ANALYTICAL CURRENTS

Network geometry influences enzymes

Owe Orwar and colleagues at the Chalmers University of Technology (Sweden) have demonstrated that the dynamics of an enzymatic reaction can be manipulated inside a network of vesicles and nanotubes. The results could provide insight into biological catalytic reactions and aid in the design of wet chemical nanodevices.

Vesicle 1 was initially filled with alkaline phosphatase and vesicles 2–4 with fluorescein diphosphate. The enzyme molecules diffused throughout the network and sequentially converted fluorescein diphosphate into fluorescein in vesicles 2–4. Scale bar = 10 μm.

Orwar and colleagues studied the dynamics of fluorescently labeled alkaline phosphatase as it converted fluorescein diphosphate into fluorescein inside a vesicle–nanotube network. Because the vesicles had average radii of 5–10 μm and the nanotubes were 100–300 nm in diameter and had lengths of tens of micrometers, the reactants and product moved and mixed by diffusion. The reactions were monitored by confocal laser scanning microscopy.

The investigators manipulated the enzymatic reaction by changing the arrangement of the vesicles and nanotubes within the network. They first demonstrated that a solution of 100 μM fluorescein diffused down its concentration gradient and filled a network of vesicles with an even distribution. However, when a vesicle filled with alkaline phosphatase was introduced into a network containing fluorescein diphosphate, a different pattern developed that consisted of waves. The wave pattern was attributed to the increase in the fluorescein signal as the enzyme started to enter the substrate-filled vesicles and react with the fluorescein diphosphate. As the fluorescein either photobleached or left the network through the walls, its signal started to decrease.

Orwar and colleagues showed that the directional, temporal, and spatial coordinates of the waves could be controlled by changing the configuration of the network. By placing the enzyme-filled vesicle in different positions relative to the substrate vesicles, the investigators determined that the rate-limiting step was not the movement of the enzyme down the length of the nanotubes but the low probability of the enzyme finding the entrance to the nanotube from the vesicle. By placing two enzyme-filled vesicles in different positions, Orwar and colleagues also showed that counter-propagating waves of fluorescein formation could be generated within the network. (*Nano Lett.* **2006**, *6*, 209–214)

QD-aptamer biosensor

By coupling aptamers with quantum-dot (QD) nanocrystals, Joseph Wang and colleagues at Arizona State University, Aarhus University (Denmark), and the U.S. Naval Research Laboratory have developed a highly sensitive and selective electrochemical biosensor for the simultaneous detection of multiple proteins. The researchers have used the device for the dual-analyte detection of thrombin and lysozyme, and they suggest that it could be expanded for the measurement of numerous proteins simultaneously.

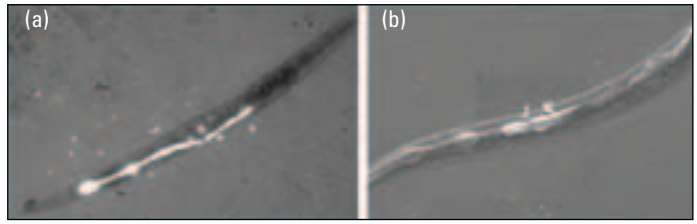
In the new approach, several thiolated aptamers and their corresponding QD-tagged proteins are immobilized on a gold surface. A protein sample, which displaces the tagged proteins, is then added, and the displacement is monitored via electrochemical detection of the remaining QDs.

One of the key features of the new sensor is its subpicomolar (attomole) detection limits. This remarkable sensitivity is possible thanks to the enormous amplification feature of the QD-based electrochemical stripping measurements. The new approach allows for the detection of samples with protein concentrations 3–4 orders of magnitude lower than those detected with previously reported aptamer biosensors. (*J. Am. Chem. Soc.* **2006**, *128*, 2228–2229)

ANALYTICAL CURRENTS

Imaging upconverting phosphors in worms

Shuang Fang Lim and colleagues at Princeton University have synthesized and characterized nanoparticles made out of upconverting phosphors (UCPs). They introduced the particles into the digestive system of the roundworm *C. elegans* and imaged them by IR excitation and scanning electron microscopy (SEM).



(a) Two-photon image of a *C. elegans* worm fed UCP nanoparticles. (b) SEM image of nanoparticles in the worm gut.

