umn, which contained 1.1 mg of aptamer, was tested using purified L-selectin receptor globulin (LS-Rg). The column captured 80% of the total protein load. Elution was carried out using a linear EDTA gradient and two other methods, based on increasing ionic strength, and yielded fractions with very different LC peak shapes. The EDTA scheme produced the sharpest peaks.

The column was tested with an unpurified LS-Rg sample containing 694 mg total protein. Purity and recovery were determined by ELISA. Assays revealed 63% purity and a recovery of 83% in the elution fraction. Overall, a 1500-fold purification was achieved with the aptamer affinity column. (*J. Chromatogr., B* **1999,** *731,* 275–84)

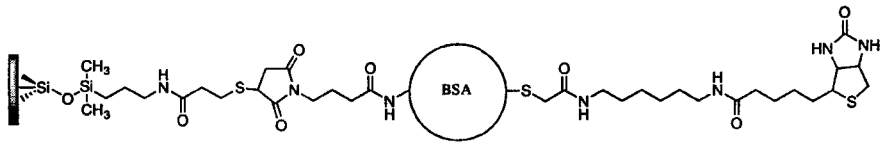
Characterizing a porous silicon optical biosensor

An optical biosensor made with thin films of porous silicon—a nanocrystalline material that can be etched into crystal silicon substrates—has been reported previously. Now, Michael J. Sailor, Keiki-Pua S. Dancil, and colleagues at the University of California–San Diego report on the specificity, stability, and reversibility of this sensor.

The porous silicon biosensor uses the Fabry–Perot transduction method to detect an analyte on the surface. Interference fringes arise from the reflection off the top and bottom of the film. When an analyte binds, the effective optical thickness (EOT) of the layer increases and the fringes shift. Thus, the measurement is made on the volume of sample contained within the porous matrix. Unlike surface-sensitive techniques, such as surface plasmon resonance, the sensor's signal response does not decrease as the distance between the surface and analyte increases, which makes the sensor more versatile—for example, allowing longer linkers to be used.

The researchers report that the porous silicon films are sensitive to oxidation and that results previously believed to indicate extreme sensitivity to antibody–antigen binding were, in fact, signal drift due to oxidation. They corrected this problem by oxidizing the film with ozone before making biochemical modifications. A further improvement in sensitivity was achieved by incorporating bovine serum albumin (BSA) into the linker, thus reducing nonspecific binding.

The EOT of the device was shown to correlate with the size of the bound analyte. With streptavidin (67 kDa), the EOT increased by 17 nm. When biotinylated protein A was added on top of streptavidin (109 kDa for the complex), the EOT increased by 31 nm. This linear increase continued as the complex grew, with the final EOT increasing by 65 nm for a complex of 259 kDa. Finally, reversibility of binding was demonstrated by adding and removing human immunoglobin G. (*J. Am. Chem. Soc.* **1999,** *121,* 7925–30)



A BSA incorporated linker.

Classifying coronary artery tissue

Raman spectroscopy is finding its way into many clinical applications, including the characterization of human tissue. Tissue composition is important for monitoring diseases, such as coronary artery disease, in which the chemical composition of artery tissue changes as the disease progresses. Michael S. Feld and co-workers at Massachusetts Institute of Technology, Emory University, Leiden University Medical Center (The Netherlands), University Hospitals of Cleveland, Case Western Reserve University, and the Cleveland Clinic Foundation report on the use of principal component analysis (PCA) for correlating near-IR Raman spectra of artery tissue with histopathology. The method is capable of classifying coronary artery tissue into three classes: nonatherosclerotic, noncalcified plaque, and calcified plaque.

The algorithm was developed on the basis of 97 samples and tested on 68 samples. Six principal components accounted for the majority of variance in the data. Results were comparable with those obtained using a previously developed biochemical-based model for classifying near-IR Raman spectra, in which artery tissue was modeled by the superposition of the spectra of key chemicals, such as cholesterol and triglycerides.

The authors plan to create a larger database. With more data, they may be able to discriminate between more than three categories of artery tissue. PCA could also be applied to other tissues, such as breast cancer samples. (*Appl. Spec.* **1999,** *53,* 938–42)

SCIENCE

"Mild" fragmentation of peptides

O-Glycosylation (Ser- and Thr-linked) sites in peptides are notoriously difficult to characterize. Direct MS determinations with postsource decay and collisionally activated dissociation often yield weak or even no signals for glycan-carrying ions because glycosidic bonds are more labile and fragment more easily than polypeptide bonds. In the October 15 issue of *Analytical Chemistry* (p 4431), Roman A. Zubarev, E. Mirgorodskaya, and P. Roepstorff of the University of Southern Denmark/Odense University show that an alternative MS/MS technique—electron capture dissociation (ECD)—fragments polypeptide bonds without breaking the labile bonds, making it well suited for the structural analysis of O-glycosylated polypeptides.

Just as electrospray and MALDI are "mild" ionization techniques, ECD is a "mild" fragmentation technique. Zubarev views ECD as "bridging the gap between the mild ionization techniques and the possibilities of MS/MS." In conventional MS/MS techniques, glycan loss occurs before backbone fragmentation. The opposite is true for ECD—backbone fragmentation occurs faster than glycan loss.

ECD, which was originally developed by Fred W. McLafferty's group at Cornell