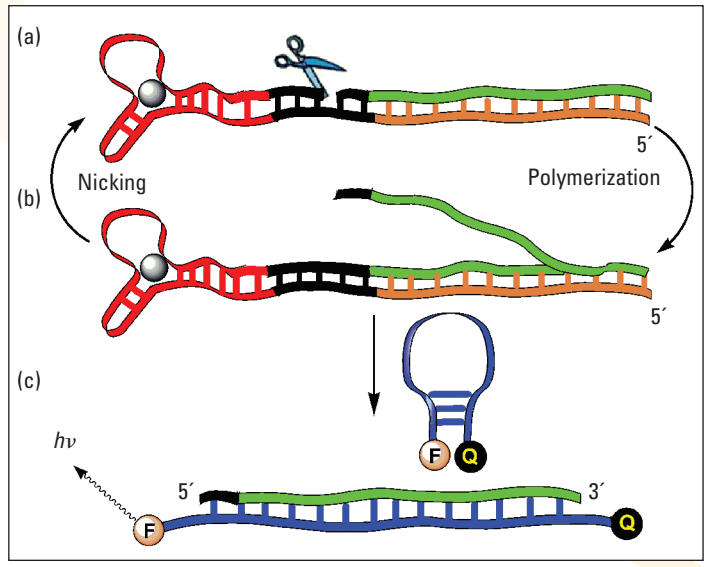
ANALYTICAL CURRENTS

## Detection of cocaine by an aptamer-based machine

Itamar Willner and co-workers at the Hebrew University of Jerusalem (Israel) have designed an aptamer-based machine to detect cocaine. To date, attempts to use DNA aptamers as the recognition element in both optical and electrochemical sensors have been moderately successful, but most aptamers suffer from poor detection limits. In this case, the detection limit was improved by a signal amplification method akin to PCR.

In the presence of cocaine, the DNA folds over onto itself to produce a seven-base duplex structure that serves as a primer for replication. DNA polymerase extends the double strand, revealing a nicking site for the endonuclease Nt.BbvC I. After the DNA is nicked, displacement of a single strand reveals a new polymerase extension site.

The polymerase extension step is repeated ad infinitum and produces multiple copies of the single-stranded DNA. These strands are complementary to a reporter hairpin DNA strand that has a donor–acceptor fluorescence resonance energy transfer (FRET) pair attached. When the single strand binds to the reporter, the hairpin opens and disrupts the FRET pair, and the signal increases at the donor's emission wavelength.




The mechanism of the aptamer-based machine. (a) DNA polymerase already has extended the second strand to reveal a recognition site for an endonuclease. (b) The single strand is displaced and (c) binds to the reporter hairpin. When the hairpin opens, the FRET pair separates; this is seen as an increase in the strength of the donor's signal.

The researchers note that the signal follows S-shaped kinetics over time because the single strand of DNA must accumulate to a concentration high enough to open the hairpin at a reasonable rate. After an amplification time of 60 minutes, the investigators detected cocaine at a concentration of $5 \times 10^{-6}$ M, which is a better detection limit than current methods offer. (*J. Am. Chem. Soc.* **2007**, *129*, 3814–3815)

## Watching single organic molecules move

Imaging individual small molecules has been difficult. But now, Eiichi Nakamura and colleagues at the Japan Science and Technology Agency, the National Institute of Advanced Industrial Science and Technology (Japan), and the University of Tokyo have come up with a way to observe them.

The investigators trapped molecules with a characteristic long hydrocarbon chain individually inside single-walled carbon nanotubes. By transmission electron microscopy, they observed whether the molecule was moving or sitting still inside the nanotube. The hydrocarbon chain helped the investigators to distinguish between the head and tail regions of the molecule and to correlate its motion relative to the nanotube.

When Nakamura and colleagues introduced a molecule into a 0.9-nm-diam nanotube, the molecule was observed to be immobilized in a fully stretched conformation. When molecules were placed inside 1.2-nm-diam nanotubes, some of them appeared to get their hydrocarbon tails stuck to the wall of the nanotube, whereas others went through a series of conformational changes in a stepwise fashion over a period of several seconds. (*Science* **2007**, doi 10.1126/science.1138690)

## ANALYTICAL CURRENTS

### Analyzing DNA by multinanopore force spectroscopy

Clinical applications require new technologies for cheap DNA analysis. To meet the need, Andre Marziali and Carolina Tropini at the University of British Columbia (Canada) have developed an array of nanopores that quickly tests DNA duplexes for sequence specificity. The detection is done electronically, which means the approach could lead to an inexpensive, disposable device for rapid clinical detection of polymorphisms.