UCPs are ceramic materials that contain rare-earth atoms. They absorb IR radiation and upconvert to emit radiation in the visible region. Lim and colleagues synthesized UCP nanoparticles with diameters ranging from 50 to 250 nm. They characterized the emission spectrum of the 200-nm-diam particles and found that particles with higher Er dopant concentration levels had red spectral lines. Those with lower Er dopant levels had green spectral lines.

The investigators introduced UCP nanoparticles into live *C. elegans* worms and demonstrated that the particles could be imaged inside the worm intestines by two-photon microscopy. The UCP nanoparticles could be monitored up to 24 h in the worms without any blinking or bleaching in the signal. When the worms were deprived of food, the UCP nanoparticles remained in the intestines. When the worms were fed, the nanoparticles could be tracked as they moved out of the digestive system in <2 h. The worms appeared to be unaffected by the ingestion of the nanoparticles; this suggests that the particles are nontoxic, nonbleaching, and biocompatible.

UCP nanoparticles inside worms were also studied by SEM. The particles glowed inside the worm in images taken at a 20-kV acceleration voltage. Lim and colleagues also dissected a worm fed with nanoparticles in cross-section by focused ion-beam milling, and they imaged the cross-section by SEM. The particles appeared to be unaffected by the sample preparation and focused ion-beam milling. (*Nano Lett.* **2006**, *6*, 169–174)

Measuring deflections in cantilever sensors

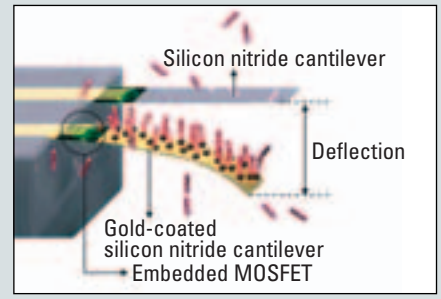
Gajendra Shekhawat, Vinayak Dravid, and colleagues at Northwestern University have developed a new way to measure deflections of cantilever sensors in response to biomolecular interactions. The method offers low noise, high sensitivity, and direct readout.

Label-free detection of biomolecular interactions with microcantilever sensors involves the measurement of cantilever bending. As ligands bind to immobilized receptors on the cantilever surface, a change in surface stress causes the cantilever to bend. The bending, or deflection, of the cantilevers can be measured in several ways, including optical-, piezoresistive-, and capacitance-based methods. But each approach has its limitations with system integration, detection sensitivity, and localized measurement of surface stress.

In the new approach, Shekhawat, Dravid, and colleagues embedded metal-oxide semiconductor field-effect transistors (MOSFETs) into each cantilever in a 2D array. The MOSFETs were directly integrated onto the individual cantilevers by conventional complementary metal-oxide semiconductor (CMOS) fabrication techniques. The transistors were placed at the base of cantilevers, where the surface stress is usually the highest.

When a cantilever was bent, a decrease in the drain current was measured by the MOSFETs. The transistors were sensitive to cantilever deflections as small as 5 nm.

The investigators used the MOSFET-embedded cantilevers to study biomolecular interactions. Streptavidin was immobilized on the surface of the cantilevers, and solutions of 100 fg/mL, 100 pg/mL, and 100 ng/mL of biotin were tested. A decrease in the drain current was measured as the biotin concentration was increased from 100 fg/mL to 100 pg/mL. Similarly, when a solution of 0.1 mg/mL of goat antirabbit IgG was introduced to cantilevers coated with rabbit IgG, a change in the drain current of almost 2 orders in magnitude was measured. (*Science* **2006**, doi 10.1126/science.1122588)



Embedded MOSFETs measure changes in surface stress when ligands bind receptors immobilized on cantilevers. (Adapted with permission. Copyright 2006 American Association for the Advancement of Science.)

