

Microwaves make faster and brighter fluorescent assays

Technique could one day quickly diagnose heart attack

Researchers at the University of Maryland Biotechnology Institute in Baltimore have demonstrated that metallic nanostructures combined with low-power microwave heating can boost the fluorescence signal from a surface-based assay nearly tenfold while cutting the time to drive the reaction to completion from tens of minutes to tens of seconds.

Traditional fluorescence-based assays have been limited in both their rapidity and sensitivity. Typically, these assays involve antigen-antibody binding, with one or both labeled with a fluorophore. The speed of the process is governed by the antigen-antibody recognition and binding step, which often takes more than 10 minutes at room temperature to complete. The sensitivity is determined by the quantum yield of the tagging fluorophore and instrumentation capabilities.

Research by Chris D. Geddes and his group at the institute, as well as others, had revealed that metallic nanoparticles enhance fluorescence signals when the fluorophores are closer than about 10 nm to the noble metal nanoparticle. Also, the increase is more pronounced for fluorophores with a low quantum yield. Thus, the signal-to-noise ratio can be increased substantially by using weak fluorophores and metallic nanoparticles.

Their new results have shown that the intensity of the fluorescein-labeled protein avidin (FITC-avidin) increased sixfold when binding to biotinylated bovine serum albumin (BSA) sitting atop a silver island nanoparticle film as compared with the same construct sitting atop glass. The spectral signal of the fluorophore remained the same despite the increase, with the peak wavelength unchanged and the normalized intensity curve of glass and the silvered surface similar.

Geddes noted that manufacturing techniques result in nanoparticles of nearly the same size and that different shapes are available. "You can make rod-shaped particles, triangular-shaped particles as well as spheres and islands. So there's a whole range of different types of structures that you can make," he said.

In the everyday world, a metallic object, such as a spoon, will spark when heated by microwaves in an oven. The

microwaves change polarity, leading to a twisting motion and heat in polar molecules such as water. In the case of a metallic spoon, the changing electromagnetic field causes charge to separate on the surface. That creates voltage differences large

enough to cause dielectric breakdown of the surrounding air or water, and then sparks occur.

The same happens in the microworld, down to particles that are a few hundred nanometers in size. But below that range,

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Albert Szent-Gyorgi, Nobel Prize, Biochemistry, 1937

Rat kangaroo kidney (PTK2) epithelial cells. Photograph by Michael Davidson, Florida State University.

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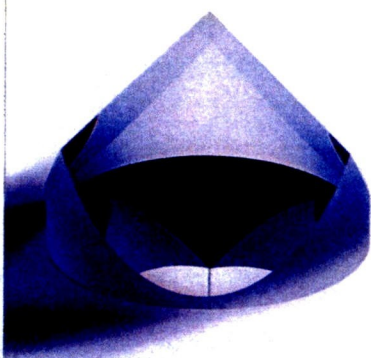
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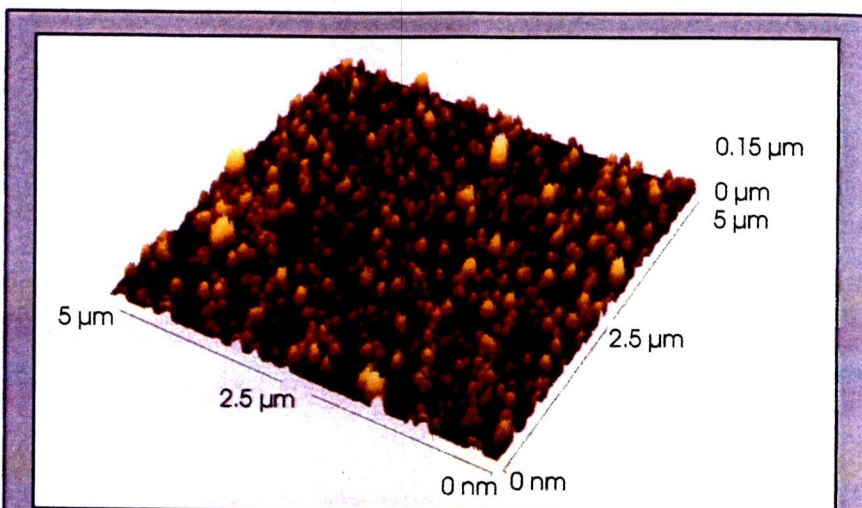
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Metallic nanostructures, such as this bumpy silver film, combined with low-power microwave heating can boost the fluorescence signal from a surface-based assay while cutting the time to drive the reaction to completion.

