

ANALYTICAL CURRENTS

Sorting rare cells

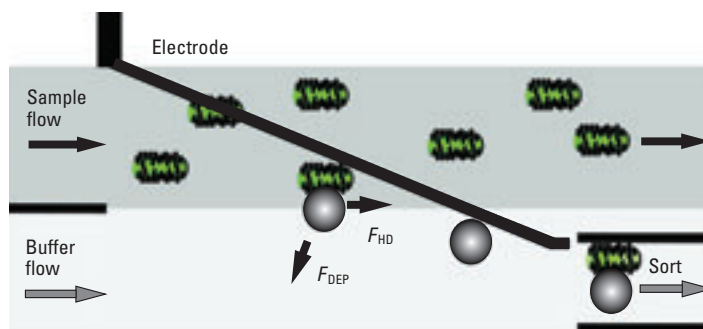
The effectiveness of cell-sorting methods hinges on three parameters—throughput, cell purity, and rare-cell recovery. Of the two most prevalent technologies, magnetic-activated cell sorting offers high throughput but limited purity and recovery, and fluorescence-activated cell sorting offers high yields of pure cells but comparatively lower throughput.

To optimize each of these parameters, Hyongsok Soh, Patrick Daugherty, and colleagues at the University of California, Santa Barbara, developed a microfluidic sorting system that relies on dielectrophoresis (DEP). In DEP-activated cell sorting (DACS), target cells are labeled with polymeric beads, which maximizes their DEP response over that of background cells.

The researchers developed a chamber in which matching electrodes line the top and bottom walls of a microchannel such that the highest field gradient is nearest the walls. Cell suspensions are introduced

along the walls, around a buffer stream that leads to a collection tube. When labeled cells experience the DEP current, they are deflected into the buffer stream, and the remaining cells flow to the waste channel.

Initially, the researchers sorted a sample in which target cells represented 0.02% of the cell population. After a single round of DACS, however, the frequency of target cells increased to 5%. A second round of DACS increased the frequency to 65%. Sorting rates of $>10^7$ cells/h, which are comparable with those of conventional cell sorters, were achieved even without wash steps.



In DACS, cells are deflected into a buffer stream for collection only if they are tagged with a dielectrophoretically responsive label. F_{DEP} = DEP force and F_{HD} = viscous drag force. (Adapted with permission. Copyright 2005 National Academy of Sciences, U.S.A.)

Because of the simplicity of the microfabrication process, the researchers believe that the system easily can be tailored to other cell types. They expect that integration of parallel sorting stages within the microchip will increase throughput and could lead to automated cell sorting in disposable chips. (*Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15,757–15,761)

Versatile mass spectrometer for proteomics

By combining the triple quadrupole (specifically, qQq) front end of a commercial instrument with an FT ion cyclotron resonance (FTICR) mass analyzer, Peter O'Connor and colleagues at the Boston University School of Medicine and MDS Sciex (Canada) created an electrospray FTICR mass spectrometer for biological applications. The new instrument was designed with proteomics in mind, in particular for studying posttranslational modifications and for top-down analysis of protein samples.

The front end contains a focusing rf-

only quadrupole, a resolving quadrupole, and a LINAC quadrupole collision cell (Q2). When these are combined with an FTICR mass analyzer, the system can detect low-abundance species. In addition, it is compatible with a wide range of fragmentation techniques, including nozzle-skimmer fragmentation, Q2 collisionally activated dissociation, multipole storage-assisted dissociation, and electron-capture dissociation.

To demonstrate the performance of the instrument, the researchers used it to analyze phosphorylated peptides. They also

performed top-down sequencing on several proteins, including commercially available and biologically derived ones. The top-down approach allowed for the unambiguous identification of the human E2 ubiquitin-conjugating enzyme, UbcH10.

According to the researchers, one of the key features of the instrument is its versatility for addressing numerous biological problems. Other advantages include increased selectivity of precursor ion species, sensitivity, and speed. (*J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1985–1999)

ANALYTICAL CURRENTS

A sense of structure

The two main methods for determining protein structure—NMR spectroscopy and X-ray crystallography—each have distinct limitations and benefits. To determine whether one method is preferable to the other or whether the two can serve complementary purposes, two groups involved in the Northeast Structural Genomics Consortium screened >400 proteins by both methods.

