MEETING AFTER THE GENOME IV

New Ways to Probe the Molecules of Life

JACKSON HOLE, WYOMING—Almost 200 years after Lewis and Clark first glimpsed the Grand Tetons, a posse of about 80 scientists gathered here from 10 to 14 October for an exploration of their own. At the annual "After the Genome" meeting, they discussed how to get from genomic information to an understanding of biology. Highlights include powerful computer programs for modeling human diseases and new techniques for protein analysis.

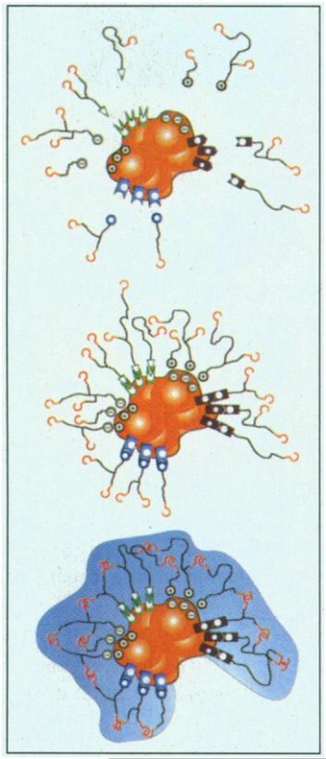
Making Coats for Molecules

For humans, Halloween is over, and the witches, Monicas, and Bill Clintons have taken home their prizes for best costume and packed their gear away until next year. But a team at the biotech start-up company Alnis, in San Leandro, California, has devised ingenious costumes for proteins and other molecules that they could wear all year long.

Alnis's scientific founder, David Soane, and his colleagues have found a way of trapping individual molecules inside polymer coatings a single molecule thick. Although the method is in its infancy, researchers can envision a wealth of applications for it. "This is a clever idea and the method has real scope," says Alexis Bell, a chemical engineer at the University of California, Berkeley, and an adviser to the company. The coatings could stabilize nature's biological catalysts, the enzymes, enabling them to tolerate organic solvents and high temperatures, and protect therapeutic proteins, such as insulin, from digestion so that they could be taken by mouth.

Other investigators are also devising stabilizing coats for proteins, but the Alnis method boasts the advantage that it can be adapted to a wide variety of biological molecules and solvent systems. What's more, because the polymer coats retain the impression of the target molecule's shape even after it is removed, they could be used for molecular detection both in the body and in biological samples, such as blood or biopsy materials. "It's potentially a very exciting technology, particularly for detecting small molecules and perhaps even proteins," says Frances Arnold, a chemical engineer at the California Institute of Technology in Pasadena.

Soane's method is a twist on molecular imprinting, a technique that has been around for several decades. In molecular imprinting, the target molecules are embedded in a material that polymerizes around them to produce a three-dimensional block bearing the targets' impressions. The block can be used for a variety of applications. By breaking it into chunks, for example, researchers can generate a chromatographic material that grabs onto the target molecules, allowing their isolation from complex mixtures.



Entrapment. After the coat molecules bind to specific sites on the target, they are linked together.

Instead of forming a polymer block, Soane generates a molecular glove that perfectly fits a protein or other molecule. He accomplishes this by exposing the molecule to custom-designed, polymerizable building blocks with distinctive heads and tails. The heads, for example, may carry positive or negative charges that allow them to bind to oppositely charged amino acid residues in the protein, while the tails, which are hydrophobic and tend to congregate with each other, are designed to link together.

Once the heads of the chemical have bound to the target molecule, Soane uses treatments such as ultraviolet light to link the chemicals into a shell, dubbed a synthetic polymer complement (SPC), around the protein. It's also possible to construct the SPC coat in such a way that an enzyme protein retains its catalytic activity. One way of doing this is to protect the enzyme's catalytic site with a molecule, such as one of the enzyme's own substrates, that binds to the site and can be removed once polymerization is accomplished. As a "proof of principle" test, Soane has shown that an SPC coat made the enzyme chymotrypsin far more durable at high temperatures in an organic solvent while still allowing it to be active.

