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Single-cell enzyme monitoring?

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A new technique to rapidly detect enzyme activity published online August 8 in Nature Biotechnology is sensitive enough to identify reactions from as few as 500 molecules, according to researchers at the University of Strathclyde, Glasgow, who say their method could potentially detect multiple enzyme activities simultaneously and at levels found within single cells (*Nat Biotechnol* 2004, DOI:10.1038/nbt1003).

"We think we can probably apply the technology to most enzyme classes," researcher Barry Moore said of the method, which employs surface-enhanced resonance Raman scattering (SERRS). In SERRS, the target compound is adsorbed onto a roughened metal surface, producing an enhanced vibrational spectrum of the target, characterized by multiple sharp peaks, that serves as a fingerprint. The research team used a suspension of citrate-reduced silver particles roughly 40 nanometers in diameter as their metal surface.

The key to detecting enzyme activities at ultra-low levels is a newly devised class of substrates covered by a University of Strathclyde patent. Each consists of three components - an enzyme recognition site, a benzotriazole azo dye, and an enzyme-cleavable linker that stably joins the other components. When free, the dye has a strong penchant for adsorbing to silver nanoparticles by displacing their citrate surface layers, and when this happens it can generate an increase of up to 10 to 14 times in the SERRS signal intensity, enough that near single-molecule detection levels of such dyes are observed.

The substrates proved susceptible to hydrolysis by a wide range of hydrolases, including lipases, esterases, and proteases. In experiments, the substrates acted rapidly, screening for activity and enantioselectivity for 14 enzymes in less than 30 seconds. Extrapolating this productivity gives a potential throughput of roughly 100,000 enzymes per day per instrument, comparing favorably with other screening techniques, the investigators said.

"You can measure SERRS on any standard Raman spectrometer," researcher Duncan Graham said. He noted no high-throughput SERRS techniques are currently employed, although his group has suggested high-throughput SERRS techniques previously for genomics.

The technique detected 0.025 micrograms/milliliter of lipase from *Pseudomonas cepacia* after reaction for 10 minutes, corresponding to at most 0.8 picomoles of enzyme in the 1-milliliter sample volume. Given the microscope lens SERRS uses only interrogates a small portion of the sample, a conservative estimate puts the actual sample volume at picoliters, whereas more realistically it is closer to femtoliters, the researchers wrote in their report. This suggests they likely sampled reactions arising from only 500 or so molecules of enzyme at most, they add. "In the long term, you could think of measurements directly in vivo, where you would get the nanoparticles into the cells," Moore told us.

The investigators are currently synthesizing new substrates to monitor other major enzyme classes, such as phosphatases and p450s. They suggest the system could be optimized so that SERRS can be used to monitor single-enzyme kinetics, as has already been done with fluorescence. Also, so far, standard Raman optics have been used, but the authors note there is great scope for miniaturization.

Graham noted his group was developing a microfluidics device to monitor enzyme selectivity and activity.

Because each dye produces a characteristic SERRS spectrum - akin to a fingerprint - that can easily be identified and quantified in a mixture, synthesizing substrates with different enzyme recognition sites coupled with different dyes could make it possible to monitor the action of multiple enzymes simultaneously, the investigators suggest. They note that it is very difficult to achieve by alternate techniques and is expected to provide a pathway to a wide range of new bioanalytical assays.

"One application of this technology is in the development of new diagnostic methods, such as in detecting specific enzyme biomarkers of disease states. For example, enzymes such as metalloproteinases are known to be involved in cancer initiation and progression," Moore said.

"It's ingenious work," said Chad Mirkinof Northwestern University in Evanston, Ill., who did not participate in this research. "It's a technique that's going to allow you to look at a variety of molecular biotechnology processes with high sensitivity with a technique that looks like it's fairly straightforward to implement.

But Mirkinsaid that calibration would be "one of the big issues with this."

"One of the main problems with SERRS is changing SERRS responses, since every surface is slightly different, with hot spots and cold spots, and calibration is often a difficult issue to deal with," Mirkin said.

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