In AC Research has been discontinued. Please see the research contents on p 1C instead.

ANALYTICAL CURRENTS

Subtractive proteomics nets nuclear envelope proteins

For Laurence Florens, John Yates, and colleagues at the Scripps Research Institute, subtractive proteomics adds up to a comprehensive look at nuclear envelope (NE) proteins. With this approach, the researchers find all 13 known integral NE proteins and dozens of previously uncharacterized open reading frames that are predicted to have membrane-spanning regions. The genes for more than a third of these putative proteins were mapped to chromosome regions associated with various types of dystrophy.

To examine only NE proteins, the researchers prepared both NE fractions and microsomal membrane fractions from rodent liver tissue and analyzed them separately. The microsomal membrane fractions did not contain NE proteins, but they did contain mitochondrial membranes and other contaminants of the NE fraction. Subtracting the proteins in the microsomal membrane fractions thus yielded a clean set of NE proteins. The researchers analyzed four times more microsomal membranes to boost the representation of minor endoplasmic reticulum proteins.

The researchers took advantage of the previously described MudPIT technology, which couples multiple LC steps with tandem MS, to analyze very large numbers of peptides. In this case, >30,000 peptides were analyzed, resulting in 2391 protein identifications. Of greatest interest were 67 previously uncharacterized open reading frames that represented potential integral NE proteins. For eight of these frames, proteins were made from cDNAs and tested, and all of them were targeted to the NE. The genes for 23 of these putative proteins were mapped to chromosome regions linked to 14 dystrophies.

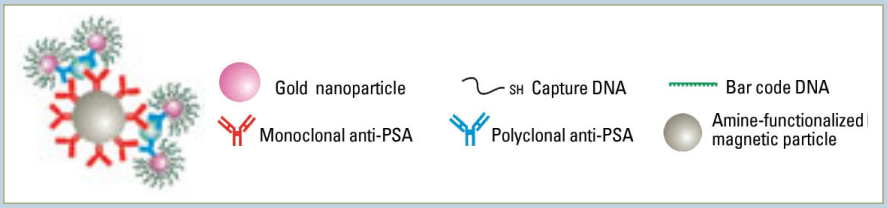
The researchers suggest that they found considerably more proteins than were found in an earlier proteomic analysis because of the sensitivity of the tandem MS and because they avoided gels, and thus the losses associated with gel extractions, and used whole tissue instead of a cell line. (*Science* **2003**, *301*, 1380–1382)

Attomolar detection of proteins

Chad Mirkin and colleagues at Northwestern University have developed a new ultrasensitive method that can detect prostate-specific antigen (PSA) at 30 aM. Adding a PCR step to the protocol drives down the limit of detection to 3 aM, which the researchers say is 6 orders of magnitude more sensitive than commercial assays.

The researchers use magnetic microparticles (MMPs) with bound monoclonal PSA antibodies in addition to gold nanoparticles (NPs) with bound polyclonal PSA antibodies and hybridized oligonucleotides, which are called bar codes. When the modified MMPs and NPs are added to a sample, both types of antibodies bind to PSA molecules to form a sandwich. To remove the MMP–PSA–NP complexes, Mirkin and colleagues apply a magnetic field to the reaction container. Washing the complexes dehybridizes the bar codes, which are left behind after another application of a magnetic field.

The residual single-stranded bar codes are analyzed in one of two ways. The researchers devised a PCR-less method, in which the bar code is sandwiched between a chip-bound oligo that is complementary to half of the bar code sequence and an NP-bound oligo complementary to the other half of the sequence. Mirkin and colleagues perform a scanometric assay in which the sandwich is exposed to a silver amplification solution and imaged on a flatbed scanner. This approach allows the researchers to quantitate the amount of recovered bar code DNA. Alternatively, PCR can be performed on the bar code DNA prior to scanometric detection to increase sensitivity. (*Science* **2003**, *301*, 1884–1886)



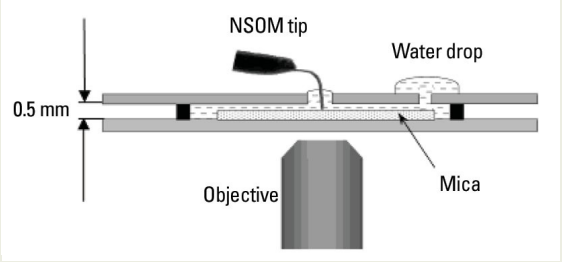
Magnetic microparticle–prostate-specific antigen–gold nanoparticle sandwich. (Adapted with permission. Copyright 2003 American Association for the Advancement of Science.)

