Ecstasy

Fifteen hundred tablets in a single shipment of "ecstasy" seized by authorities in Northern Ireland were found, by Raman spectroscopy, to have vastly different makeups, according to a study conducted by Queen's University, Belfast; Avalon Instruments, Ltd.; and the Forensic Science Agency of Northern Ireland (*Analyst* **2003,** *128,* 1331–1335). Although all the tablets contained some degree of MDMA (methylenedioxy-*n*-methylamphetamine) as the active element, the other ingredients seemed to vary with each tablet.

The current method of chemical analysis of ecstasy tablets is gas chromatography-mass spectrometry (GC-MS), which identifies any illegal phenethylamine derivatives present. The main problem with GC-MS, according to the researchers, is that the tablets must be dissolved and derivatized before analysis, which tend to be time-consuming and destructive. Because of this, it was possible to analyze only a few tablets from a single seizure; and in view of the fact that seizures can garner upwards of 200,000 tablets, this slow-going approach may give a misleading picture of the composition of the whole batch. The alternative of homogenizing a large number of tablets gives a more representative sample but removes the possibility of measuring within-batch variability.

The researchers, using Raman profiling to look at ecstasy from different sources, found that there were not ample differences in composition in the general sample population to make any significant matches between batches of tablets taken from different seizures. Despite the many different batches of tablets examined in this study, only two examples of indistinguishable sets of tablets were found, and in only one of these had the two batches of tablets been seized at different times. The study showed random variations of ingredients within tablets, but the researchers believe that with more data, it may be possible to recognize the "signature" of tablets prepared by major illegal manufacturers.

The scientists assumed that all samples were simple hydrochloride salts of the free base, because that form is easy to prepare and is the "standard" form. However, several batches of tablets showed a significant phosphate signal, possibly because the salts were prepared by extracting into aqueous phosphate buffer.

Raman profiling of tablet composition is a simple and powerful method of rapidly analyzing statistically significant numbers of seized tablets. In addition, the technique could be used to analyze batches from the pharmaceutical industry. It displays a high degree of discrimination, cannot be misled by tableting the drugs in different forms, and provides data that can easily be transferred between testing laboratories, allowing comparisons of seized tablets from different administrative regions or even different countries.

—FELICIA M. WILLIS

Herpes research and Alzheimer's disease

Researchers at Brown University in Providence, RI, and the Marine Biological Laboratory (MBL) in Woods Hole, MA, have found a physical connection between the herpes simplex virus (HSV) and the amyloid precursor protein (APP), a major component of the amyloid plaques present in the brains of people with Alzheimer's disease.

APP breaks down to form β-amyloid, which researchers believe is the underlying cause of Alzheimer's. The Brown–MBL researchers discovered an interaction between the HSV and APP while examining how the HSV travels to the lip area to form a recurring blister, even after remaining dormant for a length of time. They found that the HSV interacted with APP, a putative motor receptor that recruits a microtubular motor, kinesin, for transport through neurons. This was the first time scientists had observed a physical interaction between HSV and APP.

Protease inhibitor domain of Alzheimer's disease APP.

Elaine Bearer, senior research scientist and associate professor in Brown's department of pathology and laboratory medicine, said in a press release, "It's as if the virus hijacks a car—which in this case would be the kinesin—and the APP is the driver. The virus takes the APP where it wants to be, not where the APP wants to be."

The researchers conducted the study in the giant axon of squid, a model used extensively in research because it has a diameter of almost a millimeter, which is about 1000 times as thick as a human axon. Researchers can insert matter into the giant axon and then examine the behavior of those substances through high-powered microscopes.

Joseph A. DeGiorgis, a doctoral candidate at Brown, says, "At this point, of course, we don't yet know whether herpes plays a causal role in Alzheimer's disease, but our research does provide some interesting new insight into both diseases."

