out 2 trillion operations per second.

And GeneProt will offer its partners something its competitors don't: synthesized proteins. Not only will this strategy help jump-start drugmaking efforts, asserts Rose, but it may also help GeneProt researchers dodge patent disputes. Companies such as Incyte Genomics in Palo Alto, California, and Human Genome Sciences in Rockville, Maryland, claim rights to certain genes that can be used to make proteins in bacteria. By synthesizing proteins directly, Rose asserts, GeneProt can navigate around those claims. "The genomics companies thought they would stake out acres of virgin land," Rose asserts. "I'm not sure that will cover chemical protein synthesis."

If GeneProt's technology is as powerful as its executives claim, the first drug targets, and even drug candidates, should show up over the next year. "Expectations are very high," says Loiret-Bernal. "People are really looking at us to see if we are going to be successful or fail." Whatever the outcome, it's likely to serve as a bellwether for other firms looking to cash in on industrial protein analysis. **–ROBERT F. SERVICE**

PROTEOMICS
PROTEIN CHIPS

Searching for Recipes for Protein Chips

Protein arrays could be the basis for new diagnostics and research tools, but the technology has been slow to develop

When medical visionaries talk about the future, many offer up the image of a computer chip or CD-ROM that stores your complete DNA sequence. Interested in your odds of getting Huntington's disease or breast cancer? Just have your doctor scan your DNA.

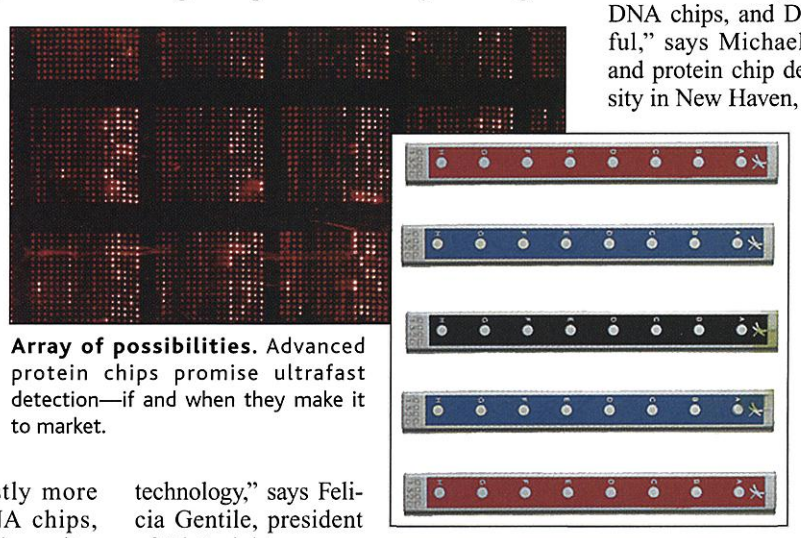
In most cases, however, we want to know what we've got right now, not what we might face in 30 years. DNA and genes won't always provide immediate answers, but looking at proteins just might. That's because proteins reflect the chemistry taking place inside cells, chemistry that is altered in potentially diagnostic ways by different diseases. The problem is that such diagnoses depend on technology that does not exist today: chips that can spot hundreds or thousands of distinct proteins at a time from a sample, say, of blood or urine.

Both academic and commercial labs around the globe are furiously competing to perfect such next-generation biochips, postage stamp–sized devices that would track many proteins in a single step. Rudimentary versions that spot a handful of proteins are already on the market. But making more complex versions is vastly more complicated than creating DNA chips, popular research tools for analyzing suites of genes involved in everything from cancer to normal cell development.

Despite the difficulty, a handful of academic groups and adventurous companies, from small start-ups to research powerhouses, are pursuing the technology. "Everyone is working on this so aggressively because it's so potentially useful," says Larry Gold, who recently stepped away from a decade of mixed success chasing biotech drugs to launch a protein biochip start-up called SomaLogic in Boulder, Colorado.

So far there hasn't been much to show for these efforts. But two recent studies offer hope that protein arrays will succeed. "I think it's virtually a sure thing," says Pat Brown, a Stanford University biochemist and pioneer of both DNA and protein chips. "But what will be the best technology, and how soon, remains to be seen."

If and when protein chips hit the market, they stand to make a big impact. "We believe there is a pent-up demand for these things. People are anxiously awaiting the technology," says Felicia Gentile, president of BioInsights, a market research firm in Redwood City, California. BioInsights predicts that the market for protein chips will grow to $500 million by 2005; other market watchers put that number as much as 10 times higher.



Array of possibilities. Advanced protein chips promise ultrafast detection—if and when they make it to market.

