RESEARCH PROFILES

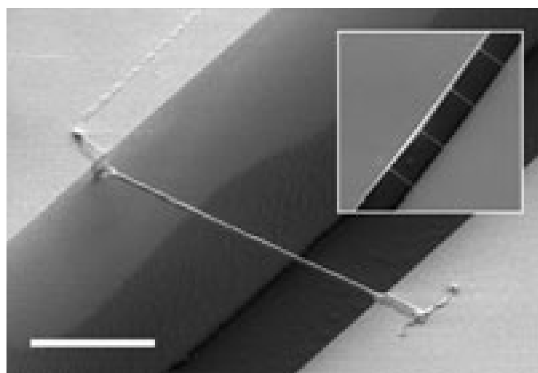
Location, location, location

Savvy realtors know that location is everything. In biological environments, the exact locations of chemical reactions can be just as important to cells in culture. Jason Shear and colleagues at the University of Texas at Austin have developed a new method that allows researchers to control where chemical reactions take place in biological matrixes. The method, which is described in the August 15 issue of *Analytical Chemistry* (pp 5089–5095), can be used to develop biosensors and to produce chemical gradients on demand.

In the new approach, 3-D cables, microparticles, and scaffolds are fabricated by cross-linking proteins in a process called multiphoton excitation (MPE). A laser is shone onto a protein sample in the presence of a biocompatible photosensitizer, which absorbs the laser energy and promotes the cross-linking of protein residue side-chains. Cables and scaffolds are produced by moving the sample stage through a beam of pulsed laser light, whereas microparticles are created by holding the sample at a stationary position in a beam of continuous-wave laser light. The continuous laser mode also optically traps the microparticle once it is formed, so that it can be manipulated.

The 3-D structures can be fabricated from a variety of proteins, and enzymes can be attached to the structures either by direct cross-linking or by biotin–avidin linkages. "There are different reasons and circumstances [for which] you might want to do [the fabrication] in slightly different ways," says Shear. "We show that there are a variety of ways that [the fabrication] could be done."

To test for enzymatic activity on the protein structures, Shear and his colleagues used a fluorescence assay based on alkaline phosphatase (AP) activity. AP cleaves a phosphate from the reagent 4-methylum belliferyl phosphate (4-MUP) to produce the fluorescent molecule 4-methylumbelliferone. The researchers constructed biotinylated bovine serum albumin (BSA) cables across a gap between two glass coverslips, added avidin, and then biotinylated AP. When 4-MUP was flowed in, fluorescence was observed at the cable and along the coverslip edges.



A scanning electron micrograph (SEM) of a BSA cable fabricated between two glass coverslips. Scale bar = 50 µm. The inset shows a low-magnification SEM of four parallel BSA cables.

The researchers obtained similar results when they performed the assay with biotinylated AP bound to avidin microparticles in capillaries. Nonspecific fluorescence along capillary walls was observed in addition to specific fluorescence at the microparticle. "Right now, the biggest challenge is overcoming issues related to nonspecific adsorption," says Shear. "Fortunately, the structures are retained extremely well, so we can wash many times and quite vigorously." The researchers are currently developing approaches for more completely passivating the surfaces to reduce this effect.

The group also fabricated BSA microparticles in neuronal cell cultures and used optical trapping forces to move them into cells. Microparticles easily passed through the cell membrane because of the high power of the optical trap and the treatment of the cells with special hypotonic media. Shear says that moving a catalytically active particle into a cell will allow them to study how cells respond to changes in intracellular chemical concentrations.

To see whether they could create chemical gradients on demand in biological environments, Shear's team fabricated avidin scaffolds in cell culture and added biotinylated AP and 4-MUP. A PDMS channel was placed over the cells and the protein structure. When a stream of 4-MUP was flowed into the channel, a fluorescent plume, in which fluorescence was brightest at the scaffold, was observed. Cells in the wake of the plume were also fluorescent.

Finally, the researchers created on-column nanoreactors in a capillary with the MPE cross-linking method. BSA and glutamate dehydrogenase (GDH) were directly cross-linked to form several lines within a capillary as a reactor for glutamate. GDH oxidizes glutamate to α-ketoglutarate in a reaction that is coupled to the reduction of NAD^+ to NADH, which is a fluorescent molecule. The researchers flowed in a small plug of the reagents and monitored the reactions by fluorescence detection.

According to Shear, he and his colleagues are thinking of new applications for the 3-D catalytic protein structures. "We've shown [previously] that we can place barriers in front of cells and have them respond to the physical limitations," says Shear (*Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16,104–16,108). "Now we can create chemical gradients in cell environments, so we really want to marry these together and create situations in which structures . . . interact both physically and chemically with cells." He adds that the team also plans to develop biosensors for use in cell cultures. Depending on the design, the biosensor could function both as a reporter for chemicals in the cellular environment and as a barrier to diffusion for chemicals, such as neurotransmitters, that are secreted by neural cells. ◾

—Katie Cottingham