—FELICIA M. WILLIS

ILLUSTRATION: SCOTT NEITZKE, PROTEIN DATA BANK

Guiding pharmacogenomics

Putting the fruits of the Human Genome Project to work to bring new treatments to market came a step closer to reality in November, when representatives of the FDA and pharmaceutical industry met in Washington, DC, to discuss a recently released FDA draft guidance (*www.fda.gov/cder/guidance/5900dft.pdf*) on pharmacogenomics data submission.

Although microarray analysis has become a widespread tool in biomedical research, genomic biomarkers have not significantly made their presence felt in the clinical development and market approval processes of new therapies. Industry has expressed reluctance to embark on programs of pharmacogenomics testing during these FDA-regulated phases of drug development because of uncertainties in how the data will be used by the agency.

The draft guidance, which is based on more than a year of discussions between the FDA and industry (see *Modern Drug Discovery,* October 2003, pp 55–56), seeks to clarify the FDA's support for pharmacogenomics in drug development and recognize its potential significance.

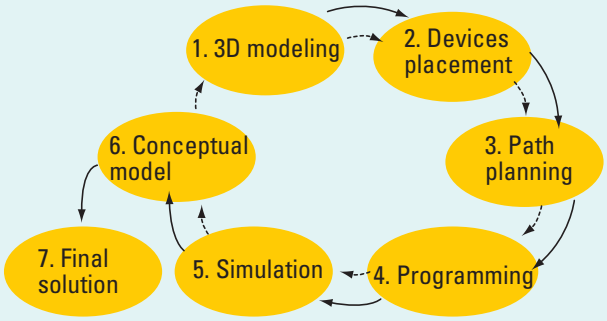
Specifically, the document defines various protocols for submission of pharmacogenomics data. It highlights situations in which such data fits into already codified laws that would require its submission for regulatory deliberation. These include cases in which the data will be used for decision-making within a clinical trial or to support scientific arguments about safety or efficacy.

Many current pharmacogenomics testing programs are exploratory in nature, intended to develop the knowledge base necessary to establish biomarker validity. For this type of data, the guidance strongly encourages voluntary submission as a means of enhancing the FDA's understanding of relationships between gene expression and responses to drugs and helping the agency prepare itself as the technology matures.

Concerns about genomic data submission do not die easily, however. Despite Woodcock's repeated assurances that the FDA will not use voluntary genomic data submission (VGDS) information for regulatory decision-making, one of the "breakout" workshops that followed the presentations reported feedback that there was "uncertainty about what FDA would do with [the VGDS] data." Furthermore, there is apprehension about the process by which test results might become "valid biomarkers", based on the data from multiple voluntary submissions (potentially, from different companies) to the FDA.

There was a sense of optimism at the November conference about the ongoing communications between the FDA and industry on this issue.

—DAVID FILMORE



Go with the flow. Researchers use iterative workflow analysis to design and implement a robot image-acquisition solution. (Adapted with permission from Bäck, P.; et al. *J. Proteome Res.* **2003,** *2,* 662–664.)

Image in that

Proteomics is quickly taking its place as a clinical tool to facilitate diagnostics and prognostics. But in doing so, the pressures are increasing to make proteomic methods easier to use and more reproducible.

In a typical proteomics workflow, dozens of 2D gels might be performed, and each gel requires several images to be taken and analyzed. The gels are then passed through a robotic handling station on which protein spots are excised, destained, digested, and placed onto a MALDI target. The target plates are then loaded into a mass spectrometer for protein fingerprint analysis or sequencing.

In analyzing this workflow, Peter James and colleagues at Lund University (Sweden) determined that gel image acquisition represented the greatest bottleneck and, in their words, "becomes a very labor-intensive and extremely boring undertaking." Thus, they developed an automated method to facilitate high-throughput gel image acquisition (*J. Proteome Res.* **2003,** *2,* 662–664).