Much of the allure surrounds the diagnostic tests these chips might make possible. Proteomics companies are working overtime to find novel protein and peptide biomarkers whose expression correlates with particular diseases. If they succeed, a single scan of a drop of a patient's blood or urine could reveal whether the person is making proteins linked to cancer, arthritis, or heart disease. DNA chips are limited as diagnostic tests, in part because most diseases don't have a distinctive genetic signature.

Beyond the doctor's office, protein chips might also help reveal the web of protein-protein interactions in different cell types, thereby enabling researchers to work out the complex chains of chemical communication inside cells. Versions of the technology might illuminate how much of a given protein is expressed at a given place and time, offering insights into, say, cellular development or aging. And drug screening could be thrown into overdrive if researchers are able to quickly test whether new compounds bind to particular proteins immobilized on chips. "Protein chips will be orders of magnitude more useful than DNA chips, and DNA chips are very useful," says Michael Snyder, a biochemist and protein chip developer at Yale University in New Haven, Connecticut.

Second wave

Protein chips are made in much the same way as DNA microarrays. Researchers dot a glass or plastic surface with an array of molecules designed to grab specific proteins; the grabbers can be other proteins such as antibodies or even snippets of DNA. Then fluorescent markers or other detection schemes reveal which spots have snagged their prey. Because researchers keep track of the identity of each protein-grabbing molecule as it's laid down in the grid, when they see that a particular spot on the grid lights up, they know which protein has been captured.

It sounds simple enough. But getting all the elements to work is far more difficult than with DNA arrays. "Measuring nucleic acids [in an array] is a simple and stream-

CREDIT (LEFT TO RIGHT): H. ZHU *ET AL.*; CIPHERGEN BIOSYSTEMS INC.

lined system," says Brown. That's because nucleic acids have the distinct advantage of complementary binding, in which one strand of DNA or RNA binds specifically to another with the corresponding sequence of bases. To make an array, you simply synthesize strands of nucleic acids complementary to the sequences you are looking for and attach them in a grid pattern to a substrate. Companies such as Affymetrix of Santa Clara, California, now sell DNA chips that screen for as many as 60,000 genes and gene fragments at once.

Proteins, in contrast, bind to their targets based on the three-dimensional shape of each, as well as a myriad of chemical interactions. Thus, for each spot on an array, researchers must come up with a unique and specific molecule to capture a desired protein target. "To measure a protein is a new problem every time," Brown laments. Moreover, the biochemistry of proteins varies widely. Soluble proteins found in blood, for example, typically have water-friendly hydrophilic groups near their surface, whereas proteins embedded in cell membranes are often coated in fatty hydrophobic groups. A biochip surface that is chemically treated to bind hydrophilic proteins won't usually work well with hydrophobic ones.

Even so, researchers are starting to tame protein arrays. In one recent report (*Science*, 14 September, p. 2101), Snyder's group at Yale and colleagues at North Carolina State University in Raleigh created a protein chip that, when presented with copies of a particular yeast protein, highlights almost all of the other yeast proteins to which it binds. This feat required the researchers to clone as many yeast genes as possible—they succeeded with 5800 out of about 6200—by inserting the genes into other yeast cells, coaxing the bugs to overexpress the proteins, and then laboriously purifying and collecting them. They used a now-standard DNA array robot to dab tiny samples of each yeast protein in more than 200 rows atop a glass microscope slide. To find out what these yeast proteins bind to, the team spritzed the slide with solutions containing various test proteins and labeled the spots where they bound.

Snyder and his colleagues rapidly identified the proteins that interacted among the thousands of arrayed yeast proteins, they reported. That revealed a wealth of details about the network of communication channels yeast use to survive. For example, the team discovered 33 new proteins that bind calmodulin—a widespread protein involved in calcium sensing—and 52 proteins that bind phosphotidylinositides, cell membrane proteins involved in growth, differentiation, and cytoskeletal rearrangements. "This is a biochemist's dream, to be able to look for any activity over the entire proteome," says Eric Phizicky, a biochemist at the University of Rochester Medical Center in New York state.

SOME PROTEIN CHIP COMPANIES

Company	Location	Approach
Ciphergen Biosystems	Freemont, CA	Antibodies
Zyomyx	Hayward, CA	Antibodies/ Antibody fragments
Biacore	Uppsala, Sweden	Antibodies
Phylos	Lexington, MA	Antibody fragments
SomaLogic	Boulder, CO	Aptamers
Oxford GlycoSciences/ Packard Biosciences	Oxford, U.K. Meriden, CT	Hydrogel arrays
HTS Biosystems	Hopkinton, MA	Antibodies/ Antibody fragments
Large Scale Biology/ Biosite	Vacaville, CA San Diego, CA	Antibodies

The work is a coup, says Phizicky, because Snyder's team managed to get so many different proteins to stick to a surface and remain active. The team accomplished this by engineering each of the proteins to contain a nickel-binding group, coating the microscope slide in nickel, and dabbing the proteins on top. Snyder has launched a company, called Protometrix, to commercialize the technology.