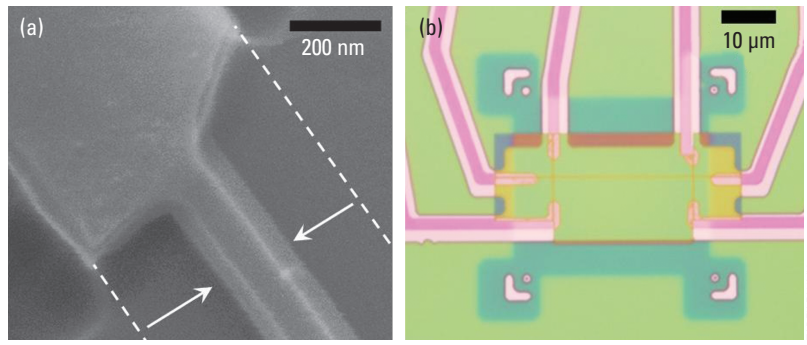
Marziali and Tropini incorporated hundreds of α-hemolysin protein molecules into a lipid bilayer. The protein acted as the nanopore. Probe DNA strands had an avidin–biotin anchor on one end and 14 nucleotides at the opposite end that were complementary to an analyte's sequence. The investigators inserted the probes into the pores by applying 200 mV. The avidin anchors prevented the probes from completely translocating through the pores, leaving the 14-nucleotide sequences sticking out of the other side.

Analyte DNA strands were allowed to interact with the protruding parts of the probes. Marziali and Tropini next decreased the applied voltage so that any unbound probes were released from the nanopores. They then reversed the polarity of the voltage so that a force was applied to the analyte-bound probes. Duplex DNA can't pass through nanopores. As the investigators increased the applied force, the duplexes dissociated and the probe strands were pulled out of the nanopores.

By using a special model, Marziali and Tropini correlated the applied force to the binding energy of the duplex. From their calculations, they could distinguish analytes that differed from the probe sequence with single-base resolution. To further improve the technique, the investigators suggest replacing the α-hemolysin molecules with more robust synthetic pores. (*Biophys. J.* **2007**, *92*, 1632–1637)

### CMOS-compatible nanowires as detectors

Semiconducting nanowires can function as sensors for low concentrations of species such as microorganisms, small molecules, and proteins. Now, Mark Reed, Tarek Fahmy, and colleagues at Yale University have come up with a new approach for building nanowires that's compatible with complementary metal oxide semiconductor (CMOS) field effect transistor technology. The method eliminates the need for hybrid fabrication techniques and permits system-scale integration of the sensors with signal processing and information systems.




(a) Scanning electron micrograph and (b) optical micrograph of a semiconducting nanowire device.

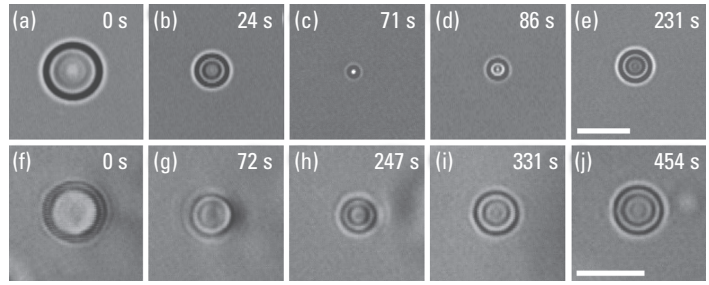
The investigators fabricated semiconducting nanowires with an anisotropic wet etch. Tetramethylammonium hydroxide etched away particular planes in ultrathin silicon-on-insulator wafers ~100× more slowly than the rest. The etching kept the pattern defined by a masking oxide layer and smoothed out edge imperfections. This resulted in nanowires with trapezoidal cross sections and an exposed silicon surface amenable to functionalization. The investigators say the approach easily integrates with CMOS technology.

Reed, Fahmy, and colleagues tested the sensors by monitoring the real-time activation of T lymphocytes, which occurs by antibody-mediated cross-linking of ligands on the cellular surface. One consequence of the activation is the release of acid. When the investigators added antibodies against specific T-lymphocyte ligands, they detected changes in pH, as a result of cellular responses, within ~10 seconds.

