

ANALYTICAL CURRENTS

Counting rare tumor cells in vivo

Quantitating the levels of circulating tumor cells is difficult, but this information helps physicians determine whether a patient requires chemotherapy after tumor surgery. Now, Philip Low and colleagues at Purdue University and the Mayo Clinic Rochester have developed an in vivo method that noninvasively counts the tumor cells in the bloodstream.

The researchers labeled cells with folate-dye conjugates instead of antibodies, because many human carcinomas are thought to express folate receptors. In addition, antibodies cause problems with background fluorescence and are cleared out by phagocytosis; this causes an underestimation of the number of circulating tumor cells. The conjugates specifically labeled circulating tumor cells, leaving normal cells untouched.

Low and colleagues imaged the labeled cells by multiphoton microscopy. By opting for a 1D line-scanning method rather than the conventional 2D scanning approach, they increased the rate of data acquisition 250-fold.

The researchers detected leukemia cells circulating in the bloodstream of mice. And when they implanted lung cancer cells in mice to mimic cancer patients, they found that the number of circulating tumor cells increased exponentially over time with tumor growth.

Next, the investigators tested their method on blood samples obtained from 12 ovarian cancer patients at various stages of the disease. Except for those patients who were



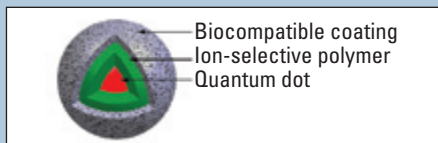
The specificity of in vivo labeling of leukemia cells in the bloodstream of mice. (Adapted with permission. Copyright 2007 National Academy of Sciences, U.S.A.)

at very early stages, all the patients had circulating tumor cell counts greater than background. Similar samples from healthy donors didn't contain the marker cells. (*Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11,760–11,765)

Nano-optode sensors are brighter than QDs and biocompatible

Heather Clark and colleagues at the Charles Stark Draper Laboratory incorporated quantum dots (QDs) into an ion-selective polymer to form enhanced Na^+ nano-optode sensors. The sensors also have a biocompatible coating that allows them to measure intracellular ion concentrations.

The polymer matrix contains a light-absorbing pH indicator (chromoionophore) and an ion-binding molecule (ionophore). As a positive ion binds to the matrix, a hydrogen ion is released, and the pH of the polymer changes; this, in turn, changes the properties of the chromoionophore.



An idealized schematic of an ion-selective nano-optode.

The QD in the nano-optode is chosen carefully so that its emission wavelength overlaps the absorption wavelength of the ion-selective polymer. At low Na^+ concentrations, chromoionophore absorption at this wavelength is high, and the QD's emission fluorescence is absorbed, leading to little visible light. As Na^+ binds, chro-

moionophore absorption decreases, and the QD's fluorescence is not absorbed, so it can be seen by the naked eye.

The researchers found that the dynamic range of their sensor was 1 mM to 1 M, and they observed minimal photobleaching. After adjusting the components of the nano-optode, they could tune the resolution of their sensors to 80 μM at the typical intracellular Na^+ concentration of 17 mM. They also note that a different combination of QD and ion-selective polymer could lead to sensors for other ions. (*J. Am. Chem. Soc.* **2007**, *129*, 8418–8419)

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Optical microcavities for detecting single molecules

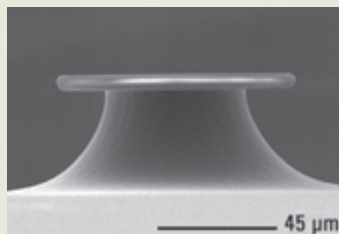
Kerry Vahala and colleagues at the California Institute of Technology have developed a single-molecule detection approach based on optical microcavities in which the target molecule doesn't have to be labeled.

Planar arrays of microtoroid resonators were fabricated in silica. The microtoroids were coupled to a tunable laser and a tapered optical-fiber waveguide and immersed in water. Light inside the microcavities circulated constantly, although a portion of the optical field evanesced into the surrounding liquid.

The investigators functionalized the

surfaces of the microcavities with either biotin or antibodies, and when target molecules were captured, the circulating light sampled them many times. The binding interaction produced red shifts of the resonant wavelength that were monitored in real time.

With this approach, Vahala and colleagues tracked single molecules of interleukin-2 (IL-2), a marker for immune-



A scanning electron micrograph of a microtoroid optical resonator. (Adapted with permission. Copyright 2007 American Association for the Advancement of Science.)

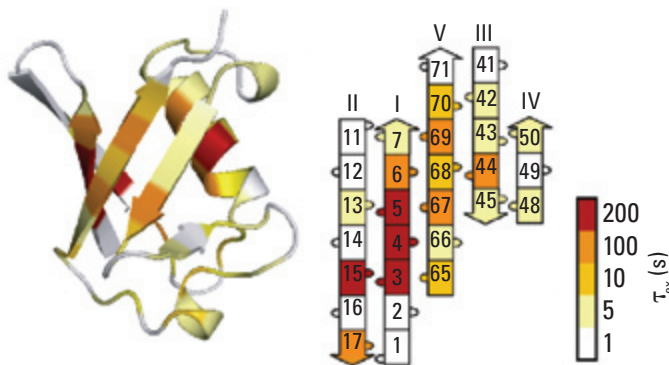
system activation, and determined that the sensor had a working range of 10^{-6} to 10^{-18} M. When the investigators tested serum solutions with 300, 600, or 900 aM of IL-2, the total resonant wavelength shift increased proportionally to the concentration of IL-2, and

the individual binding events were resolved. (*Science* 2007, DOI 10.1126/science.1145002)

Protein folding by fast 2D NMR spectroscopy

NMR can provide a wealth of structural information about stable protein forms but is often too slow to give useful data about dynamic processes, such as folding and unfolding. Conventional 2D NMR experiments require ~100 scans and can record only very slow kinetic events with time constants of minutes to hours. Now, Bernhard Brutscher and colleagues at the Institut de Biologie Structurale Jean-Pierre Ebel and the Institut de Recherches en Technologies et Sciences pour le Vivant (both in France) have developed band-selective optimized flip-angle short-transient (SOFAST) real-time 2D NMR spectroscopy to study protein folding and unfolding on the timescale of seconds to minutes.

