

ANALYTICAL CURRENTS

Size-selective lipid bilayer for binding assays

Paul Cremer and colleagues at Texas A&M University have developed a way to measure protein–ligand binding in a supported lipid bilayer while filtering out other proteins on the basis of size. All of this is done in microfluidic channels. The investigators say future chip-based sensors could incorporate such thin films to select analytes of the appropriate size from a mixture.

The supported bilayer contained two kinds of lipids: One type was modified with a ligand recognized by incoming protein analytes, and the other was conjugated to poly(ethylene glycol) (PEG). PEG acted as a size-exclusion filter because the conformation of its polymer chains prevented analytes larger than a certain size from reaching the ligand in the bilayer.

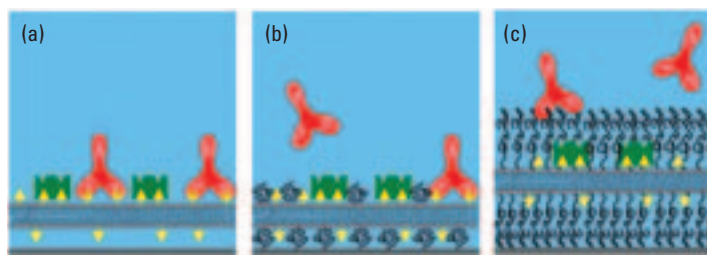
Cremer and colleagues tested bilayers in which biotin was the ligand and the amount of PEG was varied from 0 to 1.5 mol%. Solutions of fluorescently

labeled streptavidin or antibody–biotin immunoglobulin G (IgG) were flowed over the bilayers. Streptavidin's binding to biotin

wasn't appreciably altered by PEG because of the protein's small size (52.8 kD). But IgG is much larger (150 kD), and its binding to biotin in the presence of 0.5 mol% PEG dropped to ~60% of the value obtained in the absence of PEG. At 1.5 mol% of PEG, IgG's binding to biotin was completely inhibited.

The investigators further tested PEG's filtering capabilities by flowing a mixture of streptavidin and IgG over a biotin-modified bilayer containing 1.5 mol% PEG. As controls, solutions of

streptavidin or IgG were introduced into biotin-modified bilayers that didn't contain PEG. Although the concentration of streptavidin bound to the biotin was indistinguishable from the mixture to the control, the amount of bound IgG was reduced by ~2 orders of magnitude in the mixture. Cremer and colleagues point out that the filter can be applied to a variety of receptor–ligand pairs. (*J. Am. Chem. Soc.* **2006**, doi 10.1021/ja062010r)



A lipid bilayer (a) without and (b) with PEG. (c) PEG acts as a size-selective filter and prevents large proteins from binding to the ligand (yellow).

Probing biological noise

Quantitative analysis can be muddled by the intercellular variability—biological noise—that comes from differences in protein abundance due to an organism's life-cycle stage and other factors. Many theories have been proposed to explain this noise. Now, two independent research groups have examined the biological factors systematically and offer some explanations for the variability.

Both groups used flow cytometry and fusion proteins with green fluorescent protein to quantify intercellular and intraculture variability in strains of the yeast *Saccharomyces cerevisiae*. Jonathan Weissman,

John Newman, and colleagues at the University of California, San Francisco, and the California Institute for Quantitative Biomedical Research examined >4000 strains grown in rich and minimal media. Nava Barkai, Yitzhak Pilpel, and colleagues from the Weizmann Institute of Science (Israel), Harvard University, and the University of Cambridge (U.K.) studied 43 strains under 11 growth conditions.