The researchers produced ^1H - ^{15}N heteronuclear single-quantum correlation (HSQC) spectra of each protein, believing that well-resolved HSQC spectra indicated folded proteins that were amenable to NMR spectroscopy. They then categorized the results as excellent, good, promising, or poor and/or unfolded on the basis of parameters such as spectral dispersion, line widths, and number of resolved peaks. Similarly, they screened each protein for its ability to produce diffraction-quality crystals and, when possible, they determined 3-D structures.

Of the 420 proteins studied, the researchers could determine the structures of only a handful by both methods. Furthermore, although good HSQC spectra did indicate how well a protein was folded, the spectra were poor indicators of whether the protein could form crystals or whether its structure could be determined by X-ray crystallography.

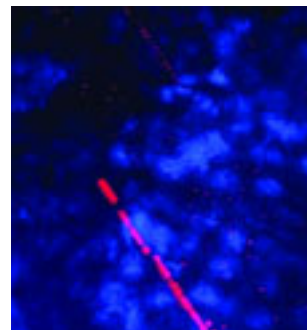
At the same time, although each method was applicable to only a subset of the proteins studied, the researchers found that a concerted approach with both methods increased the likelihood of a 3-D structure being calculated for a given protein. Thus, the groups recommend that a parallel approach with both NMR spectroscopy and X-ray crystallography be pursued to maximize the success rate of any large-scale structural study. (*J. Am. Chem. Soc.* **2005**, *127*, 16,505–16,511; 16,512–16,517)

Imaging with gold nanorods

Because visible light does not penetrate tissues well, researchers have been attempting to develop imaging agents that can be excited by light at other regions of the spectrum, such as near-IR. One group, led by Alexander Wei and Ji-Xin Cheng at Purdue University, has developed gold nanorods that can be imaged in vivo by two-photon luminescence (TPL) microscopy. The nanorods are excited in the near-IR region; this makes them well suited for imaging tissues.

The researchers characterized the excitation and emission properties of the nanorods. The intensity of the signal depended on the polarization of the excitation light. The greatest intensities were observed when the polarization angle of the beam was aligned along the long axis of a nanorod. The TPL emission spectrum, however, was depolarized. Wei, Cheng, and co-workers also compared nanorod luminescence with the fluorescence of rhodamine. They determined that a single gold nanorod is $\sim 60\times$ brighter than a single rhodamine molecule. On the basis of these data, the researchers conclude that the TPL signal intensity of gold nanorods is comparable with that of quantum dots.

Finally, gold nanorods were injected into the tails of mice to determine whether the nanorods could be used as in vivo imaging agents. Individual nanorods were visible by TPL microscopy in two earlobe vessels of a mouse 5 min after injection. The signal was no longer detected after 30 min. According to the researchers, the properties of gold nanorods suggest that the imaging agents could be useful for in vivo, cell-specific labeling studies in the future. (*Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15,752–15,756)



Gold nanorods (small red dots) are visible in mouse blood vessels. (Adapted with permission. Copyright 2005 National Academy of Sciences, U.S.A.)

Cystic fibrosis breath analysis

Cystic fibrosis (CF) patients often develop bacterial infections in their lungs. Because many bacteria produce sulfide gases, the presence of sulfides in breath could potentially be used as a noninvasive marker for cystic fibrosis (CF). To test this idea, F. S. Rowland and co-workers at the University of California, Irvine; the University Hospital of the Rheinisch-Westfälische Technische Hochschule (Germany); and Long Beach Memorial Medical Center analyzed the levels of carbonyl sulfide (OCS), dimethylsulfide (DMS), and carbon disulfide (CS_2) in the breath of CF patients and healthy controls. The gas compositions were determined by ultra-trace GC/MS.

The researchers did not find a statistically significant correlation between increased sulfide gas concentrations and positive bacterial cultures in the CF group. A wider range of exhaled OCS, DMS, and CS_2 concentrations was observed for the CF group than the control group. OCS and CS_2 levels were higher in samples from some CF patients than in those from other CF patients or healthy controls. Poor pulmonary function was correlated with high OCS concentrations only. (*Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15,762–15,767)

Chiral separations on chip

To facilitate the use of plastic microchips for applications such as proteomics, Baohong Liu and colleagues at Fudan University (People's Republic of China) modified the surface of PMMA microchannels with an alumina gel-derived protein network. The modified microchannels can encapsulate target biomolecules, such as bovine serum albumin (BSA); this makes them well suited for on-chip analysis of chiral interactions.