The researchers first used apo- α -lactalbumin as a model system to illustrate



Color-coded H/D exchange rates of human ubiquitin at pH 11.95 derived from SOFAST data. (Adapted with permission. Copyright 2007 National Academy of Sciences, U.S.A.)

the SOFAST method. The folding of this protein has been studied in the past by line-shape analysis of individual 2D NMR cross-peaks in a single spectrum, but the technique is limited to slow kinetics and is prone to experimental errors of up to 25%. The team initiated a refolding event by increasing

the pH from 2 to 8 quickly and then recorded a series of SOFAST-heteronuclear multiple quantum coherence spectra at a rate of 0.1 per second. They quantified refolding kinetics for 92 of 121 backbone amide sites in the protein and calculated time constants for folding that agreed well with previously measured values.

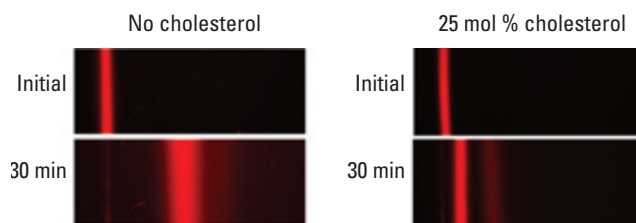
To further demonstrate the potential applications of SOFAST, the researchers measured the unfolding kinetics of ubiquitin by H/D exchange methods. H/D

exchange rates of solvent-accessible sites at high pH are typically too fast to measure by conventional 2D NMR methods. With SOFAST, the investigators quantified H/D exchange kinetics at sites that were not previously detectable by NMR. (*Proc. Natl. Acad. Sci. U.S.A.* 2007, 104, 11,257–11,262)

Separation by solid-supported bilayer electrophoresis

It's tough to separate, purify, and detect lipids and membrane-bound proteins, because it's easy to inadvertently change the native structure of the molecules or even lose them. So, Paul Cremer and colleagues at Texas A&M University have devised a new way to separate components of lipid membranes that doesn't harm the molecules.

The investigators created a solid-supported lipid bilayer out of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and cholesterol. The lipid bilayer acts as a separation medium for electrophoresis, much like the way cross-linked acrylamide or agarose works as a gel for separations in conventional electrophoresis. Molecules that would otherwise disintegrate in a nonlipid en-



A comparison of the band broadening in epifluorescence micrographs of fluorescently labeled lipids migrating in pure POPC (left) and in POPC with 25 mol % of cholesterol (right).

vironment can be introduced into the solid-supported lipid bilayer and migrate via electrophoresis for separation.

After comparing the migration of a fluorescently labeled lipid in pure POPC or POPC with cholesterol, Cremer and colleagues found that the cholesterol was necessary to limit band broadening during the electrophoresis. In the POPC-cholesterol bilayer, the band

resolved into two distinct chromatographic features with an area ratio of ~70:30. After testing the migration of each pure isoform, the investigators confirmed that they were electrophoretically separating the two isomers (which exist in a 70:30 mole ratio) from each other.

A mixture of lipids, tagged with two different fluorescent labels, was next separated in the POPC-cholesterol bilayer to prove that complex mixtures could be resolved. Cremer and colleagues suggest that the method can be extended to the separation and purification of membrane-bound proteins as well as label-free imaging of freeze-dried lipid bilayers. (*J. Am. Chem. Soc.* **2007**, *129*, 8072–8073)

Raman microspectroscopy of mitochondria

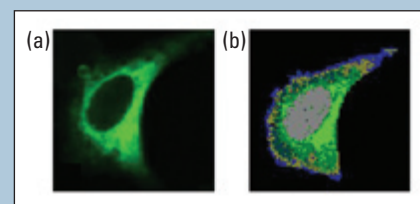
Max Diem and colleagues at Northeastern University have used confocal nonresonant Raman microspectroscopy to track mitochondrial distribution in cells. Mitochondria typically are visualized by electron microscopy or fluorescence-labeling methods. Both of these techniques are at least somewhat invasive, and researchers wanted to find a method that could track mitochondria yet would minimally disrupt the intracellular environment.

In Raman spectra, the changes between regions of a cell are subtle and usually not easily discernible by the naked eye, so the investigators relied on multivariate methods to analyze their spectra. They focused on the spectral range of 1200–1800 cm^{-1} , a region where

protein peaks are abundant, and used hierarchical clustering to divide the spectra into five clusters. Each cluster corresponded to a biochemically distinct region and was pseudocolored to create an image of the cell.

After collecting Raman spectra, the investigators stained the cells with a fluorescent dye specific for mitochondria. When they compared the fluorescence signal from the dye to their Raman images, they found a distinct overlap of one cluster with locations of high mitochondrial distribution.

The researchers note that although their study was carried out on fixed cells, the use of a water-immersion objective would allow the same procedure



(a) A fluorescence image of a HeLa cell after staining with green, mitochondria-specific fluorescent dye. (b) A five-cluster Raman map of the same cell. (Adapted with permission. Copyright 2007 Biophysical Society.)

to be carried out on live, unlabeled cells noninvasively and in their native environment. Diem and colleagues believe that their method could eventually be used to track mitochondrial processes such as migration in real time. (*Biophys. J.* **2007**, *93*, 668–673)