But as valuable as it is for spotting protein-protein interactions, the Snyder team's chip won't help much with diagnostic tests. That's because these tests must be able to fish out particular proteins present in low concentrations in fluids chock-full of other proteins, some of which can cross-react with the chip's sensors to give off false signals.

At Stanford, Brown's team is hotly pursuing a technique for diagnostic chips. Instead of using an array of everyday proteins to capture other proteins, the team is developing arrays that use antibodies, which capture specific proteins even at low concentrations. Researchers have decades of experience dotting such antibodies on surfaces for one-at-a-time assays. But Brown's team has more ambitious goals. The researchers arrayed hundreds of antibodies atop microscope slides that had been specially treated with a polymer called poly-L-lysine and other compounds to promote the binding and stability of the antibodies. They then tested how well they could detect samples of protein. The results were far from perfect: Only 20% of the arrayed antibodies could provide accurate measurements of proteins at low concentrations, the team reported in the February issue of *Genome Biology*. But Brown insists that the study marks an important first step toward making useful antibody arrays. Snyder agrees, calling the work "a good start."

Complex diagnostic arrays, however, could be years away. As Brown and others have found, working with antibodies is tough. They are large, weighing about 150,000 daltons compared to just a few thousand for typical probes that capture DNA. Separate probes therefore must be placed farther apart, limiting the number that can fit into an array. And even though antibodies harbor small active sites that are more specific in their binding than those of many other proteins, they contain large protein-based supporting structures that can cross-react with proteins other than those to which they are designed to bind, confounding results.

Race to market

Brown and Snyder are leading the academic race for protein chips. But plenty of industrial competitors are hot on their heels: More than a dozen companies are working to bring protein chips to market. The biggest battle is over which molecules to lay down in the array to best capture proteins of interest. Like Brown, some companies are developing arrays that use antibodies to identify specific proteins. For example, Zyomyx of Hayward, California, is gearing up to begin precommercial tests with a relatively small antibody chip designed to screen for 30 cytokines, proteins known to play a key role in inflammatory diseases such as arthritis and heart disease. Still, other protein-grabbing strategies abound.

Cambridge Antibody Technology (CAT) in Cambridge, U.K., and Dyax in Cambridge, Massachusetts, for example, are making smaller versions of antibodies using an established technique known as phage display. Smaller proteins potentially mean less trouble, because they minimize the chance that a nontarget protein will interact with the antibody's supporting structures, says Larry Cohen, CEO of Zyomyx, which is also working with both CAT and Dyax to develop protein chips using antibody fragments. Antibody fragments can also be produced far more quickly than full-sized antibodies by growing them on the surface of viruses that infect bacteria and make more copies. With this technique, CAT can screen about 20,000 different antibody fragments per month. The downside, however, is that in some cases these molecules don't bind as tightly to their

protein targets as do full-sized antibodies.

Phylos, a biotech company in Lexington, Massachusetts, has its own twist on the technology. Its founders developed a system to create libraries of small antibody-mimic proteins. These mimics are as easy to produce as antibody fragments made by phage display, and they are more stable, says Albert Collinson, who heads the company's business development. Phylos also has a scheme for arraying the capture proteins in high density and with a common orientation. Because of these advantages, "Phylos appears to have the most sophisticated protein capture technology," according to the market research firm BioInsight's most recent review of the protein-chip field. Collinson says the company hopes to begin testing its chips for diagnostics and other uses early next year.

But using a protein, antibody or otherwise, to capture another protein has its drawbacks. This approach makes it tricky to detect where target proteins bind on a chip: Both the capture molecules and the targets are proteins, so a simple protein-staining technique would light up each spot. That forces many companies to use more complex assays, such as creating fluorescent compounds that have to bind to target proteins to light up. SomaLogic's Gold says a better solution is changing the probe molecules laid down on the grid to "aptamers," short stretches of nucleotides that can twist, fold, and bind to target molecules much like proteins do. A key advantage, Gold says, is that once an aptamer binds to a protein, researchers can forge a tight covalent bond by hitting it with ultraviolet light, allowing them to wash excess protein off the chip surface and scan for the tight binders that remain. SomaLogic, Gold says, doesn't plan to make chips itself but is in discussions with about 10 other companies that might market aptamer-based chips.

