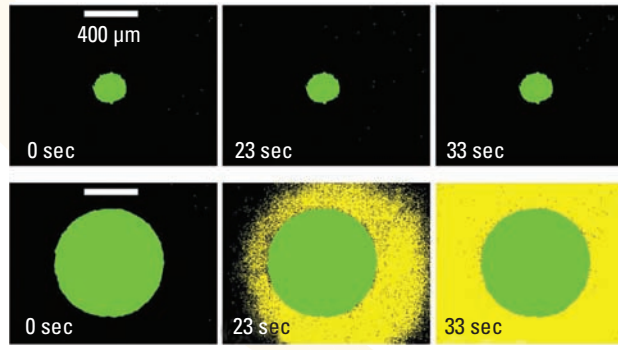
ANALYTICAL CURRENTS

Merging chemistry and microfluidics to study complex biological networks

Although studying biological networks on the level of the individual reaction is useful, systems as a whole must be analyzed to understand the spatial dynamics and the global mechanisms driving the various functions. In a recent paper, Rustem Ismagilov and colleagues at the University of Chicago review their group's work of combining chemical concepts and microfluidic devices to gain insights into network-based biological processes, such as blood clotting.

The first step in the team's dual approach is to simplify the complex network by dividing it into function-based modules. Then, they substitute a single reaction for each module to construct a chemical model of the complex system. After the chemical model is used to formulate and test hypotheses regarding the mechanism driving the biological network, the results are confirmed in experiments with the actual biological components. The microfluidic device provides the spatial control that is needed to analyze and perturb these systems. Using this amalgam of chemistry and microfluidics, the group studied two previously unanswered questions—why blood clots stop growing at a certain point and how severe an injury has to be to activate the clotting cascade. (*Acc. Chem. Res.* **2008**, DOI 10.1021/ar700174g)



The chemical precipitation model predicted the existence of a threshold patch size necessary to initiate blood clotting. Time-lapse fluorescence microphotographs show that the chemical reaction mixture "clots" on large patches but not on small ones (patches are green). Yellow indicates clotting, whereas black indicates no clotting. (Adapted with permission. Copyright 2006 National Academy of Sciences, U.S.A.)

New method to print protein arrays from DNA arrays

Previously, researchers developed in situ protein synthesis methods for spotted protein arrays to get around some of the problems of producing or obtaining the requisite amount of purified protein and short shelf life. These in situ methods, however, produce proteins colocalized with the DNA, and the DNA templates cannot be reused. Michael Taussig and colleagues from the Babraham Institute (U.K.) and Uppsala University (Sweden) have come up with a new approach in which the synthesized proteins are immobilized on a separate slide and a single DNA template array can be used multiple times.

In the DNA array to protein array (DAPA) technique, a hydrophobic, permeable membrane sandwiched between two slides enables the creation of the protein array. PCR-amplified fragments that encode for a set of single-chain, histidine-tagged proteins are covalently bound to an epoxy-coated slide in an array configuration. The membrane is soaked in cell-free lysate capable of performing coupled translation and transcription. As the newly synthesized proteins diffuse across the membrane, they are immobilized on a slide coated with Ni^{2+}-nitriloacetic acid, which captures the histidine tags. The group designed a simple apparatus to incubate the slide pairs.

Initially, the team produced an array of histidine-tagged green fluorescent protein (GFP) and estimated the amount of protein generated by comparing the signal intensities of the DAPA with those of directly spotted GFP standards. Arrays of >20 different proteins were synthesized with DAPA, and researchers could make 20 protein arrays from a single DNA template with no noticeable performance degradation. The proteins did undergo some diffusion, resulting in spots that were bigger than the template DNA spots, and the technique didn't work well for proteins >120 kDa. The authors report that DAPA requires about the same effort as performing a western blot, and they intend to use the method for functional analysis of the proteome. (*Nat. Methods* **2008**, *5*, 175–177)

ANALYTICAL CURRENTS

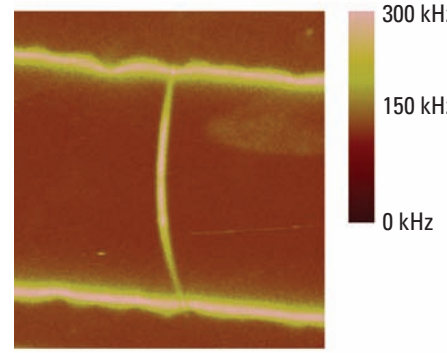
Optical waveguides for biodetection

As optical biosensors become more sensitive, they are also getting smaller, but the materials that make up an optical "nano" detection system aren't always compatible with the biological molecules they are designed to study. To address this problem, Donald Sirbuly, Aleksandr Noy, and colleagues at Lawrence Livermore National Laboratory have coated nanowires with a lipid membrane that can trap biomolecules for interrogation.