Initially, the researchers developed a system that could simulate robotic movements using a program originally developed to control arc-welding machines. They found that it was easier to work in the absence of physical instruments and that this method allowed them to maximize robot placement and movement efficiency.

The researchers then created a system whereby gels are positioned in a vertical rack and can be moved systematically to a scanner, where they are identified by a bar-code reader. The gels are first given a low-resolution scan to obtain an overall fluorescence reading. To ensure that roughly the same number of spots is examined in each gel, the researchers found that they had to empirically determine the correct photomultiplier tube settings by randomly picking two gels from each batch and making the appropriate adjustments. They found that this greatly facilitated subsequent gel matching. Once the proper settings have been established, the system performs a detailed scan for main data acquisition.

"The main problem now is cleaning the gel plates," the authors write, "so an automatic dishwasher is next on the automation agenda."

—RANDALL C. WILLIS

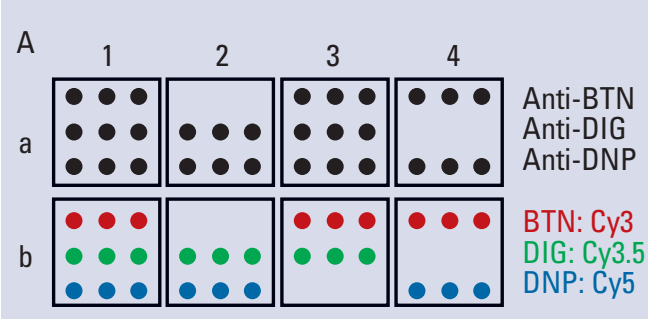
PHOTO: PHOTODISC

Protein probes

Protein microarrays have become fashionable in recent years because of their ability to map thousands of proteins in a high-throughput manner, improving our understanding of cell, tissue, and organ biology, whether for clinical diagnosis or basic research. Typically, these arrays rely on fluorescent tags attached to the protein or its target, but the broad emission and narrow excitation spectra of most fluorophores can complicate attempts to multiplex the assay (i.e., use several different tags). To get around this problem, Chad Mirkin and colleagues at Northwestern University developed a nanoparticle-based system that is coupled to surface-enhanced Raman scattering (SERS) spectroscopy for the multiplex screening of intermolecular protein interactions (*J. Am. Chem. Soc.* **2003,** *125,* 14676–14677).

To study protein/small-molecule interactions, the researchers modified 13-nm gold particles with oligonucleotides that were terminally capped with the small-molecule target on one end and a Raman dye on the other. Alternatively, to study protein–protein interactions, they replaced the small-molecule moiety with the target protein. The researchers then exposed a glass slide that had been spotted with solutions of the test proteins to the beads and looked for specific interactions using SERS spectroscopy.

In a proof-of-concept experiment, the scientists screened three unrelated small molecules—biotin, digoxigenin, and dinitrophenyl—each linked to a specific Raman dye or dye combination (Cy3, Cy3.5, and Cy5, respectively), against a slide spotted with solutions of their respective antibodies. After a buffer wash and treatment with a silver enhancement solution, the triple-dot array was clearly visible. Then, by analyzing the Raman spectra of each spot, the researchers were able to clearly identify each of the small-molecule–antibody pairs and found that the interactions were very specific. The researchers then screened three similarly labeled proteins—mouse IgG, ubiquitin, and human protein C—against a slide carrying their respective antibodies. Again, they found highly specific, easily identified interactions.



Bead there, probed that. By coating gold particles with proteins or small molecules and Raman dyes, researchers hope to detect their protein partners on glass slides. (Adapted with permission from Cao, Y. C.; et al. *J. Am. Chem. Soc.* **2003,** *125,* 14676–14677.)

Although the experiments had been designed to work with glass slides, the scientists also achieved similar results when they probed polymer substrates such as nitrocellulose or PVDF membranes with the nanoparticles, suggesting that the Raman labeling technique might be suitable for Western blotting experiments.

