Pinning a label

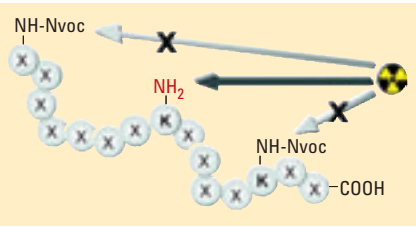
Defined and effective radiolabeling of synthetic peptides is a significant concern because of the growing use of such molecules in bioactivity studies. If the specific activity of the peptide produced is not high enough, the sensitivity of a binding assay is likely to be compromised. If the wrong amino acid site is labeled, assurance that the appropriate molecules are binding in the assay becomes suspect. Although numerous synthetic methods exist for labeling peptides produced using standard solution synthesis, to date chemistries relying on solid-phase synthesis have been less successful. Because solid-phase methods are an important facet of biomedical research, this is a large deficiency.

Resin-based bead systems cannot be manipulated at nanoscale resolutions, so a tremendous amount of radioactivity is necessary to obtain sufficient selective labeling. This is especially problematic because subnanomolar amounts of the peptide may be biologically relevant in binding studies. In addition, the impurities occurring during solid-phase peptide synthesis may be labeled, confounding the problem.

Using a two-stage process, researchers Norman Koglin and colleagues at the University of Leipzig (*www.uni-leipzig.de*) in Germany recently reported on a method of labeling a single distinct amino group (*J. Med. Chem.* **2003,** *46,* 4369–4372). They did this by blocking other competing reactive amino groups in the peptide with a photolabile Nvoc (6-nitroveratryloxycarbonyl) protecting group.

The specific lysine residue to be radiolabeled was primed (and protected from Nvoc labeling) by acylation of the free N-terminal amino group with Boc (*tert*-butyloxycarbonyl). Upon finishing synthesis, the final peptide was cleaved from the resin and the acid-labile Boc protectant removed. The now-unprotected target amino group was then radiolabeled with tritiated hydrogen, giving a 1:1 label-to-peptide stoichiometry. Subsequent removal of the photolabile Nvoc residues by UV irradiation created a "normal" peptide with a single radiolabeled lysine group in a defined location. This method, say the researchers, could be of tremendous utility for a variety of needs ranging from pharmaceutical-related receptor testing to general metabolic studies.

—MARK S. LESNEY

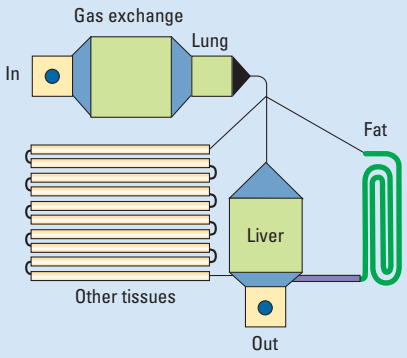


Radioactive landing. Selective radioactive labeling of Nvoc-protected peptide. The radiolabeled peptide can be isolated with a single HPLC purification step. (Adapted with permission from Koglin, N.; et al. *J. Med. Chem.* **2003,** *46,* 4369–4372.)

Animal-on-a-chip

Scientists at Cornell University (*www.cornell.edu*) recently constructed a simple in vitro model of a mammalian organ system (*Biotechnol. Prog.* **2003,** ASAP), which they say is a promising step toward the early determination of drug candidate ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties without the cost, time-intensiveness, and limited predictive power of animal studies.

The microscale cell culture analog (μCCA) system (see figure), designed using standard lithography techniques on a silicon substrate, included four organ-mimicking chambers: "lung", "liver", "other tissue", and "fat". The lung and liver sections contained living cells—a rat lung cell line (L2) and a human hepatocyte cell line (C3A), respectively—whereas the other two compartments had no cells but mimicked physiological fluid distribution.



Anatomy approximated. Schematic of the 1 in. × 1 in. μCCA design. (Adapted with permission from Viravaidya, K; et. al. *Biotechnol. Prog.* **2003,** ASAP.)

