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Protein Chips Offer Powerful Method for Probing Protein Function

Using microscope slides, precision robots and other off-the-shelf equipment, researchers have created protein microarrays that can measure the function of thousands of proteins simultaneously. These "protein chips" which are counterparts to the much publicized "gene chips" that reveal the activity of thousands of genes will propel the next wave of proteomics research.

According to the researchers, the technique will enable rapid screening of thousands of small-molecule drug candidates to determine their potential to affect specific proteins. And ultimately, the technique will allow scientists to create protein "snapshots" of cells profiling the massive number of enzymes and other proteins in their various forms.

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— **Stuart L. Schreiber**

In an article published in the September 8, 2000, issue of the journal *Science*, Howard Hughes Medical Institute investigator Stuart L. Schreiber and Gavin MacBeath, both at Harvard University, reported that they had successfully developed and tested protein microarrays. Each microarray contained more than 10,000 spots of protein that were robotically deposited on the surface of a common glass microscope slide. The technique preserved the function of the delicate proteins, which the researchers demonstrated by showing that the deposited proteins reacted with other proteins and small molecules.

"We took our cue from the DNA microarrays that are being used so successfully to measure gene activity on a genome-wide basis, in the form of messenger RNA levels," said Schreiber. "But then the question arises how much are we missing by only looking at RNA levels? And clearly the answer is that there's a great deal going on in terms of the proteins in cells."

To develop a microarray method for measuring proteins that could be used easily in other laboratories, MacBeath and Schreiber employed equipment and materials readily affordable by academic laboratories. "We are particularly proud that we were able to develop a technique that can be carried out in a typical university environment under conditions compatible with a typical university research budget," said Schreiber.

In creating the protein chips, the scientists used a contact-printing robot developed earlier by HHMI investigator Patrick O. Brown at Stanford University. The robot precisely delivers tiny droplets of liquid protein—each the width of a human hair—to microscope slides. The robot placed liquid protein samples on microscope slides at a density of 1,600 spots per square centimeter. The protein samples were made to adhere to the glass slides by coating the slides with an aldehyde-containing reagent that attaches to primary amines, chemicals that are commonly found in proteins. The scientists also took measures to prevent evaporation and denaturation of the proteins, thereby ensuring that the proteins on the slide would retain their natural shape and activity.

The scientists performed three kinds of experiments to demonstrate that their protein microarrays could be used to determine the functionality of proteins. In one set of experiments, the researchers showed that the arrays could detect protein-protein interactions. They created microarrays of proteins and treated those microarrays with fluorescently labeled proteins that were known to attach to the proteins on the slide. The fluorescent spots that were clearly visible on the slides indicated the proteins had attached to one another.

In another set of experiments, the scientists showed that the microarrays could reveal interactions between enzymes and their substrates, molecules upon which the enzymes act. The researchers treated an array of kinases with radiolabeled kinase substrates. When the treated microarrays were "developed" in a photographic emulsion, the radiolabels were detectable as spots on the microarrays.

In a third type of experiment, the scientists demonstrated that the protein microarrays could be used to detect small molecule-protein interactions by incubating the protein microarrays with small molecules in solution. Earlier, the scientists had created arrays of small molecules (small molecule microarrays) using a technique called diversity-oriented organic synthesis. When the arrays were treated with fluorescently labeled proteins that contained target receptors that interacted with the molecules, the microarray spots revealed that there was normal binding.

"We believe that both protein and small molecule microarrays can be used for two fundamentally different purposes," said Schreiber. "And these initial experiments demonstrate the simplest one analyzing the functionality of proteins such as binding.

"This is only a starting point," he emphasized. "The most important future application of this technique will be in profiling the proteins in cells under different conditions, just as RNA profiling reveals the relative levels of RNA

present in a cell.

"Profiling proteins will be invaluable, for example, in distinguishing the proteins of normal cells from early-stage cancer cells, and from malignant, metastatic cancer cells that are the real killers." However, noted Schreiber, protein profiling will prove far more difficult than RNA profiling.

"The proteome that is, the cell's array of proteins is more complex than the genome," he said. "Although one gene may encode one protein, those proteins are modified in many ways after they are constructed. So, each gene product may result in dozens of proteins that have been rearranged, fragmented or chemically modified to produce a slightly different activity. And there is every reason to believe that these modified proteins are going to be key elements to understanding function and eventually physiology.

"We are optimistic that in a short time we will meet the technical challenges that will enable protein profiling with this technique," said Schreiber.