Both groups found that although the overall abundance of a given protein correlated inversely with noise levels, the greatest impact seemed to be caused by stochastic fluctuations in mRNA levels;

these fluctuations were caused by either changes in mRNA half-life or gene activation. The investigators also noted that genes physically located within the same region of the chromosome exhibited similar noise levels; this suggested a possible role for chromatin structure. A strong correlation was seen at the level of gene regulation—different genes controlled by the same transcription factors exhibited comparable protein noise patterns. The researchers suggested that this intercellular variation may offer an evolutionary advantage. (*Nature* **2006**, doi 10.1038/nature04785; *Nat. Genet.* **2006**, *38*, 636–643)

ANALYTICAL CURRENTS

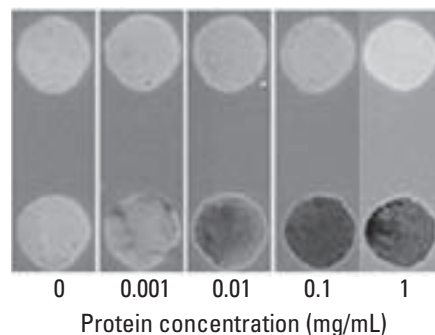
Quantitative imaging of proteins on chips

When bombarded by electrons, metal surfaces release secondary electrons, the intensity and number of which are influenced by the chemical nature of the metal surface. Ralph Nuzzo and colleagues at the University of Illinois, Urbana–Champaign, harnessed these emissions to quantitatively study protein adsorption.

The researchers used soft lithography, microcontact printing, and microfluidic patterning to generate self-assembled monolayers on the surfaces of gold chips. By using scanning electron microscopy, they determined that electron image intensity decreased as the thickness of the surface layer increased when they added functionalized acrylamide polymers. They also noted that

the nature of the acrylamide conjugates affected the degree of the intensity variation—the sulfur atom of a thioether moiety attenuated secondary electrons differently than molecules of poly(ethylene glycol).

Nuzzo and colleagues then adsorbed different proteins, such as bovine serum albumin, lysozyme, and fibrinogen, to the surface, either directly or via biotin–streptavidin interactions. They noted that each of the proteins bound to the surface to a different extent and that they could monitor this process quantitatively from the secondary electron response. Image contrast correlated with not only surface thickness but also the molecular weight of the proteins tested; this suggests that secondary elec-



Changes in protein adsorption to a gold chip were monitored by secondary electron imaging in a scanning electron microscope.

tron imaging could feasibly be used as a quantitative protein affinity assay. (*J. Am. Chem. Soc.* **2006**, doi 10.1021/jac060248+)

Crystal monitors live cells in real time

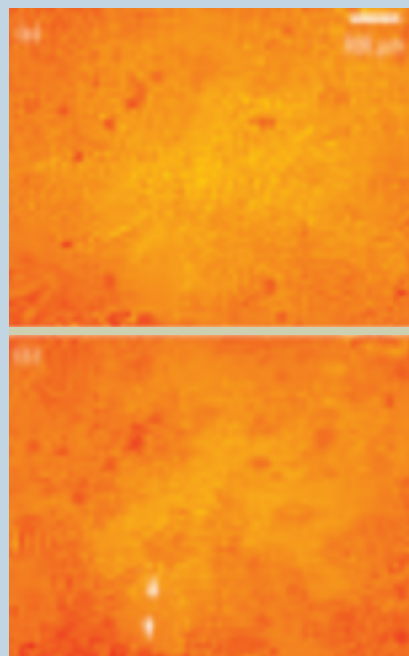
Biosensors that use cells often have trouble converting changes in cellular activity to real-time electronic signals. To overcome the problem, Michael Sailor and colleagues at the University of California, San Diego, have developed a photonic crystal for which the intensity of the light scattered by the crystal follows physiological changes in cells in real time. The investigators suggest that crystals could allow cellular libraries to be multiplexed for high-throughput screens.

Sailor and colleagues filled a porous silicon film with polystyrene and treated it with O₂ plasma to turn the surface hydrophilic. Rat liver cells were grown on the surface. Light scattered by the crystal significantly increased when the cells were exposed to a toxic dose of Cd²⁺; corresponding optical images verified changes in the cell morphology in response to the toxin. The investigators demonstrated that the

changes in scattering intensity from the crystal were picked up ~2 h before a conventional cell-staining method began to show a loss in cellular viability.

Sailor and colleagues next monitored the responses of cells grown on such crystals when the cells were exposed to 10- and 40-mM doses of *N*-acetyl-*p*-aminophenol (APAP; also commonly known as acetaminophen). They compared the data to those obtained by a standard ethidium live/dead cell assay and phase-contrast microscopy.

