BIOCHEMISTRY

Protein Arrays Step Out of DNA's Shadow

As technical obstacles yield, en masse protein testing is poised to take one of biochemistry's most exciting techniques into the heart of cellular chemistry

As scientific tools go, DNA microarrays are the ultimate in multitasking, allowing researchers to track the activity of thousands of genes at once. That's made them wildly popular for tracking how patterns of gene expression change in diseases such as cancer (see p. 1670). It's also led to spin-offs, particularly efforts to create similar arrays of proteins lined up on surfaces, which among other things could be used to test the activity of potential drugs against thousands of protein targets all at once. Early progress has been slow; proteins are harder to synthesize than DNA, and plunking them down on solid surfaces tends to cause them to unfold and thereby lose their activity. Now, however, those barriers appear to be crumbling.

On page 1760 of this issue, researchers at Harvard University report creating arrays of over 10,000 proteins on a piece of glass just half the size of a microscope slide. They then used their arrays to study a variety of protein functions, work that included identifying members of the array that bind to other free-floating proteins and to small, druglike molecules.

"This is fantastic. I'm envious," says biochemist Eric Phizicky of the University of Rochester in New York, who is also developing protein arrays. Phizicky says the Harvard team's work holds the promise of vastly speeding up drug development by enabling pharmaceutical companies to quickly screen potential drugs against protein targets, while ensuring that the candidates don't react with secondary proteins that can cause side effects. So it's a sure bet, he says, that other groups will race to exploit the new technology. "This is going to be the new way of doing things," Phizicky says.

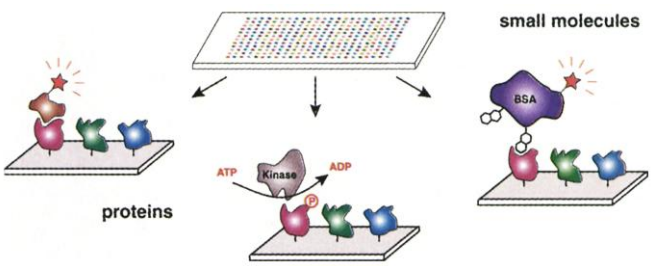
Phizicky and others point out that protein arrays might even have some advantages over DNA arrays. After all, it's proteins, not DNA or RNA, that carry out the vast majority of chemical reactions in cells. What's more, DNA arrays detect either the messenger RNAs (mRNAs) made by active genes or DNA copies of the mRNAs, and the amount of mRNA in a cell often shows no correlation with the amount of protein that gets produced by the cell. Even more troubling, proteins can undergo innumerable slight chemical changes that can profoundly alter their activity. The bottom line, say protein researchers, is that if you want to know what's happening to a cell's proteins, you have to study the proteins themselves.

That realization has sparked a number of early versions of protein array technology in recent years. In work reported in the 10 February issue of *Nature*, for example, biologist Stan Fields and his colleagues at the University of Washington, Seattle, devised something resembling a test tube version of an array, in which each test tube was a tiny well in a plate containing specially engineered yeast cells. This allowed the researchers to test the interactions of all 6000 yeast proteins with many of the others. And in the February issue of *Analytical Biochemistry,* Andrei Mirzabekov and colleagues at Argonne National Laboratory in Illinois and the Russian Academy of Sciences' Joint Human Genome Program in Moscow described a technique for creating arrays of proteins immobilized inside tiny gel packets dotted across a surface. But although the new techniques opened the door to making arrays of proteins, they still weren't as simple to use as DNA arrays.

That's where the new work by chemical biologist Gavin MacBeath and chemist Stuart Schreiber comes in. To make their arrays, MacBeath and Schreiber used a robot originally designed to synthesize DNA arrays. The robot dips a quill-like tip into a well containing a single purified protein and then turns to a glass slide, where it spots down a tiny 1-nanoliter drop onto the slide. After the robot washes and dries the tip, it then repeats the procedure with a different protein, and so on to build the array.

But the real key was getting the proteins to stick to the surface without denaturing. To accomplish that trick, MacBeath and Schreiber first coated their glass slide with a layer of a protein called bovine serum albumin (BSA), which provides a water-friendly surface that prevents the denaturing of proteins dropped on top. Next, they used the robot to make their array of protein droplets, each of them containing billions of protein copies. Finally, to anchor the proteins to the BSA surface, the researchers carried out a chemical reaction that caused lysine amino acids on the array proteins to bind to lysines in the BSA. Because lysines are present throughout the entire length of a protein, some copies always wind up binding with their chemically active regions open to the surface.

With their arrays in hand, MacBeath and Schreiber demonstrated that they could test the actions of various proteins with ease. For example, they could easily detect the binding of a small, druglike molecule bearing a fluorescent tag to particular proteins in an array, which could help reveal novel drug targets. In hopes of using the protein arrays to speed drug discovery and other applications, MacBeath says that he and colleagues at the Massachusetts Institute of Technology and the University of California, San Francisco, are launching a new company, Merrimack, to commercialize the technology.



Strength in numbers. Arrays speed efforts to identify proteins that bind to other proteins (left), drug molecules (right), and protein kinases (center), which chemically modify protein targets.

Studying the functions of proteins in arrays isn't the only possible application of the technology, MacBeath says. Researchers also hope to array antibodies that bind to specific proteins. That would enable them to see which proteins are actually being produced in various tissues and, presumably, offer further clues to what causes various diseases. That goal, too, is fast approaching. Mirzabekov says his team has arrayed 10 antibodies in gel-pack–based chips and is now looking to scale up the technology. And MacBeath says he and collaborators have preliminary results showing that they should be able to pull this off with their new arrays. So while DNA arrays may have gotten a jump in the biochip business, it's a safe bet that protein chips won't be far behind.

–ROBERT F. SERVICE

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