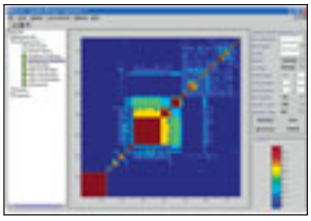
The model toxin naphthalene was flowed through the lung chamber to the rest of the system and then through an output, where it was looped back to the lung chamber. Levels of glutathione (GSH), a major target for certain toxic naphthalene metabolites, decreased in both cell lines over a period of 6 h to about the same level, although not at the same rates.

Control experiments, in which L2 cells were used in both the lung and liver chambers, or where the liver chamber was kept blank, clearly indicated that naphthalene metabolism was taking place exclusively in the cytochrome P450-active C3A cells. Thus, GSH depletion in the lung chamber results solely from toxic metabolites that are circulated there from the liver chamber. This finding agrees with previous models of naphthalene metabolism.

Probing of the L2 cells, isolated from the μCCA system, with four different naphthalene metabolites revealed that naphthalene diol and, to an even greater extent, naphthoquinone were the major metabolites causing GSH depletion (and significant loss of cell viability) in the lung.

The researchers concede that for real applications, these simple cell lines are poor mimics of actual organs, but, they assert, the system can be easily redesigned to accommodate more accurate tissue-engineered constructs, which are an area of intense research.

—DAVID FILMORE



Survey says. A cluster diagram of powder X-ray diffraction pattern similarity. (Reproduced with permission from Almarsson, Ö.; et al. *Cryst. Growth Des.* **2003,** *3,* 927–933.)

Polymorphs in parallel

The ability to identify and characterize drug compound polymorphism early in the discovery process is critical because of the significant impact that crystal form can have on a drug's chemical and medicinal behavior. Unfortunately, traditional methods used to generate and analyze crystals involve manual processes that are time-, resource-, and labor-intensive. Recently, however, researchers at TransForm Pharmaceuticals, Inc. (*www.transformpharma.com*), developed an automated method for high-throughput surveys of compound polymorphs (*Cryst. Growth Des.* **2003,** *3,* 927–933).

Using multiple 96-well aluminum blocks holding borosilicate tubes, the researchers performed high-throughput crystallization trials of two test compounds—an angiotensin II receptor antagonist (**1**) and sertraline HCl (**2**), the active ingredient of the selective serotonin reuptake inhibitor Zoloft (sertraline). They analyzed the resulting crystals using Raman spectroscopy to group them into polymorphism families and capillary powder X-ray diffraction to identify the specific polymorphs.

Over 7 days, 1500 crystallization trials of **1** resulted in 186 different crystal forms, which represented 18 polymorphs—double the number noted in the literature for this compound. Compared with traditional methods, the high-throughput method allowed researchers to perform 15 times as many trials in one-eighth the time, which represents a 120-fold efficiency improvement and also required less than half of the material needed.

In a similar study with **2**, the researchers found fewer polymorphs than described in the literature. But they identified the pharmaceutically relevant forms over a period of weeks rather than the years required for manual techniques. Importantly, the polymorph that is the active ingredient in Zoloft tablets was identified.

The researchers recognize that their method is not necessarily suitable to all situations, but they suggest that "at the first indication of polymorphism of a given compound, a multimode screening strategy can be employed to generate the most comprehensive data set of physical forms possible, to lessen the risk of future discoveries of polymorphs" that could slow down the development process.

—RANDALL C. WILLIS

The soy matrix

Because of its high availability, thermal stability, noncytotoxicity, and biodegradability, soy protein has been shown to be a viable matrix for applications in the polymer, food, agriculture, and cosmetic fields. These properties also present favorable possibilities for the use of soy as a drug delivery matrix, and in a recent study, Cláudia Vaz and colleagues from the University of Minho in Portugal (*www.uminho.pt/english*) and the Agrotechnological Research Institute in The Netherlands (*www.agrotechnologyandfood.wur.nl*) have developed a soy polymeric delivery matrix by using melt-based processing technology (*Biomacromolecules* **2003,** *4,* 1520–1529).