ANALYTICAL CURRENTS

NSOM turns over a new leaflet

Imaging biological samples in aqueous environments is a challenge with current near-field scanning optical microscopy (NSOM) probes. That's why L. J. Johnston and co-workers at the National Research Council Canada fabricated their own bent optical probes using a two-step chemical etching technique and focused ion beam milling. Other scientists have fabricated and used bent probes for imaging in fluids; however, the probes suffered from poor reproducibility. The new probes create more reproducible, high-contrast images and have transmission efficiencies that are 2 orders of magnitude greater than those of comparable pulled probes.

Phospholipid monolayers and bilayers deposited onto a mica sheet by Langmuir–Blodgett transfer were imaged with the new probe. The samples contained dipalmitoyl–phosphatidylcholine/dilauroyl–phosphatidylcholine (DPPC/DLPC) mixtures. A dye-labeled lipid was added to visualize the fluid and gel phases.



Schematic of the NSOM setup.

DPPC/DLPC monolayers looked similar when examined by both NSOM and atomic force microscopy (AFM), but the stripes of aligned DPPC molecules measured ~100 nm wide with AFM and ~200 nm wide with NSOM. The NSOM resolution was lower because the NSOM probe aperture had a wider diameter than the AFM tip. Both leaflets of the phospholipid bilayers could be independently imaged with either technique. The researchers also noted that the NSOM tip does not rupture the bilayer.

Because upper and lower leaflets of DPPC/DLPC bilayers look different when the opposite leaflet has a homogeneous composition, Johnston and co-workers wanted to see if the configuration of the bottom leaflet could determine the appearance of the upper leaflet. They created bilayers in which both leaflets were mixtures of DPPC and DLPC. Indeed, the researchers observed stripes of DPPC in the upper leaflet, which leads them to conclude that the bottom leaflet serves as a template when the upper leaflet is transferred to form a bilayer. (*Langmuir* **2003**, *19*, 9246–9254)

Seeing SNPs

David Ward and colleagues at Yale University School of Medicine; ThermoBioStar, Inc.; the Oklahoma City Veterans Affairs Medical Center; the University of Oklahoma Health Sciences Center; and the Denver Veterans Affairs Medical Center have developed a single-nucleotide polymorphism (SNP) multiplex assay on a thin-film biosensor chip. Under optimal conditions, as little as 100 amol of DNA was detected in <45 min.

To discriminate between alleles, pairs of oligonucleotides (P1) are attached to the chip surface by their 5′ ends in an array. The 3′-terminal sequences differ by one base at the site of the SNP. P2 probes, which include a biotin moiety, are added. Together, the P1 and P2 probes form the sequence complementary to target DNA, which is amplified by PCR from a patient.