All three assays showed that a 10-mM dose of APAP wasn't toxic. However, the scattering intensity of the crystal increased within 2 h of the addition of the 40-mM dose; the largest change was observed after 8 h of exposure. In contrast, microscopic images revealed cell death within a few hours, and the ethidium assay showed first signs of cell death after ~12 h of APAP exposure. (*Langmuir* **2006**, doi 10.1021/la060420n)



Optical images of rat liver cells grown on a polystyrene-filled silicon crystal demonstrate the changes in morphology (a) before and (b) after exposure to a toxic dose of cadmium chloride solution.

Combined IRMPD and ECD for proteomics

IR multiphoton dissociation (IRMPD) and electron capture dissociation (ECD) provide different types of sequence information. If the two methods could be performed simultaneously, increased sequence coverage could be obtained. So, Ron Heeren and co-workers at the FOM Institute for Atomic and Molecular Physics (AMOLF) and the Utrecht Institute for Pharmaceutical Sciences (both in The Netherlands) have designed an FT ion cyclotron resonance (FTICR) mass spectrometer with overlapping electron and IR beams. With the new configuration, the researchers obtained

higher sequence coverage with the combined method than with either one alone; this feature increases the confidence in peptide and protein assignments.

Previous attempts to combine IRMPD and ECD on FTICR mass spectrometers have failed to completely overlap the electron and IR beams, both of which have been introduced from the rear of the instrument. To overlap the beams in the new design, Heeren and co-workers use a pneumatic probe and an IR mirror to direct the IR beam from the front of the instrument to the ion cloud in the ICR cell. The electron

beam originates from the rear of the mass spectrometer.

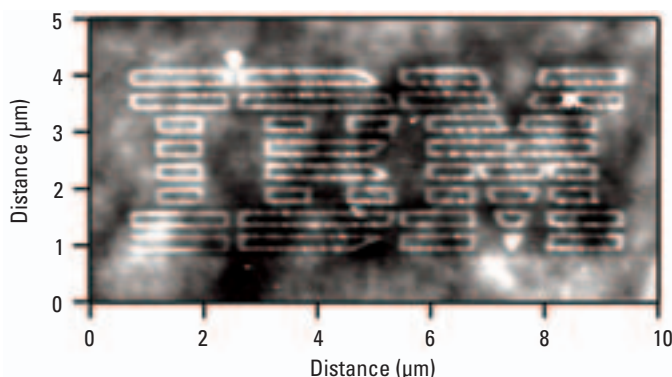
The researchers tested the new design by fragmenting substance P and mellitin. For both molecules, the sequence coverage was higher when simultaneous IRMPD and ECD were performed than with either method alone; the combined approach also produced fragments in a shorter period of time. Because the timing of the combined method is compatible with LC, the researchers say that simultaneous irradiation is ideal for proteomics experiments. (*Rapid Commun. Mass Spectrom.* **2006**, *20*, 1838–1844)

AFM takes on electrophoresis

No longer limited to imaging and measuring forces, atomic force microscopy (AFM) has now tackled electrophoresis. H. Kumar Wickramasinghe and colleagues at the IBM Almaden Research Center have tweaked the AFM cantilever so that DNA oligonucleotides can migrate electrophoretically on it within milliseconds.

In the new setup, the molecules moved down a sharp pyramidal probe, which measured 11.2 μm from base to tip. The probe sat at the end of a cantilever that raster-scanned over a surface, just as in a conventional AFM setup. Wickramasinghe and colleagues created a trench, 150 nm wide and 0.8 μm deep, around the base of the probe; this acted as a reservoir for the molecules.

The probe was covered in a hydro-



AFM image of a 5-mer DNA oligonucleotide patterned onto a surface. (Adapted with permission. Copyright 2006 American Institute of Physics.)

philic poly(ethylene glycol)silane coat so that a 2-nm layer of water condensed on it. The top of the cantilever and the surface were modified with conductive coatings so that they acted as electrodes. When an electric field of $\sim 10^6$ V/m was applied between the cantilever and the

surface, the molecules ran from the reservoir toward the tip of the probe through the water layer. When the molecules reached the tip, they jumped from the probe onto the surface. By scanning the probe, the investigators could pattern molecules onto the surface, much like in dip-pen lithography.