Vaz and colleagues prepared the matrix material with soy protein isolate, hydroxylapatite filler, glyoxal, and the drug theophylline. They used extrusion processing to convert the premixed components to plastic materials with encapsulated theophylline, and followed this step with injection molding to form the matrix into the desired shape. Drug-release kinetic experiments were conducted on the moldings by HPLC in isotonic saline solutions that were buffered to various pH levels.

The scientists found that theophylline was completely released within 56 h in a pH 5.0 buffered solution with constant diffusion coefficients and boundary conditions that remained stationary, which indicated that the system was drug-diffusion-controlled. However, at pH 7.4, drug release from the matrix material was more variable. Because polymer solubility was higher at this pH, there was a greater dependence on dissolution rate, swelling, and drug diffusion. Simple changes to the formulation, such as increasing cross-linking through heat treatment, decreasing the net charge through extrusion at a lower pH, or adding matrix reinforcements, had notable effects on the release rates in the pH 7.4 solution, but they caused no significant variations in the pH 5.0 conditions.

The two-step process of extrusion and injection molding provides a simple route to a drug product in a single piece of equipment, according to the researchers, and the advantages of soy protein, they say, make it a highly favorable process for straightforward industrial-scale production.

By in situ modification of the matrix by cross-linking, changing the net charge, or adjusting the filler properties, the researchers have shown possible mechanisms for manipulating drug release patterns. The observed pH sensitivity, in particular, points to the potential for the application of systems similar to this one for controlled delivery devices in the biomedical field.

—KIMBERLY S. CLEAVES

SOYBEAN PHOTO: DEPARTMENT OF AGRICULTURE

PHOTO: CENTERS FOR DISEASE CONTROL AND PREVENTION

Reducing mom-to-baby HIV transmission

A death sentence for an unborn baby in the developing world may no longer be inevitable when the mother is HIV-positive. Researchers in Baltimore and Uganda studied HIV-positive pregnant Ugandan women and their newborns for 18 months after birth (*Lancet* **2003,** *362,* 859–868). They found that a simple, safe, and inexpensive regimen of the HIV drug nevirapine reduces the risk of mother-to-baby transmission by 41% compared with a more complicated approach that is currently in limited use.

The director of pathology at the Johns Hopkins School of Medicine (*www.hopkinsmedicine.org*), J. Brooks Jackson, said in a press release, "This use of nevirapine, if widely implemented, has the potential to prevent several hundred thousand new infections every year."

According to UNAIDS (the Joint United Nations Programme on HIV/AIDS) and the World Health Organization, approximately 800,000 babies are infected with HIV each year by mother-to-baby transmission during gestation or birth, or through the breast milk of their infected mother.

The study, which was funded by the U.S. National Institute of Allergy and Infectious Diseases, the HIV Prevention Trials Network, the National Institute on Drug Abuse, the National Institute of Mental Health, and the National Institutes of Health's Office of AIDS Research, included 311 mothers and their babies who were given nevirapine and 308 pairs who received zidovudine (AZT).

An AZT regimen given to mothers during pregnancy and labor and to infants for six weeks after birth is a common and effective approach used in more developed countries to prevent transmission. But this strategy is too complex and expensive for resource-poor countries like Uganda.

In this study, the mothers in the AZT group received 600-mg doses when labor began and 300-mg doses every three hours until delivery. The babies then received twice-daily doses for a week. The nevirapine regimen involved only two doses—a single 200-mg dose at the beginning of labor for the mothers, and a small dose within three days of birth for the babies. By the age of 18 months, 75 babies in the AZT group were HIV-positive, compared with only 47 in the nevirapine group.

Neither the babies nor the mothers given nevirapine showed any serious adverse side effects. When nevirapine is used for an extended time in high doses, and combined with other antiretroviral drugs, rashes and liver problems have been reported, but the one-time doses used for this study showed none.

According to Jackson, "Access to HIV testing and counseling remains a huge obstacle. Fortunately, the recent availability of funds for HIV prevention and treatment for Africa from the Bush AIDS relief plan will likely make a huge difference in the implementation of this nevirapine regimen."