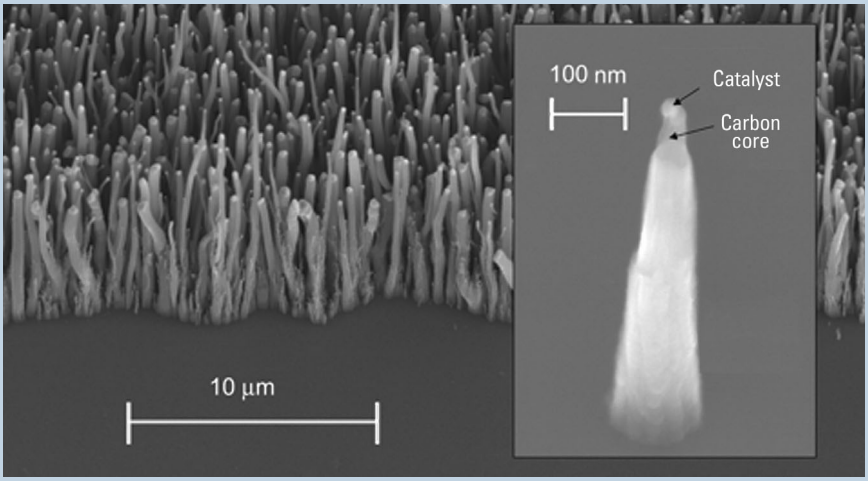
Ligation of P1 to P2 and hybridization of the target sequence occur simultaneously. The researchers use a mutant DNA ligase that preferentially joins perfectly matched sequences. Thus, only completely complementary target DNA molecules remain bound to P1 and P2 after the chips are washed. Immobilized P2 is detected by adding an enzyme, coupled to antibiotin, that acts on a precipitable substrate. The precipitate causes the chip surface to change from gold to blue or purple, which can be visualized by eye or by a digital imaging system.

Ward and colleagues apply the new assay to the detection of SNPs from people at high risk for venous thromboembolism (VTE). An array of three known SNPs that contribute to VTE was spotted, and 100 target DNA samples were analyzed. The results were identical to those obtained by the Third Wave Invader system, a more costly method.

In another experiment, the researchers tested samples from 50 African-American individuals for 20 SNPs on chromosome 17. Four of these SNPs were previously analyzed by other methods. The results from the new assay differed for three of the SNPs. When researchers reexamined the prior restriction fragment length polymorphism (RFLP) data, they determined that these data were erroneous. Thus, Ward and colleagues conclude that their chips are more accurate than RFLP analyses. (*Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11,559–11,564)

Carbon nanofiber electrodes

Although conventional glassy carbon, carbon paste, pyrolytic, and graphite electrodes are all well characterized, the electrochemical nature of newly developed carbon fibers is not. Timothy McKnight and colleagues at the Oak Ridge National Laboratory, the University of Tennessee, and Kansas State University examine the impact of certain fabrication methods on electrode response and show that the large surface area that results from aligning carbon nanofibers vertically has some beneficial effects.



Scanning electron micrograph of a carbon nanofiber forest and an isolated carbon nanofiber (inset).

Nanofibers are cylindrical to conical in shape, but unlike nanotubes, they have internal caps within the hollow core. In this study, densely packed, randomly spaced nanofiber "forests" were grown on either a 5-mm-diam area or on discrete 250-μm-wide electrodes.

Unmodified 5-mm-diam nanofiber forest electrodes were tested after 5 months of storage in a common wafer container. Even without special precautions—the container allowed extended exposure to air and airborne contaminants—and without activation, the electrodes exhibited fast electron-transfer kinetics and near-ideal cathodic and anodic peak separations. The researchers suggest that activity was preserved during the long storage because the surface area is ~10× greater in the forest electrodes than in planar electrodes. Thus, the electrodes operate in an extreme diffusion-limited regime, in which the extra surface area compensates for any inactivation that may have occurred.

Discrete electrodes were not as highly active as their forest counterparts, which the researchers suggest may be because of the additional processing steps—particularly refractory metal reactive ion etching—needed for their fabrication. On the other hand, partial passivation of the electrode surface was achieved by covering it with an oxide coating. Doing this actually improved activity, most likely because surface hydrophilicity increased, which improved wetting, and surface contaminants were removed. Oxygen-reactive ion etching, which is used to improve conventional carbon electrode activity, also increased activity on the discrete nanofiber electrodes.