Nanowires act as waveguides, which propagate light in a single direction. Up to 30% of the optical energy actually travels outside the waveguide, and this energy can be used to optically probe molecules in its path.

The researchers created their device by coating SnO_2 nanowires with a 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) lipid bilayer. To ensure that the DOPC uniformly coated the target surface, they incorporated a fluorescently labeled lipid marker. Optical images of the waveguide confirmed that the lipids were evenly spread across its entire surface.

To demonstrate the biomolecule-sensing capabilities of their system, the scientists monitored real-time hybridization of two complementary strands of DNA. They attached single-stranded DNA (ssDNA) to the membrane-coated waveguide via a cholesterol tag. The ssDNA strand was also labeled with PicoGreen dye, which shows a strong fluorescence enhancement on binding to double-stranded DNA. Using their system, the researchers could detect hybridized DNA pairs in <10 seconds by monitoring the optical signal generated by the waveguide. They also note that their device is easy to clean and that membranes can be rapidly exchanged multiple times. (*ACS Nano* **2008**, *2*, 255–262)



Confocal fluorescence image of a nanowire coated with the lipid bilayer and ssDNA probe.

Ultracentrifugation separates racemates from enantiomers

Yitzhak Mastai, Antje Völkel, and Helmut Cölfen of Bar-Ilan University (Israel) and the Max Planck Institute for Colloids and Interfaces (Germany) have used density gradient ultracentrifugation to separate a racemate from excess enantiomer crystals. Density gradient techniques are commonly used to separate mixtures of biopolymers, such as single- and double-stranded DNA, but they had previously not been applied to the separation of chiral compounds.

Solid racemic compounds differ in density from their enantiomerically pure counterparts, and as a proof of principle the scientists chose alanine. They mixed D/L-alanine with either the pure D- or L-enantiomer and spun their samples with a density gradient in a preparative ultracentrifuge. After 21 hours of centrifugation at 16,000 rpm, the pure enantiomers clearly separated from the racemate. The researchers could recover 75% of the L-alanine and 90% of the D-alanine and say that the <100% recovery rate is likely due to problems with the fraction recovery technique rather than the separation. (*J. Am. Chem. Soc.* **2008**, *130*, 2426–2427)

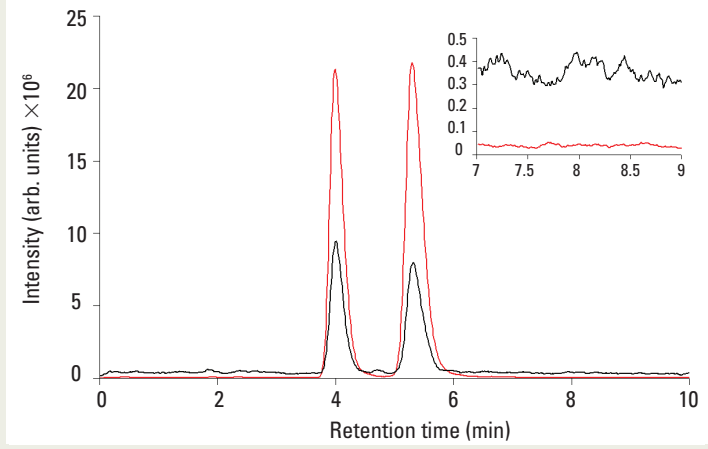
Microfluidic devices for terahertz spectroscopy

Brian Kirby, Farhan Rana, and colleagues at Cornell University have constructed a microfluidic device for measuring terahertz (THz) spectroscopy of biomolecules. Proteins, RNA, and DNA all have vibrational modes in the 0.1–5 THz frequency range, but THz spectroscopic analysis of these molecules has been limited because water also has a strong absorption in this region. Previous experiments have been limited to dry or partially hydrated samples and required a high-power free-electron laser source.