For now, all of these approaches are having trouble getting out of first gear to make products that compete with rudimentary protein chips already on the market. In 1990, Biacore in Uppsala, Sweden, began introducing sensor chips that use a technique known as surface plasmon resonance to investigate which proteins interact and to monitor the speed of such reactions. Ciphergen Biosystems of Freemont, California, sells a chip that screens samples for the presence of up to eight different proteins. But with both chips, researchers can look at no more than a few different proteins at one time. Ciphergen president Bill Rich is quick to admit that most researchers want more and that these chips are just the earliest examples of what is to come.

Which technology will prevail is unclear. But Zyomyx's Cohen says it's safe to assume that the nascent field will go through a shake-out in the next couple of years. Even with some success, protein chips will not match the complexity of DNA chips anytime soon, says Ruedi Aebersold, a proteomics expert at the Institute for Systems Biology in Seattle, Washington. He thinks companies will start with a limited approach, making chips to test for the presence of just tens to hundreds of proteins. Still, Aebersold and others believe even such modest gains could make the chips useful diagnostic tools. If so, protein chips could take an opposite course from that of DNA chips and be useful in the clinic long before they make a big impact in the research lab. **–ROBERT F. SERVICE**

PROTEOMICS
PATENTS

Gene and Protein Patents Get Ready to Go Head to Head

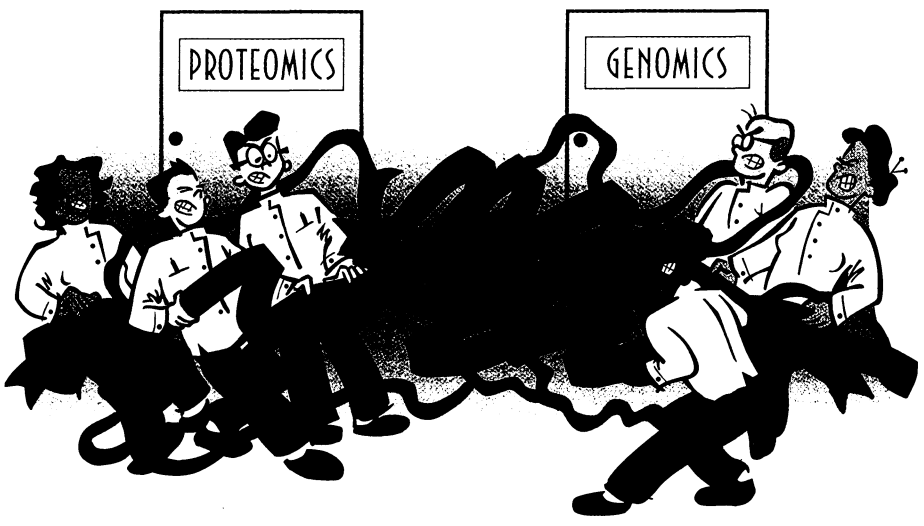
Genomics companies thought they had genetic medicine to themselves. Now proteomics firms are staking a claim

When dueling teams unveiled the near-complete human genome last February, among those cheering the loudest were companies racing to patent proteins.

Humans, the sequencers told us, may have only 30,000 to 40,000 genes, far fewer than the previous estimate of 100,000. But with proteins, the more they look, the more they find: Researchers now believe that we have as many as 2 million. Not only does this finding demolish the dogma that each gene encodes a single protein, it also throws a wrench in the business strategy of many firms that have spent the past decade furiously locking up patents on key genes involved in disease. Those patents cover what were thought to be the single proteins those genes encode—which means that any other proteins the genes give rise to may be ripe for patent lawyers' pickings. "The patent game isn't closed by any means," says Raj Parekh, chief scientist at Oxford GlycoSciences, a proteomics firm in the United Kingdom. That may be good news for protein-hunting companies like Oxford GlycoSciences, but it's likely to produce a confusing landscape of competing gene and protein patent claims, perhaps setting the stage for legal battles for control over the future of genetic medicine.

Genomics powerhouses such as Human Genome Sciences (HGS) in Rockville, Maryland, and Incyte Genomics in Palo Alto, California, have collectively filed more than 25,000 DNA-based patent applications (a number that includes both full-length genes and gene fragments). If any pharmaceutical company wants to use a patented gene and protein to develop new drugs, the reasoning goes, it has to pay royalties. This strategy makes sense as long as one gene produces one messenger RNA (mRNA) that in turn codes for one protein, as the textbooks say. But genes clearly don't tell the whole story.

Recent studies have revealed that cells often splice mRNAs together in a variety of ways to make different versions of a protein. These "splice variants" can perform separate functions in the body. One mRNA variant,



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