the situation changes. The metallic nanoparticles still build up charge, but their small size limits the charge separation and the resulting voltage. Sparks, therefore, don't happen as the polarity of the microwaves flips back and forth. Instead, the nanoparticles heat up.

It's the result of a lucky coincidence in which metallic nanoparticles bombarded by microwaves heat up without sparking, Geddes noted. "Mother Nature's being very nice to us in the fact that the size of the particles that this occurs for is the same as utilized for the metal-enhanced fluorescence."

Differences between water and metal mean that they don't heat at the same rate, he said. "The dielectric constant of water is about 78. The dielectric constant for silver is much higher. So when you put silver in water, the microwaves preferentially heat the silver nanoparticles."

It is this differential that Geddes and his group exploited. As detailed in the Dec. 15 issue of *Analytical Chemistry*, they first subjected silver island films to heating in a microwave cavity with a 140-W power setting. They used a similar power level for later assay work. After confirming that exposure to the microwaves didn't affect the silver nanoparticles, they covered half of a glass slide with a silver island film and coated both glass and silvered surfaces with biotinylated BSA. Previous work had shown that this produces a monolayer on both substrates that positions the fluorophore in FITC-avidin at more than 4 nm above the surface, an ideal distance

for metal-enhanced fluorescence.

The silver island films are essentially silver bumps measuring 100 to 500 nm across and nearly 60 nm high, with some aggregates. They cover perhaps 40 percent of the surface area of the slide on which they are deposited.

The scientists exposed the antibody to the antigen, waited for the reaction to complete, washed the slides to remove unbound fluorophores and measured the resulting fluorescence. Using a 500-nm-long-pass filter, they verified that the expected intensity increase for the metallic surface had occurred without a shift in the emission spectrum of the fluorophore. The time for completion of the reaction for all surfaces was about 30 min.

They used microwave heating for 20 s and compared the resulting emission with that of an unheated sample. The results showed a ninefold increase in intensity of the heated metallic nanoparticles as compared with the heated glass. The unheated slide, in contrast, had almost no fluorescence signal, indicating that the reaction was incomplete. Further tests showed that the low-power heating caused neither conformational changes in proteins nor nonspecific binding.

As for what this microwave-heated metallic-enhanced fluorescence assay platform could be used for, Geddes pointed to his work on a myocardial infarction detection system. Currently, such detection is done by running an assay looking for specific cardiac markers in blood after

serum separation. The process takes an hour or so, during which time it is unclear whether a patient being tested has had a cardiac event. With the kit developed by Geddes and his group, whole blood can be used, and the reaction is completed in about 20 s. That could lead to a potentially lifesaving acceleration of cardiac assessment. It is also something that could conceivably be accomplished by emergency medical technicians on the scene, long before an ambulance arrives at a hospital.

More work must be done before such a possibility can become a reality. For one thing, the microwave power setting might have to be tweaked. It's important that the microwave heating process not alter any target molecule.

Certain tests, in which time is not so critical, are not ever likely to use the technique. Others — such as for pancreatitis, toxic shock and myocardial infarction — would benefit greatly from a fast and sensitive assay. The same probably would be true for bioterrorism-related tests.