In the new system, the scientists fabricated their microfluidic channels from Zeonor 1020R, a cycloolefin polymer with significantly lower water content than PDMS, the material typically used to construct these devices. The microfluidic channels were designed to avoid excess water absorption and thus required only a low-power THz source. The researchers note that measuring THz spectra in a microfluidic environment is ideal because the samples can be exchanged in real time and the microfluidic channels can be easily integrated with photonic components to create multifunctional spectroscopy platforms. (*Opt. Express* **2008**, *16*, 1577–1582)

LC/MS detection of amino acids with zero-voltage electrospray

Torben Lund and colleagues at Roskilde University and the Technical University of Denmark set out to create an LC/ESI-MS^3 method to separate and detect D- and L-aspartic acid so they could estimate the age of human teeth. Along the way, they were surprised to find that an electrospray voltage of 0 yielded a 40-fold gain in S/N and a detection limit of 0.2 ng for aspartic acid.



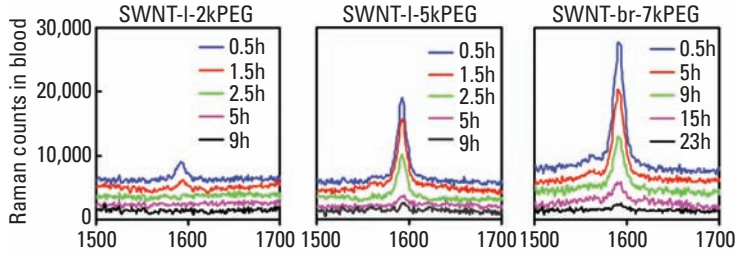
LC/MS chromatograms of L- and D-aspartic acid with electrospray voltage of 0 (red) and 4 kV (black). Inset shows improvement in noise level. (Adapted with permission. Copyright 2008 John Wiley & Sons.)

Amino acids are known to have poor sensitivity in positive-ion mode, so the group was optimizing the electrospray voltage to enhance sensitivity for aspartic acid. Once the team saw the marked S/N improvement with aspartic acid at zero voltage, they tested a variety of naturally occurring amino acids. The zero-voltage effect was highest for amino acids with ionic or polar side chains.

The group found that zero-voltage ESI is dependent on sheath-gas flow rate, eluent flow rate, and capillary temperature, similar to the case in the no-discharge atmospheric-pressure chemical ionization and sonic spray ionization (SSI) methods. The researchers hypothesize that zero-voltage ESI may be the same mechanism as SSI. However, they call the technique zero-voltage ESI because they were using the regular ESI interface rather than an SSI interface. (*Rapid Commun. Mass Spectrom.* **2008**, *22*, 455–461)

Raman reveals nanotubes' fate

Single-walled carbon nanotubes (SWNTs) show promise as drug-delivery vehicles, but little is known about the long-term fate of these structures in living organisms. Radiolabeled and fluorescently labeled nanotubes have been used to track the circulation of nanotubes in vivo, but these labels decay or dissociate from their carriers over time and thus cannot be used for long-term studies. Hongjie Dai and colleagues at Stanford University have taken a different approach and have used Raman spectroscopy to track SWNTs in live mice.



Spectra showing the decreasing Raman signal over time for three different types of functionalized SWNTs. (Adapted with permission. Copyright 2007 National Academy of Sciences, U.S.A.)

SWNTs exhibit an intrinsic Raman scattering intensity that doesn't decay over time and is unchanged by functionalization of the nanotubes with noncovalent coatings. For this study, the authors used SWNTs coated with polyethylene glycol (PEG) chains of various lengths. They injected mice with solutions of these nanotubes and drew blood at various time points over the course of 24 hours. The scientists found that the longer the PEG chain, the longer the SWNTs remained in circulation in the bloodstream.

The researchers also tracked the fate of the nanotubes over a longer time course of 3 months. They found significant SWNT accumulation in the liver and spleen and smaller accumulations in bone, kidney, intestine, stomach, and lungs of mice. Nanotubes were found in the feces but not in the urine of the mice, leading the scientists to conclude that SWNT excretion occurs via the biliary pathway. The authors note that no toxic effects were observed in the mice, in contrast with nonfunctionalized SWNTs, which have been shown to exhibit fiber toxicity in living animals. (*Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1410–1415)