However, the same processing techniques had considerably less effect on and were not consistent for the 5-mm-diam electrodes. The researchers speculate that this result may be due to the inability to reach the deep recesses of the forest while the smaller area, with larger borders, may be easier to modify. This inaccessibility may be an advantage in some cases, though, because the electrode forest is more stable and resistant to deactivation. (*J. Phys. Chem. B* **2003,** *107,* 10,722–10,728)

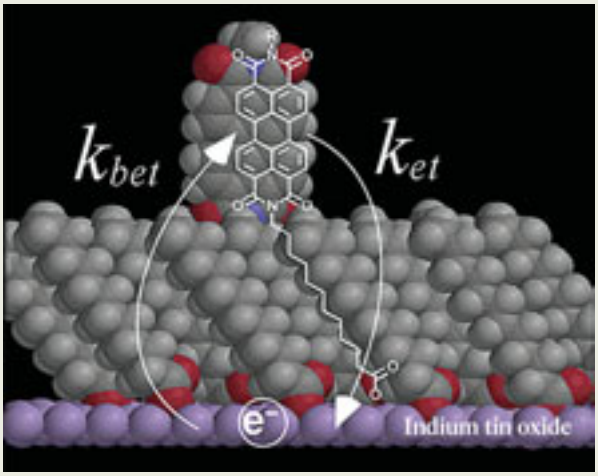
ANALYTICAL CURRENTS

SMS for molecular electronics

Molecular-scale components have been suggested as possible wiring for future computer and electronics applications. However, more needs to be known about electronic interactions between molecules. For instance, experiments on the interactions between molecules and electrodes have been conducted, but they only look at the average effect, not at single molecules. To understand the electron transfer from one molecule to an electrode, David Adams and colleagues at Columbia University are applying single-molecule spectroscopy (SMS).

The researchers use SMS to examine photoinduced interfacial electron transfer rates in a prototypical chromophore–bridge–electrode system, in which a perylene bisimide chromophore 1 self-assembles in a mixed monolayer with a carboxylic acid terminated aliphatic bridge on an indium tin oxide electrode. The chromophore typically relaxes from its excited state radiatively, though it occasionally injects a charge into the electrode and creates a radical.

Whenever the chromophore injects an electron back to the electrode instead of fluorescing, it causes a short (>20 ms to seconds) break in fluorescence. Charge recombination, or back electron transfer, leads to resumption of fluorescence. Each blink on or off represents a single electron transfer event, and the forward and back electron transfer rates were calculated from the average lifetimes of the on and off states. The latter was determined by constructing histograms of the durations of the on and off periods for individual molecules and then fitting exponential curves to the histograms to calculate the average lifetimes.



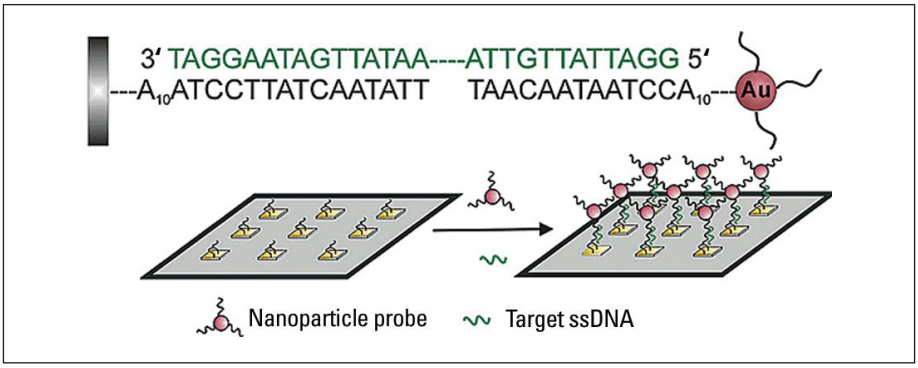
A schematic of electron transfer from a chromophore through a monolayer to an electrode, and the back transfer of an electron.

Adams and colleagues suggest that this single-molecule technique may be applicable in many systems and that future studies may examine the effects of the bridging molecules' length and of different functional groups on electron transfer rates. Both are of interest in molecular electronics. (*J. Am. Chem. Soc.* **2003,** *125,* 12,649–12,654)

Nanoparticles and diffraction gratings for DNA detection

Although nanoparticles have already been used to detect DNA, Chad Mirkin, Joseph Hupp, and colleagues at Northwestern University introduce a new real-time method that combines nanoparticles and micropatterned chemoresponsive diffraction gratings. This approach has the high sensitivity and selectivity of nanoparticle probes and the experimental simplicity, wavelength-dependent resonance amplification, and miniaturization potential of the diffraction-based technology, the researchers say.