"I see quite a few commercial opportunities here for this," Geddes said. He noted that the technique requires only standard fluorescence instrumentation, a factor that could make the method's move into the marketplace easier. □

Hank Hogan

FRET fine-tuned to see neurons

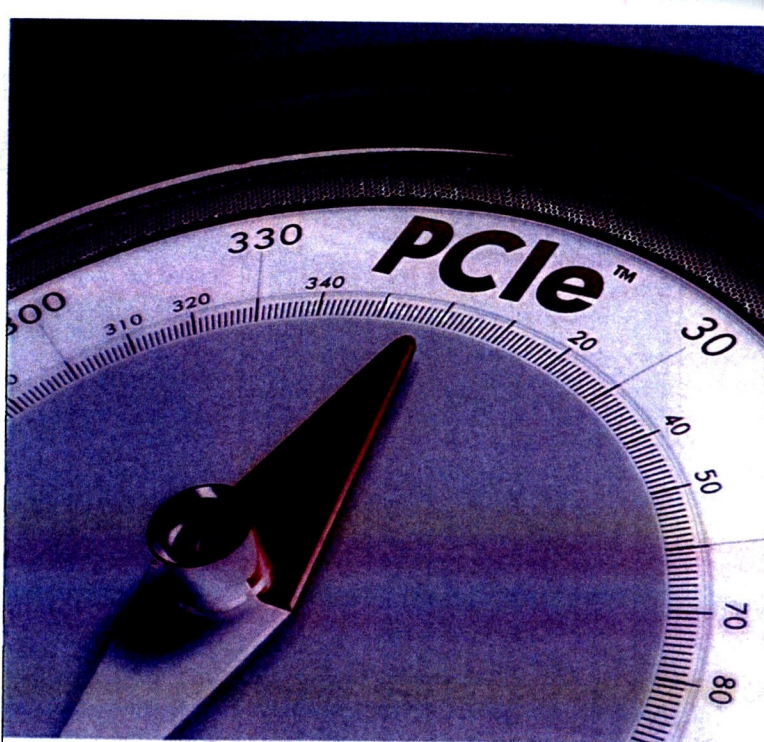
Genetically encoded calcium indicators are incorporating fluorescent proteins to image various nerve cells, especially those found in nematodes, fruit flies, zebra fish and mice. Förster resonance energy transfer (FRET) between the fluorescent protein — such as cyan or yellow — helps capture changes in cytosolic calcium within most types of cells, but current techniques are not fast enough to image these changes inside neurons.

Now scientists at Max Planck Institut für Neurobiologie in Martinsried, Germany, report in the Dec. 9 *BioFast*, the online edition of *Biophysical Journal*, that they have created a class of calcium biosensors that eschews the commonly used calcium-binding protein calmodulin in favor of variants of troponin C, a protein that enables calcium to effect contractions of skeletal and cardiac muscle.

They generated several permutations of cyan and yellow fluorescent protein and inserted them into TN-L15, a calcium sensor derived from troponin from chicken skeletal muscle. The fluorophores acted as FRET donors and acceptors within the biosensor protein. The investigators then inserted the biosensor into hippocampal neurons from rats and imaged the fluorescence initiated by calcium binding with a microscope from Carl Zeiss AG of Jena, Germany, and a CCD camera from Photometrics of Tucson, Ariz. They also tested the biosensor system in transgenic flies.

Each fluorescent protein produced a different signal strength peak when the sensor to which it was attached was exposed to increasing levels of calcium. In their best case, the investigators found that one yellow variant led to an increase in the emission ratio of up to 400 percent from no calcium present to calcium saturation.

However, when they tried the technique in human embryonic kidney cells, the maximum change in signal strength was 160 percent, which they ascribed to partial binding of TN-L15 to magnesium instead of calcium. To solve this, they induced mutations into the calcium and magnesium binding motifs of



The industry has set its course.

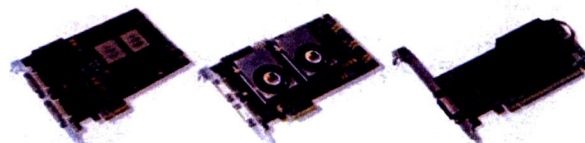
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