The investigators also tackled the direct detection of macromolecules without fluorescent, radioactive, or any other labels. With the streptavidin–biotin system, they established that the nanowire sensors had a detection sensitivity of ~10 fM. Reed, Fahmy, and colleagues also found they could discriminate between low concentrations of mouse immunoglobulin G and A proteins with commercially available antibodies. (*Nature* **2007**, *445*, 519–522)

## Dynamically changing concentrations in droplets

Daniel Chiu and colleagues at the University of Washington Seattle have developed an optical vortex trap that dynamically changes the concentrations of aqueous droplets over 4 orders of magnitude. They say that the manipulation of concentrations of nanoscale containers provides a new level of control that was previously difficult to achieve.




Shrinkage and expansion of aqueous droplets dispersed in (a–e) acetophenone and (f–j) decanol. Scale bars = 5 μm. (Adapted with permission. Copyright 2007 Wiley-VCH.)

The investigators focused a laser through a microfabricated hologram to form a special type of beam called a Laguerre–Gaussian beam. It generated the optical vortex trap, which was then focused on individual femtoliter-volume droplets. The droplets were surrounded by an immiscible continuous phase that had a slight solubility for water; the aqueous–organic interface was impenetrable to chemical species inside the droplets.

Chiu and colleagues slowly "peeled" off layers of water molecules at the aqueous–organic interface with the optical vortex trap and concentrated the molecules trapped inside the droplet. When the power of the vortex trap was lowered or turned off, water molecules that had left the droplet returned to it and increased its volume.

By switching the power of the trap, the investigators were able to perform consecutive cycles of shrinkage and expansion of the droplets. A droplet's volume could even expand beyond its original size if it accumulated the water released from an adjacent droplet.

The investigators suggest that the combination of this technique with controlled single-droplet fusion would have applications in studying fundamental chemical processes such as the effects of macromolecular crowding and protein nucleation and crystallization. (*Angew. Chem., Int. Ed.* **2007**, *46*, 1326–1328)

## Large-scale phosphoproteomics studies with ETD

Electron transfer dissociation (ETD) has attracted a lot of attention from proteomics researchers because of its ability to fragment posttranslationally modified peptides without the loss of fragile modifications. Now, two groups report the application of ETD to large-scale analyses of the phosphoproteome. Donald Hunt and colleagues at the University of Virginia, Princeton University, the U.S. National Institutes of Health, and Thermo Electron identified several phosphorylation sites in yeast, described a novel motif for phosphorylation, and conducted a network analysis.

In another study, Akhilesh Pandey and colleagues at the Johns Hopkins University School of Medicine, the University of Southern Denmark, Agilent Technologies, and the Institute of Bioinformatics (India) discovered many novel phosphorylation sites in human cells. They also compared ETD with collision-induced dissociation (CID) and reported 15 new phosphorylation motifs.

Hunt and colleagues digested an ~600 pmol sample of total yeast protein and enriched for phosphopeptides with immobilized metal-affinity chromatography. When ETD was performed with a linear ion trap mass spectrometer, 1253 phosphorylation sites were identified on 629 proteins. One of the phosphorylated proteins was Cdc10, which is involved in cytokinesis and morphogenesis. From the ETD spectrum, the researchers deduced that one of the Cdc10 peptides is phosphorylated on a histidine residue instead of serine, threonine, or tyrosine. This unexpected finding suggests that Cdc10 may be phosphorylated by Sln1, the only known yeast histidine kinase. The team also reported that phosphorylated proteins are significantly more conserved than other proteins. (*Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2193–2198)

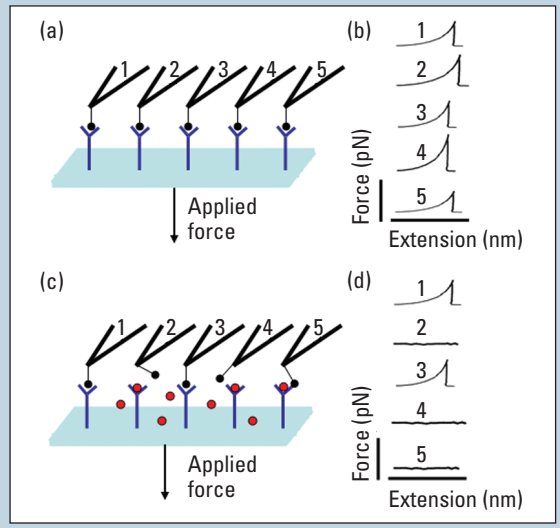
Pandey and colleagues analyzed phosphopeptides from human embryonic kidney 293T cells. Proteins were digested, and phosphopeptides were isolated with a $TiO_2$ microcolumn and analyzed on a 3D ion trap mass spectrometer by ETD. A total of 1435 phosphorylation sites were identified, and 80% of those were novel. Because the *c* and *z* fragment ions generated by ETD often were of low intensity, the researchers wondered whether ETD is actually better than CID for phosphopeptide detection. When they compared the two methods, ETD outperformed CID. However, the scientists note that the processes are complementary. (*Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2199–2204)