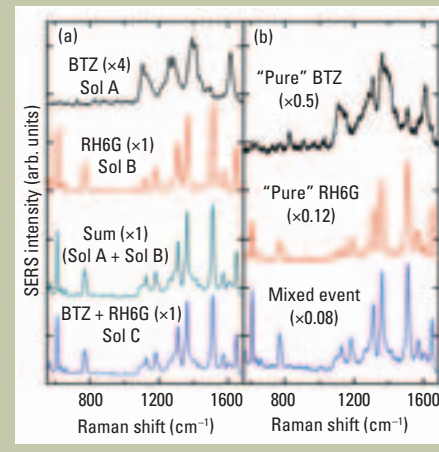
Single-molecule SERS

Although several researchers have reported single-molecule (SM) sensitivity in surface-enhanced Raman scattering (SERS), all of the studies have relied on indirect evidence. To provide more direct and convincing evidence of SM-SERS, Eric Le Ru, Pablo Etchegoin, and colleagues at Victoria University of Wellington (New Zealand) carried out SERS measurements on a two-analyte mixture. The results provide the first unambiguous proof of SM-SERS.

In the new approach, the researchers prepared a colloidal solution with equal concentrations of two dyes—BTZ and RH6G. They used a relatively high concentration (100 nM) of dyes to ensure that at least one molecule was present at most active sites. They reasoned that the SERS signal should always be a mixture of the two dyes because of the large number of molecules. Thus, if a SERS signal was observed for just one dye, it would provide evidence for a small number of molecules.

The researchers collected a series of 1000 SERS spectra from the two-dye mixture as well as from control samples containing each of the dyes alone. The control samples showed that the two dyes have clearly distinguishable SERS spectra. The mixture spectra were identical to the sum of the individual dye spectra; this suggests that the two dyes did not interact with each other.

In the mixture spectra, the researchers observed fluctuations in intensity and shape, which they attributed to changing colloid configurations in the scattering volume. They also noticed large fluctuations in the relative proportion of signal from each of the two dyes. In some scans, the signals were from only one dye. This observation, which indicates that the SERS signal is dominated by a small number of molecules, provides the first direct evidence for SM-SERS. (*J. Phys. Chem. B* **2006**, *110*, 1944–1948)

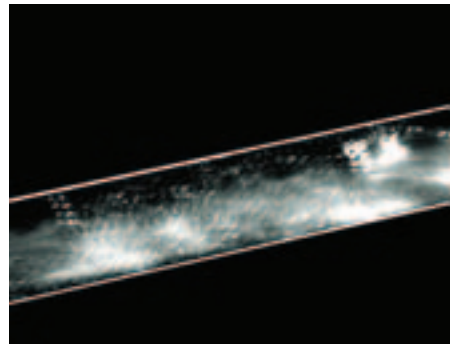


(a) Average SERS spectra from solutions A (100 nM BTZ), B (100 nM RH6G), and C (100 nM of each dye). Sum of spectra from A and B is nearly identical to the spectrum of solution C. (b) Individual spectra of solution C, showing a pure BTZ event, a pure RH6G event, and a mixed event.

Acoustic mixing in microchannels

Mixing in microfluidic devices usually occurs via diffusion because the flow is governed by low Reynolds numbers. To better control fluid mixing in such systems, A. Wixforth and colleagues at the University of Augsburg and Advalytix (both in Germany) have turned to surface acoustic waves (SAWs). They have found that the interaction between microfluidic volumes and SAWs induces streaming effects that act as internal stirrers, even at low Reynolds numbers.

To demonstrate their approach, the researchers coupled microfluidic devices of various geometries to a piezoelectric substrate that contained so-called interdigital transducers (IDTs). The IDTs generate SAWs, which are excited on the substrate and diffracted into the microfluidic device. There, the SAWs generate sound waves, which propagate through the base of the device and enter the fluid-containing channels.



The interaction between small-volume fluids and SAWs induces mixing in microchannels. (Adapted with permission. Copyright 2006 American Institute of Physics.)

The simplest geometry tested was a Y-shaped microchannel structure, in which two fluids come together into a common channel. Both inlets were filled with pure water, and fluorescent beads were added to one of them so that the induced streaming patterns could be observed with a fluorescence microscope. Near the junction, a SAW chip was coupled to the bottom of the microchannel. When a high frequency was applied, mixing occurred because of the interaction of the fluid and the SAW.

To calculate the efficiency of SAW-induced mixing, the researchers analyzed fluorescence images at various distances from the IDT. One measurement was also taken without the generation of a SAW, for control purposes. As the amplitude of the SAW increased, efficient mixing was observed at shorter distances from the IDT. (*Appl. Phys. Lett.* **2006**, *88*, doi 10.1063/1.2171482)

ANALYTICAL CURRENTS

Glycoprotein-responsive gels

Takashi Miyata and colleagues at Kansai University and the Japan Science and Technology Agency (both in Japan) have shown that proteins can serve as ligands in biomolecular imprinting. Using only a trace amount of cross-linker, the researchers prepared molecularly imprinted gels with lectin and antibody ligands. The gels underwent volume changes in response to α-fetoprotein (AFP), a tumor-specific marker commonly used in the diagnosis of liver cancer.