Soane says that the SPC covering can also be released from its target molecule, although he won't say how because the technique is proprietary. If the empty shells then encounter the molecule again, they can bind it. He's shown, for example, that empty SPCs can recognize a small molecule called esculin that contains a sugar. Eventually, the chemical molds might be used for molecular detection—in effect serving as artificial antibodies that are more stable, cheaper, and quicker to make than the real thing. For example, SPCs linked to tracers that can be detected by ultrasound might help with early, noninvasive diagnosis of cancer.

"We have the beachhead successes for the recognition and binding aspects," Soane says. Still, he adds, "it will be a long time between now and when a diagnostic or therapeutic discovery is made." But costumes as good as these seem likely to win a prize or two eventually, for utility if not for beauty.

Chips for Protein Analysis

For the past several years, the fluorescent glow of DNA chips has signaled a revolution in researchers' ability to detect nucleic acids and monitor gene activity in living cells. But developing ways to keep track of the many different proteins in a cell has been much more difficult. Although techniques like the polymerase chain reaction can amplify scarce DNA into detectable amounts, the tiny concentrations of proteins in cell extracts, blood, and other biological samples can't be boosted so easily. But a new tool might help with protein analysis: the ProteinChip technology developed by scientists at Ciphergen Biosystems Inc. of Palo Alto, California.

Because the Ciphergen method combines a tiny chip with a "sticky" surface with the sensitive analytic capabilities of mass spectrometry, it doesn't require an amplification step. Consequently, it is not only very fast but can be used with small samples—microliters instead of the milliliters of conventional methods. "They're tackling one of the core problems of analyzing proteins: looking at proteins that are present in very low abundance," says Jeff Wiseman of SmithKline Beecham Inc. in King of Prussia, Pennsylvania. The method should allow

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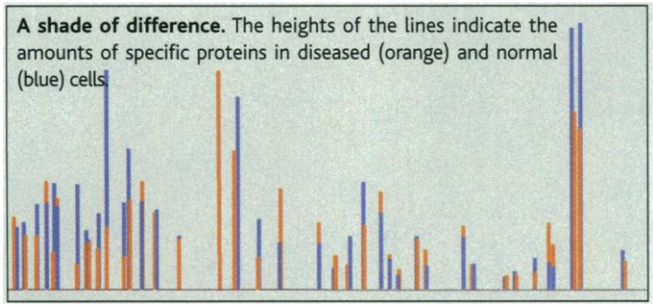
scientists to discover new proteins, purify them, measure their quantities, and discern their chemical and biological properties, all with one chip.

The technology is the brainchild of William Hutchens of Ciphergen and his colleagues. The chip, which is about a millimeter across, holds some kind of molecular bait—antibodies, carbohydrates, receptors, or any of a wide variety of smaller synthetic chemicals—that can trap many different proteins at once. To perform an analysis, a researcher applies a sample to a chip, lets the proteins adhere to it, and then washes away anything that doesn't stick.

In the next step, a laser zaps the chip surface with just enough energy to break noncovalent bonds and release the proteins. An electric field shoots these proteins to the detector of a mass spectrometer, which reads out their molecular weights. (The company calls the process Surface-Enhanced Laser Desorption/Ionization or SELDI.)



A shade of difference. The heights of the lines indicate the amounts of specific proteins in diseased (orange) and normal (blue) cells.

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Knowledge of the chemical nature of the molecular bait combined with the molecular weights of the proteins permits one particularly useful analysis: producing fingerprints of the protein composition of samples containing hundreds or thousands of proteins. By comparing closely related samples—blood serum from a healthy person and from someone with a disease, for example, or extracts of dividing and nondividing cells—scientists can detect changes in the amounts and types of proteins.

According to John Quackenbush, a molecular biologist at The Institute for Genomic Research in Rockville, Maryland, such changes can provide valuable clues to which chores the proteins are performing. In one set of experiments, for example, he compared the protein content of dividing and nondividing cells of the bacterium *Haemophilus influenzae*. The analysis picked up 600 of the 1740 proteins thought to be encoded in the *H. influenzae* genome, and for about 30 to 60 of them, the amount varied under the two conditions. "It's pretty extraordinary to be able to sit down and, in the course of a few hours, get information about 600 distinct proteins," Quackenbush says.

