleable bovine-tendon collagen that had also been cleansed. They then used this tube to replace 3-to-4-centimeter lengths of the carotid artery in 18 rabbits. The carotid is the primary artery carrying blood from the heart to the head.

Rabbits examined after 4 weeks, 7 weeks, and 3 months were free of blockages in the transplant and showed no signs of immune rejection, the group reports in the November NATURE BIOTECHNOLOGY.

The rabbits even adopted the new vessels as their own, infiltrating the tubes with cells, the scientists were surprised to find. This remodeling project consisted of lining the transplanted collagen tubes with healthy layers of smooth muscle cells and endothelial cells—both commonly found in mammalian vessels, says study coauthor Susan J. Sullivan, a cell biologist at Organogenesis in Canton, Mass. The transplant appears to serve as a temporary scaffolding on which natural vessels can develop, she says.

The rabbit cells may find the inner layer of bovine collagen more hospitable than one of pig collagen, says Timothy Scott-Burden, a cell biologist at the Texas Heart Institute in Houston.

The combination of durable porcine collagen and the more malleable bovine collagen makes the new vessel slick enough inside to inhibit blood from clotting but sturdy enough inside to withstand surgery and blood pressure, says Sullivan. In earlier experiments, vessels of porcine collagen often clogged. On the other hand, bovine-collagen tubes have weakened. "Perhaps the combination [of collagens] helps this problem," Scott-Burden says.

The study represents progress in the quest to design a bioartificial vessel that the body will tolerate, says John T. Watson, a biomedical engineer at the National Heart, Lung, and Blood Institute in Bethesda, Md. Because the researchers show the importance of how the collagen was prepared, he says, "this is a step forward."

Next, Sullivan plans to test the biosynthetic vessels in larger mammals, such as pigs or dogs. *—N. Seppa*