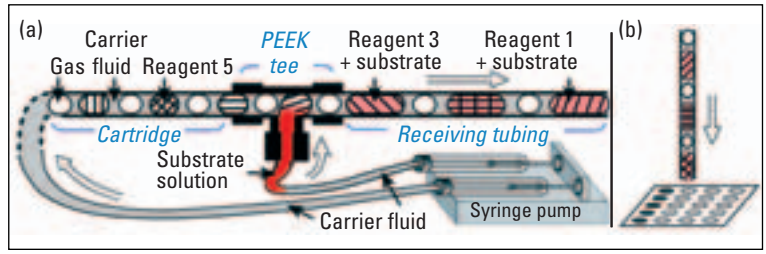
In the presence of AFP, the imprinted gels shrank because of the formation of lectin–glycoprotein–antibody complexes, which play an important role in reversible cross-linking reactions. The amount of shrinking was dependent on the concentration of AFP. Nonimprinted gels, however, swelled slightly in the presence of AFP.

To confirm that both the lectin and antibody ligands simultaneously recognized the peptide and saccharide chains in AFP, the researchers examined the swelling behavior of the gels in the presence of AFP and ovalbumin. Although ovalbumin and AFP contain similar saccharide chains, ovalbumin has a peptide chain that is different from AFP. In the presence of ovalbumin, the imprinted gels swelled, but in the presence of AFP, they shrank.

The results confirmed that the formation of lectin–glycoprotein–antibody complexes caused the gels to shrink. This shrinking behavior could be used to accurately detect glycoprotein biomarkers and could serve as the basis for new diagnostic devices in biomedical applications. (*Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1190–1193)

Testing reactions on the microgram scale

Delai Chen, Takuji Hatakeyama, and Rustem Ismagilov at the University of Chicago have demonstrated a microfluidic system that can screen organic reactions while consuming <1 μg of substrate per reaction. The system doesn't require any microfabrication and can be used to optimize reactions with limited quantities of reagents.



(a) A microfluidic system screens conditions for organic reactions and uses <1 μg of substrate per reaction. (b) The results of the screen are analyzed by MALDI MS.

The microfluidic system consists of three parts: a cartridge with preformed plugs of reagents, a mixing tee, and receiving tubing. Each plug in the cartridge contains a different reagent solution, and the plugs are surrounded by a fluorinated carrier fluid. Two syringes are connected to the system. One introduces the substrate solution into the tee, and the other pushes the fluorinated carrier fluid into the cartridge.

Syringes are used to induce the flow that initiates reactions. The reagent plugs react with the substrate in a sequential manner in the tee, and plugs containing the reaction products move into the receiving tubing. Including a long "blank" solvent plug between every two plugs of reagents prevents cross-contamination. After all the plugs have entered the receiving tubing, the flow is stopped, and the tubing is sealed. The plugs are deposited from the receiving tubing onto a MALDI plate and analyzed by MALDI MS.

To test the system, Ismagilov and colleagues studied the deacetylation of ouabain hexaacetate (Ac_6-OUA), a cardiac poison that is used in neuronal studies. Previous studies demonstrated the selective deacetylation of Ac_6-OUA to ouabain triacetate (Ac_3-OUA) with only a 22% yield. The investigators reacted 6 cartridges of 44 reagents with Ac_6-OUA in their microfluidic system and found that inorganic bases reacted best with Ac_6-OUA to give Ac_3-OUA with a yield of 85%.

To optimize the deacetylation reaction conditions, the investigators analyzed the reaction of Ac_6-OUA with amines and inorganic bases. By quenching the reactions at different times and in various solvents, they isolated unstable hydrolysis intermediates of Ac_6-OUA, ouabain pentaacetate and ouabain tetraacetate, with respective yields of 39% and 47%. The syntheses of these unstable intermediates had not been previously reported. (*J. Am. Chem. Soc.* **2006**, *128*, 2518–2519)