ANALYTICAL CURRENTS

## Cantilevers measure solution-phase thermodynamics

Eric Toone and co-workers at Duke University have developed an atomic force microscopy (AFM) method for conducting solution-phase thermodynamic measurements. Typical AFM experiments measure the force required to dissociate a bound complex, but this value cannot be directly related to thermodynamic properties such as free energy, because applying that force removes the system from an equilibrium state.

Toone and co-workers have circumvented this problem by using the probability of a binding event between two surface-functionalized moieties in the presence of various concentrations of free ligand to calculate a solution-phase association constant. In proof-of-principle experiments, the researchers immobilized His-tagged galectin-3 on quartz slides functionalized with $Ni^{2+}$/nitrilotriacetic acid and attached lactose to gold-coated cantilevers. The tip was brought into proximity with the surface and retracted 350–800× per measurement. Each retraction was classified as either a binding or nonbinding event on the basis of its force–extension plot. As expected, the greater the concentration of free ligand in solution, the smaller the probability that a binding event occurred.

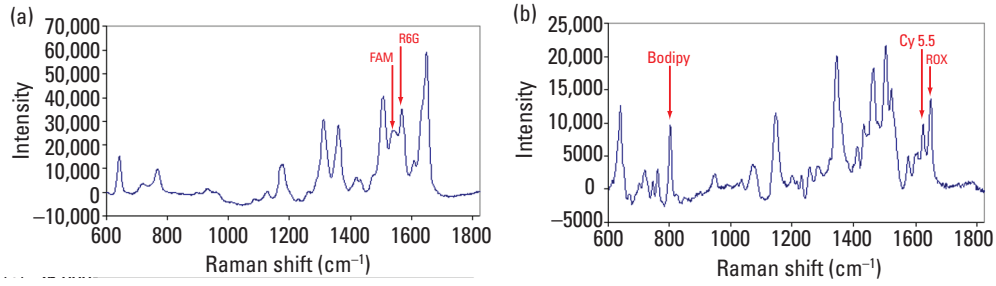



(a) Binding interactions in the absence of free ligand. (b) The associated force–extension plots all show binding events. (c) Binding interactions in the presence of free ligand. (d) Only force–extension plots 1 and 3 show a binding event.

The researchers plotted the concentration of free ligand versus the probability of a binding event and calculated a solution-phase binding constant. After correcting for the multivalency of the system, they determined a value that is in good agreement with values measured by isothermal titration calorimetry. (*Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 2579–2584)

## Dual-wavelength SERRS for multiplex DNA detection

Pity those haystack-crawling needle seekers. While they are still out there searching, the analogous problem of finding and quantifying a specific DNA sequence in a mixture of oligos has just gotten easier. Duncan Graham and co-workers at the University of Strathclyde (U.K.) have shown that they can identify up to five individual DNA strands simultaneously at concentrations as low as $10^{-12}$ M in a heterogeneous mixture with dual-wavelength surface-enhanced resonance Raman scattering (SERRS).




SERRS spectra of a mixture of five DNA–dye conjugates at excitation wavelengths of (a) 514.5 nm and (b) 632.8 nm. (Adapted with permission. Copyright 2007 Wiley-VCH.)

The researchers chose five dyes and attached them to the 5′ ends of DNA sequences. Then, they added silver colloid particles to the DNA–dye conjugates and measured SERRS spectra of each sample at two laser excitation wavelengths. At one or both wavelengths, each chromophore had a unique "fingerprint" spectrum that could still be distinguished in a mixture of all five samples. The fingerprint peak intensity was linear down to ~$10^{-12}$ M for each conjugate.

Now that the basic multiplex detection has been shown, experiments are being carried out to demonstrate applications in molecular biology. (*Angew. Chem., Int. Ed.* **2007**, *46*, 1829–1831)