—RANDALL C. WILLIS

Vaccine contract

Vaccination remains one of the most valuable tools for preventing infectious disease. But vaccine delivery systems need to be improved and developed to enhance the immune response. Significant progress has been made over the past several years in the development of vaccines; however, effective vaccines are still not available for some life-threatening diseases, including AIDS. The need for an AIDS vaccine is illustrated by the rapid spread of the varying HIV strains in developing countries and the introduction of therapies to mitigate the consequences of HIV/AIDS.

To address this problem, Becton, Dickinson and Co. (BD), a multinational manufacturer and marketer of medical devices and life science products, recently entered into a research collaboration and awarded a $1.6 million contract to The Johns Hopkins Bloomberg School of Public Health to establish the Becton Dickinson Immune Function Laboratory and Vaccine Evaluation Unit. The facility will evaluate BD devices for the delivery of new vaccines. This collaboration of academia and industry to use a multidisciplinary approach strives to develop better and safer vaccines. Expanding vaccine development and research efforts for immune cell discovery and delivery is the goal of scientists at The Bloomberg School. The goal of the Vaccine Evaluation Unit is to test the safety and efficacy of vaccine delivery, improve vaccine safety, and reduce the transmission of blood-borne diseases.

The difficulties in developing vaccines against immune diseases are complicated by the lack of a complete understanding of the types of immune responses needed for protection. For malaria, HIV, measles, dengue, and other diseases, the researchers hope to develop vaccine candidates to evaluate the quality of immune responses. Despite the obstacles, the team hopes to successfully develop vaccines by rapidly and accurately determining the quality of the immune response, which will indicate whether they are likely to protect against infection.

Private industries as well as nonprofit research institutions are responding to the threat that immune diseases pose, and the United States and developing countries are increasingly relying on biotechnology to play a significant role in public health.

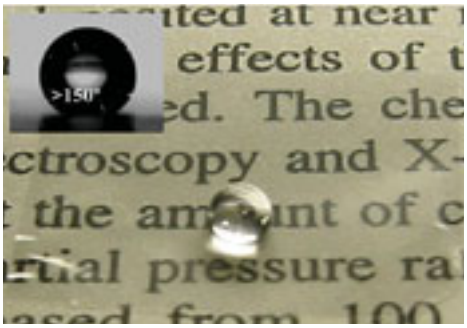
—KIMBERLY S. CLEAVES

PHOTO: PHOTODISC

Ultimate water repellent

Teflon, the commonly used fluorocarbon-based nonstick surface, is highly water-repellent, causing a drop of water to have a contact angle, the angle between a material surface and that of a water droplet, of about 120°. Materials that have this or higher water repellence are extremely useful in chemistry and biological sciences to keep surfaces from retaining or being fouled by samples and reagents. Adjusting the surface texture can make a fluorocarbon surface even more repellent, producing a water–surface contact angle of 150°. However, a problem is encountered when trying to produce optically transparent water-repellent surfaces: Glass can handle the processing required, but plastics cannot.

Katsuya Teshima and colleagues at Nagoya University and Dai Nippon Printing Co. report a process for producing a super-water-repellent surface on poly(ethylene terephthalate) (PET) without degrading its optical transparency. The process has two steps: nanotexturing the surface and then applying the fluorine compounds (*Langmuir* **2003,** *19,* 10624–10627).



Ultra-water-repellent protection. A drop of water on a PET (plastic) substrate that is transparent, allowing the text to be seen below. (Adapted with permission from *Langmuir* **2003,** *19,* 10624–10627.)

The scientists nanotextured the polymer surface by using an oxygen plasma. The untreated PET surface had a root-mean-square roughness of 1.0 nm, but after exposure to the oxygen plasma, the roughness was 9.1 nm and many protrusions were 10 nm or higher. Although the roughness increased, the surface became more hydrophilic because the plasma opened phenyl rings. Therefore, drops of water flattened out, with their contact angle decreasing from 80° on the normal PET surface to 10° after oxygen plasma.