—FELICIA M. WILLIS

Earlier is better, for MS

In the longest study to date on multiple sclerosis (MS) patients followed from the onset of symptoms, researchers at Beth Israel Deaconess Medical Center (BIDMC; *www.bidmc.harvard.edu*) found that immediate initiation of drug treatment with Avonex (interferon β-1a) in people showing signs of risk for developing MS—but before a definitive diagnosis has been made—is an effective strategy for slowing the progression of this debilitating disease.

The results of the study, which was sponsored by Biogen (*www.biogen.com*), the manufacturer of Avonex, were presented at the meeting of the European Committee for Treatment and Research in MS in September.

"The initial symptoms of MS are often nonspecific, suddenly appearing and then disappearing within a short period of time, only to recur and become obvious much later after the disease has already spread throughout the nervous system," said R. Philip Kinkel, the study's principal investigator, in a BIDMC press release.

Currently, according to Kinkel, a specific diagnosis can be made only after there is a second occurrence of MS symptoms involving a different part of the nervous system from the first, or the observation, via MRI, of new abnormalities in the brain or spinal cord. These signs, however, might only arise after severe neurological effects have already set in. Beginning treatment earlier could significantly delay these effects.

Kinkel and colleagues originally conducted a 2-year blinded placebo-controlled trial in which 383 participants who had demonstrated initial MS symptoms were treated weekly with either Avonex or placebo. Those who received the active drug experienced a 44% lower rate of developing a diagnosis-qualifying second MS attack than the control group.

The researchers followed up for an additional 3 years with 203 of the patients—half of whom had been taking Avonex from the start and continued to receive it and half of whom had been on placebo until their second attack (or for the entire initial 2-year study) but were then put on the drug.

After a total of 5 years, patients in the former group had a 35% reduction in the rate of developing a clinical diagnosis of MS and a 48% reduction in the number of relapses when compared to those who did not get immediate treatment.

The BIDMC team plans to study this group of patients for an additional five years to determine the effects of early intervention on the development of fixed MS-related disabilities. Of course, results from larger populations will also be important, because not all patients who experience MS-like symptoms from the onset will go on to develop typical MS, even without therapeutic intervention.

—DAVID FILMORE

Biotech business

Liquid chromatography will maintain its dominance for commercial biotechnology separations over the next five years, but membrane filtration methods will also contribute significantly to the growth of the market during this period, according to a recent report by Business Communications Co. (BCC; *www.bccresearch.com*; report C-073N).

BCC analysts estimate the current U.S. market for biotech separations to be $2.1 billion, and they project that it will expand to $3.6 billion by 2008, at an 11% average annual growth rate (AAGR).

Sales of LC for protein and nucleic acid separations reached over $1 billion in 2003, and the forecast is for $1.69 billion by 2008. This would preserve LC's nearly 50% share of the total market.

An adjustment in market share, however, is taking place with the rapid growth of membrane ultrafiltration and microfiltration methods, which are expected to sustain an AAGR of 14.1% through 2008. These techniques are providing competition to the centrifugation market.

In 1998, centrifugation had almost a $100 million greater market share in biotech separations than membranes, but in 2003, the two markets are about equal at $400 million. Forecasts for 2008 have membranes with almost $700 million in sales, whereas centrifuges are expected to reach only about $500 million. More easily met sanitary design regulations and greater recovery rates in membrane filters are two reasons cited for this transition.

A steady AAGR is predicted for electrophoresis, the other significant market category for biotech separations, from less than $300 million this year to $371 million in 2008. The most important change here is the erosion of the gel/slab electrophoresis market as a result of growth in capillary electrophoresis. However, according to the report, the current importance of two-dimensional gel analysis to genomic and proteomic research will help keep the gel/slab sector strong.

The final component of the market, as defined by the BCC analysts, is niche technologies, such as chip-based microarrays, magnetic systems, and supercritical fluids systems. This sector is expected to show the largest percentage growth over the next five years (15.2% AAGR) to reach almost $400 million.