In the new setup, single-stranded DNA (ssDNA) oligonucleotides are identified by attaching a complementary DNA sequence to both the diffraction grating surface and the surface of the nanoparticle. When the ssDNA targets are present, they link the nanoparticles to the surface. The plasmon absorbance of the nanoparticles anchored on the grating causes local refractive index changes,



A schematic showing how nanoparticles and target DNA attach to a patterned, functionalized gold diffraction grating.

which are easily measured as changes in diffraction efficiency. If the nanoparticle probe is heavily functionalized with complementary strands, significant signal amplification occurs, and the diffraction responses are markedly different at various laser wavelengths.

On the basis of data collected from nano- and picomolar concentrations of a target, the researchers extrapolate detection limits of 40–900 fM. In practice, the researchers say, the limit could be much worse if the target winds up spread very sparsely over a large grating area. However, they add that smaller grating areas could be used.

Mirkin, Hupp, and colleagues note that this technique's detection limits compare favorably to those of molecular fluorescence, but the new method has increased sequence specificity. They also indicate that some aspects of the concept can be compared to surface plasmon resonance, since both detect changes in refractive index. (*J. Am. Chem. Soc.* **2003**, *125,* 13,541–13,547)

Protein biochip uses force

Protein chips typically determine whether two molecules are bound. However, much more information can be gleaned if the unbinding force can be determined. For instance, specific and nonspecific binding may be differentiated by how much force is required to separate the molecules. Hermann Gaub and colleagues at the Lehrstuhl für Angewandte Physik and Center for NanoScience (Germany), Johnson & Johnson Pharmaceutical Research and Development, Ortho-Clinical Diagnostics, and nanotype GmbH (Germany) introduce a parallel assay for measuring unbinding forces on multiple single molecules.

A schematic of the force sensing capability of DNA zippers. (a) The zippers are attached, with a reporter, to the top plate, and (b) when the lower surface is brought in proximity, the antigens bind to the antibodies but not other proteins. (c) The plates are moved apart, and (d) the strong antibody–antigen bonds remain intact while the weaker DNA bonds break. (Adapted with permission. Copyright 2003 National Academy of Sciences, U.S.A.)

The technique uses DNA zippers as molecular force sensors, which has been done before, but only with atomic force microscopy tips. In the case described by Gaub and colleagues, one strand of the DNA zipper, which serves as a reference bond with a strength of 14 pN, is secured to an upper surface via a polyethylene glycol linker. The complementary strand bears a fluorescent reporter and the ligand to be tested. The ligand's binding partner, an antibody, is attached to a lower surface; when the two surfaces are brought together, the ligand binds to the capture antibody, completing a molecular chain between the two surfaces. When the two surfaces are moved away from each other, the weakest bond in the chain will break; the fluorescent reporter will remain with the stronger bond.

The researchers applied the technique to a protein–protein system and a recombinant hepatitis C antigen with several different antibodies. For protein–protein interactions, four antibodies were bound to the DNA zipper force sensors and tested against each of four antigens. Specific versus nonspecific interactions were identified. In particular, antirabbit polyclonal antibody showed rather high nonspecific interaction with green fluorescent protein and human serum albumin.

For hepatitis detection, a sandwich assay configuration was used. Detection antibodies were connected to the top surface with the DNA zipper, and two capture antibodies were bound to the lower surface separately and in conjunction to trap hepatitis antigen. As expected, the higher-affinity antibody captured more than the lower-affinity antibody, but the mixture of the two captured more than either one individually.

The researchers suggest that many more uses for this technique are possible, such as placing certain binding partners at specific positions or delivering molecular species only when certain interaction forces meet. (*Proc. Natl. Acad. Sci. U.S.A.* **2003,** *100,* 11,356–11,360)