Once an interesting protein is identified—say, one that is made in large amounts in dividing cells but not in quiescent cells—the chips can be used to characterize, isolate, and even sequence it. By systematically testing different combinations of wash conditions and chip materials, researchers can use SELDI to explore the properties of a protein, such as how strongly it adheres to a surface with positive charge and whether it binds a particular metal ion. Furthermore, Ciphergen's computer programs can identify the combination of surface and wash conditions under which the protein of interest has the fewest neighbors, opening the way to purifying the protein.

Scientists can sequence the protein on the chip by exposing it to enzymes that release its peptides one by one, so that they can be analyzed by mass spectrometry. Conventionally, Hutchens says, different tools had to be used for each step from discovering a protein to isolating and characterizing it. But with SELDI, he adds, "you use exactly the same chip [for all three steps]."

Computer Models for Drug Testing

Companies developing new drugs usually face a great leap into the unknown when they move from test tube and animal studies into clinical trials. Given that it takes at least $20 million just to get a drug into human efficacy tests, failures can be expensive. One critical choice comes early: which of the many disease-related molecules should be targeted. "If you don't make the right choice of drug target at the beginning, you can really have a big mistake at the end," says Robert Dinerstein of Hoechst Marion Roussel Inc. in Bridgewater, New Jersey. Now scientists at Entelos Inc. in Menlo Park, California, are trying to reduce the guesswork by simulating diseases—and the molecular interactions that underlie them—in a computer.

At the meeting, Tom Paterson of Entelos reported that the company had so far built models for three common diseases: asthma, obesity, and HIV/AIDS. Each one seeks to combine what's known about the molecular and cellular changes leading to the disease with the symptoms it causes. The Entelos system "links the basic processes to their consequences in the entire system," says Dinerstein, who has used the asthma program in his work on respiratory diseases. "That hasn't been done before."

Using these programs, researchers can conduct virtual experiments to pretest drugs, modeling what happens when a drug alters the activities of a specific molecule. So far the models have helped pharmaceutical companies develop new hypotheses about mechanisms of disease and evaluate existing and novel therapeutic approaches.

To construct the models, the Entelos team formulates mathematically based hypotheses about how all the components in the disease system interact. With asthma, for example, they incorporate what is known about the role of inflammatory cells and the factors they make and respond to in constricting the respiratory airways. The researchers then tune the math and the relationships between the different parts of the model until it accurately reflects the way the disease behaves. The simulation can then show what happens to any one component of the system in response to a change in another part of it—caused, say, by administering a drug or exposing the airways to allergens. "There's nothing quite this comprehensive," says one of Entelos's scientific advisers, bioengineer Douglas Lauffenburger of the Massachusetts Institute of Technology.

Dinerstein tested the asthma simulation by seeing how it responds to existing drugs. He found in the model exactly what companies had learned from clinical trials: Effective drugs decrease airway resistance, while ineffective drugs, including some that companies had pursued quite aggressively, don't.

Using the asthma program, Dinerstein's group also carried out a virtual experiment in which they blocked the activity of a certain inflammatory factor to see if it might be a good target for an inhaled form of therapy. The next asthma attack was worse because another part of the body was compensating for the decreased inflammatory response. "We hadn't really thought about the rebound effects," says Dinerstein.

In addition, the software provides information management tools with quick connections to the literature references and the mathematics on which a given part of the model rests. Researchers can also incorporate their results into the program and chronicle the evolution of their thinking.

Different parts of the model vary in reliability, depending on the information available. But as Dinerstein notes, even the gaps can help because they point out where researchers should direct their studies. Now that scientists are investing a large effort toward finding the sequence and function of all the human genes, such models are badly needed, says Lauffenburger. "The promise of this whole new field of molecular medicine requires that we get an idea of the consequences of molecular alterations. Until you can put together models like this, it's all pretty much guesswork."

—EVELYN STRAUSS

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