Liu and colleagues immobilized BSA in the modified microchannels to create a new protein stationary phase. They then used it to separate D- and L-tryptophan with electrochemical detection. The modified surface was more stable and hydrophilic than native PMMA microchannels and exhibited less nonspecific adsorption.

The general approach is not limited to the study of the interaction of an enantiomeric amino acid with BSA. According to the researchers, it could be used to study interactions with other biomolecules, such as antibodies, peptides, or whole cells. (*J. Proteome Res.* **2005**, doi 10.1021/pr050240j)

QD-based DNA nanosensor

To detect specific DNA sequences without first performing an amplification step, Tza-Huei Wang and colleagues at Johns Hopkins University developed an ultrasensitive nanosensor that uses quantum dots (QDs) linked to DNA probes. The nanosensor can detect <50 copies of DNA and has much less background fluorescence than conventional fluorescence resonance energy transfer (FRET) systems.

The nanosensor includes two oligonucleotide probes that bind to separate regions of an assayed strand of DNA (target DNA). The reporter probe is labeled with a Cy5 fluorophore, and the capture probe is labeled with biotin. The probes bind the target DNA, and a streptavidin-coated QD binds to the capture probe. This process brings the Cy5 molecule close to the QD so that FRET can occur. Because QDs have broad absorption and narrow excitation spectra, the QD-based nanosensor system can be finely tuned so that background fluorescence is negligible. QDs also concentrate the FRET signal by binding many target-probe complexes.

To detect FRET signals, the researchers developed a novel confocal fluorescence microscopy platform. The QD-target-probe complexes were continuously flowed through a microcapillary past two detectors that were specific for signals from either FRET donors or acceptors.

When the system was tested with a single-stranded target DNA, fluorescence was observed with both detectors. However, when the target was absent or when a non-complementary DNA strand was used, only donor fluorescence was observed. Wang and colleagues also compared the performance of the QD nanosensor with that of a molecular beacon, which is typically used in FRET assays. The QD nanosensor was ~100× more sensitive.

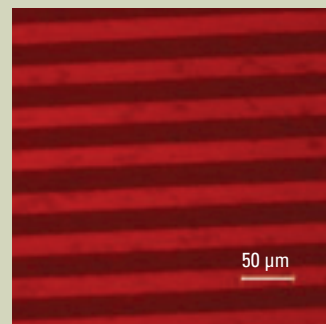
Finally, the researchers combined the nanosensor with an oligonucleotide ligation assay. This method allowed the discrimination of point mutations in DNA samples from ovarian cancer patients. According to the researchers, QD nanosensors could also be applied to non-DNA molecules, such as proteins. (*Nat. Mater.* **2005**, *4*, 826–831)

Plasma-initiated micropatterning

Biomolecular micropatterns are used in numerous applications, including biosensors, DNA microarrays, and immunoassays. Although several micropatterning methods exist, they vary in terms of cost, ease of use, reproducibility, and applicability to specific ink-substrate combinations. To overcome some of the limitations of existing methods, including slow pattern formation, Kathryn Uhrich and Bryan Langowski of Rutgers University have developed a simplified technique that creates biomolecular micropatterns of varying complexity on organic, biocompatible polymer substrates. The method is cost-effective and reproducibly generates well-resolved microscale patterns in ~8 min.

In the new technique, which is called microscale plasma-initiated patterning, a patterned PDMS stamp is used to protect some areas of the substrate from oxygen plasma while allowing other areas to be exposed. As a result, distinct microscale domains with different hydrophilicities are formed on the substrate. The different regions exhibit varying affinities for a given biomolecule.

The researchers evaluated several inks, including goat anti-rabbit immunoglobulin G, poly-L-lysine, and bovine serum albumin (BSA). Protein patterns of BSA were stable after 4 weeks of incubation at 37 °C. (*Langmuir* **2005**, *21*, 10,509–10,514)



Fluorescent micrograph of a simple protein pattern of BSA on PDMS generated by plasma-initiated micropatterning.