However, the increase in hydrophilicity provided an ideal substrate for attaching silane molecules. The researchers used fluorosilane compounds with chemical vapor deposition to coat the surface. Two of the compounds tested, *n*-octadecyltrimethoxysilane and methyltrimethoxysilane, repelled water with contact angles greater than 150° and allowed transmission of light across 90% of the visible spectrum. The researchers suggest that the high degree of water repellency is due to the combination of surface nanotexture and fluorine organosilane surface and that the low-temperature process could be applied to other polymers for the production of inexpensive, water-repellent, optically transparent devices. This would benefit the production of assay plates that could handle smaller volumes while allowing optical examination from the underside of the plate.

—MICHAEL J. FELTON ■

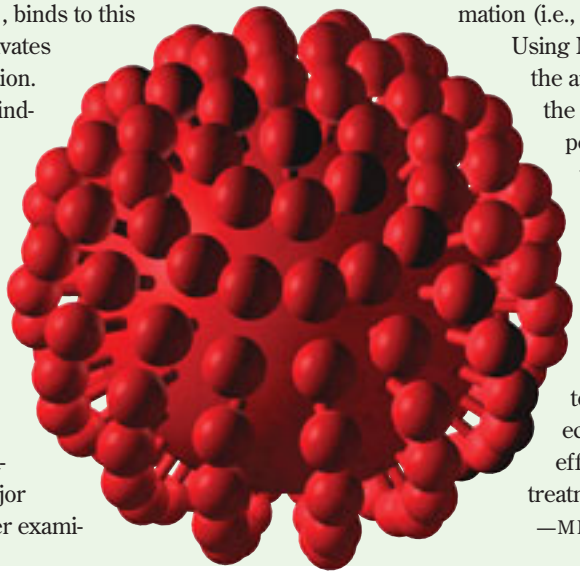
Targeting HIV with cyclic peptides

Many anti-infective agents target processes or structures that occur only in the attacking organism and not in the host. After all, infections can usually be cleared by killing the host. HIV is no exception, and Steven Runyon and Joseph Puglisi at Stanford University have developed a cyclic peptide that binds to an RNA loop unique to lentiviruses, including HIV and the bovine version of the virus, BIV (*J. Am. Chem. Soc.* **2003,** 10.1021/ja036344h).

The target of the cyclic peptide is a BIV RNA segment called TAR. A BIV protein, Tat (also unique to lentiviruses), binds to this RNA segment and activates transcriptional elongation. Preventing Tat from binding to the RNA may interfere with BIV infection.

To block Tat, Runyon and Puglisi looked at the interaction between Tat and TAR. High-resolution NMR shows that Tat has an irregular β-hairpin conformation that fits with a major groove in TAR. Further examination of Tat shows that a reverse turn incorporating residues 74 and 76, which are both glycines, is likely to be important, while the ends of the peptide are most likely not involved in binding. If the ends are not necessary, a peptide that blocks the binding of Tat could be cyclic.

Cyclic peptides are advantageous because they are more resistant to enzymatic degradation than linear peptides and are less likely to adopt alternative structures because they are constrained. Using this information, the researchers synthesized a 14-amino-acid cyclic peptide that retains the proper conformation (i.e., the β-hairpin). Using NMR spectroscopy, the authors showed that the synthetic cyclic peptide binds as tightly to TAR as a linear version of the peptide. They suggest that further work on such cyclopeptides could lead to more stable molecules that could be effective anti-HIV treatments.



HIV CELL: TONY FERNANDEZ

—MICHAEL J. FELTON

KEY TERMS: assays and screening (pp 13, 14, 17, 18), automation (pp 14, 18), clinical (pp 13, 14, 17, 18), drug delivery (p 18), medicinal chemistry (p 18), money (p 17), proteomics (pp 13, 14, 17, 18)