—DAVID FILMORE

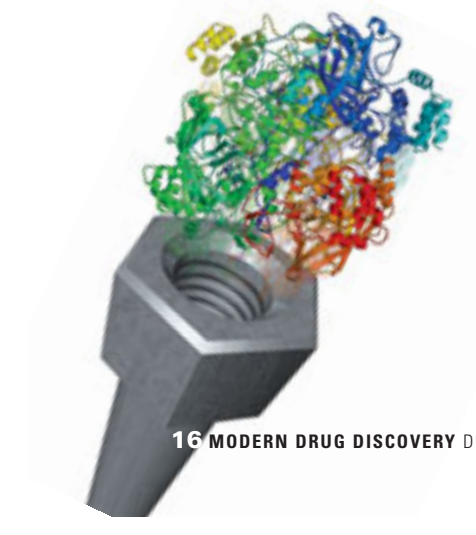
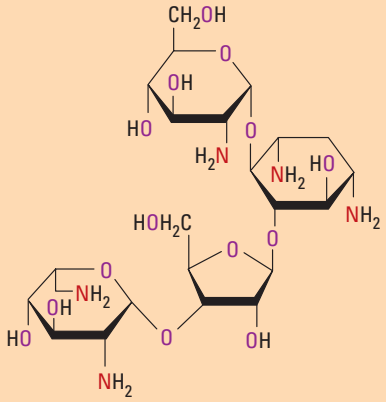


IMAGE: PROTEIN DATA BANK

Natural products aMASSed

One area that remains a largely untapped source of potential therapeutic agents is natural product research. In part, the reluctance of researchers to follow this approach is due to the difficulty of detecting active compounds that might be present in low concentrations or distinguishing these compounds from other agents that offer false positives.

To address this problem, researchers at Ibis Therapeutics, a division of Isis Pharmaceuticals (*www.isisph.com*), developed a method for multitarget affinity/specificity screening (MASS) of crude mixtures of natural products (*J. Nat. Prod.* **2003,** *66,* 1186–1190). Using MASS, which relies on electrospray ionization (ESI)-Fourier transform ion cyclotron resonance (FTICR) MS, the researchers hope to identify small-molecule ligands that form noncovalent complexes with potential drug targets.



MASS hit. Paromomycin was found to selectively bind to 16S RNA using ESI-FTICR MS.

In a proof-of-concept experiment, the scientists screened an extract from a *Streptomyces* culture against two molecules—an RNA molecule that mimics the 16S ribosomal RNA of *E. coli* (16S) and one that serves as a negative control (16Sc). If a ligand binds both RNA molecules nonspecifically, they reasoned, the peak intensity ratio 16Sc/16S should be the same value as if there were no ligand present, but if a ligand binds specifically to the 16S molecule, then the 16Sc/16S ratio should increase.

After growing a culture of *S. rimosus* sp. *paromomycinus* for four days, the researchers centrifuged the culture and fractionated the supernatant using HPLC. They combined aliquots of the fractions with the RNA molecules and subjected the mixture to ESI-FTICR MS, determining the 16Sc/16S peak intensity ratio for all 135 fractions.

The scientists initially identified one major peak that they determined was caused by paromomycin, an aminoglycoside antibiotic common to *Streptomyces*. This finding confirmed that the screening system worked. Aside from seeing other molecules that appeared to bind to both RNA molecules nonspecifically, they noted a second molecule that caused the 16Sc/16S ratio to increase, although it was 11-fold less specific for the 16S molecule than was paromomycin. Using MS/MS analysis, the researchers determined that the second molecule was composed of a core paromomycin moiety that had been modified on one or more of its rings.

Although these results are still preliminary, the authors write, "The knowledge gained in tracking molecules that bind both specifically and nonspecifically is important in the development of structure–activity studies using traditional medicinal chemistry."

—RANDALL C. WILLIS

KEY TERMS: automation (p 12), cell biology (p 11), clinical (p 15), drug delivery (p 12), high throughput (p 12), modeling (p 11), screening (pp 12, 16), technique (pp 11, 16)