The migration speeds of the molecules depended on their electrophoretic mobilities. A 5-mer DNA oligonucleotide took 15 ms to migrate to the tip, and a 16-mer took 5 ms.

Electrophoresis wasn't limited to DNA: Streptavidin, a neutrally charged protein, also could migrate through the water layer. The protein's migration demonstrated that electroosmotic flow could occur on the probe. (*Appl. Phys. Lett.* **2006**, doi 10.1063/1.2195777)

ANALYTICAL CURRENTS

3D tracking with spectroscopic readouts

Haw Yang and colleagues at the University of California, Berkeley, and Lawrence Berkeley National Laboratory have described an apparatus that tracks moving nanoparticles in three dimensions and simultaneously provides spectroscopic measurements. The researchers added an xyz piezoelectric stage and a near-IR laser to a conventional confocal microscope. To demonstrate the system, the team monitored an individual gold nanoparticle in solution. As the laser beam hit the nanoparticle, the scattered light was split into two feedback signals that guided the movement of the stage in the xy plane and brought the nanoparticle back into focus in the z direction. A green laser beam was used to collect the spectroscopic information. The system is noninvasive and has a spatial resolution of <math><210\text{ nm}</math> and a response time of <math><1\text{ ms}</math>. (*Appl. Phys. Lett.* **2006**, *88*, 223901)

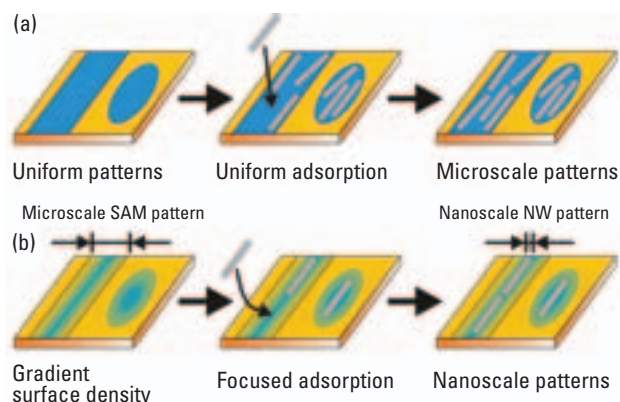
Patterning nanowires by a “lens” effect

The ability to create patterns of nanowires (NWs) on surfaces is necessary for building devices such as field effect transistors and biological or chemical sensors. However, manipulating the layout of NWs over large areas is difficult. Seunghun Hong and colleagues at Seoul National University (Korea), Northwestern University, and Florida State University have now developed a way to create nanoscale patterns of NWs on microscale self-assembled monolayers (SAMs).

The investigators directed the assembly of both V_2O_5 NWs and single-walled carbon nanotubes (SWCNTs) on two types of SAMs. One was a uniform layer of cysteamine and 1-octadecanethiol. The other consisted of regions with gradients—the surface density of cysteamine reached a maximum at the center of each region and gradually decreased toward the boundary.

Both the V_2O_5 NWs and SWCNTs assembled evenly over the uniform SAM surface. However, the V_2O_5 NWs and SWCNTs assembled at the centers of the regions on the gradient SAMs, where the density of cysteamine was the highest, and left the remaining areas empty. The researchers call this a lens effect, because the gradient directs the assembly of NWs or SWCNTs in a manner analogous to an optical lens focusing light.

The V_2O_5 NWs focused on 80-nm-wide areas in the center even though the cysteamine region in the SAM was $>2\text{ }\mu\text{m}$ wide. The investigators suggest that the negatively charged NWs were strongly attracted to the region with the highest density of positive charge. With the SWCNTs, Hong and colleagues demonstrated that they could fine-tune the adsorption so that only a few nanotubes lay down in the center of the cysteamine region of the SAM. (*J. Phys. Chem. B* **2006**, *110*, 10,217–10,219)



NWs and SWCNTs can be patterned in a controlled manner on (a) uniform and (b) gradient SAMs.