



Beyond Genome - The Future of Medicine
June 12-16, 2005 • Fairmont Hotel • San Francisco, CA

SCIENCE ONLINE SCIENCE MAGAZINE SCIENCE NOW NEXT WAVE STKE/AIDS/SAGE SCIENCE CAREERS E-MARKETPLACE HOME

Science **e-marketplace** [HELP](#) [SUBSCRIPTIONS](#) [FEEDBACK](#) [SIGN IN](#) 

[SEARCH](#) [ARCHIVES](#)

Advances In : Biochips – A Chip of the Old Protein



Protein microarrays can provide more data faster and less expensively than traditional investigative methods. But only now have proteomic researchers begun to realize their value.

by Peter Gwynne and Gary Heebner



CONTENTS

- [Protein microarraying systems](#)
- [Surface chemistries](#)
- [Low volume platforms](#)
- [High volume platforms](#)

This is the second of four special supplements this year on Advances in Biochips. The first appeared in the 4 March issue of Science and the next will appear in the 19 August issue.

Inclusion of companies in this article does not indicate endorsement by either AAAS or Science, nor is it meant to imply that their products or services are superior to those of other companies.

The term “proteome” refers to all of the proteins that a cell expresses. So identifying and measuring the quantities of proteins in a cell’s proteome becomes a major goal of proteomic research. Carrying out those tasks at a specific time is difficult enough. But specialists in proteomics face the additional complication that

ADVERTISERS

American Type Culture Collection (ATCC)

diverse materials (such as microorganisms, cell lines, and recombinant DNA materials) to media and other reagents, as well as bioscience laboratory services.

703-365-2700

<http://www.atcc.org/>

Greiner Bio-One

- [Microarray imaging and detection](#)
- [Data collection and management](#)

the makeup of proteins in a given cell varies over time, depending on the cell's health and ambient conditions.

To understand those variations, proteomics specialists focus on three main strands of research. Comparative studies, otherwise known as protein profiling, aim to measure the relative

abundance of each protein in at least two cells, one of which might be healthy and the other or others suffering from a disease state. Functional research focuses on the ways in which a particular protein behaves and/or interacts with other cellular constituents. And structural analysis seeks to determine the sequence and three-dimensional configuration that give the protein its functional properties.

International [Germany]
plasticware,
microarrays, and
microarray fabrication
products
+49 7022-948-0
<http://www.gbo.com/bioscience>

Greiner Bio-One International [USA]
407-333-2800

To help deal with those problems, researchers have begun to turn to protein microarrays. Otherwise known as protein biochips, the arrays have obvious value for research on proteins. Fundamentally, they provide a way to study proteomics in terms of what protein researchers actually need.

Arrays' Advantages

They also offer advantages over more traditional methods of proteomics research. "Protein arrays provide easier, more sensitive measurement," says Ray (Ruo-Pan) Huang, founder and interim president of **RayBiotech**. "Compared with 2-D gels and mass spectrometry, they allow you to detect protein expression in the proteome in an unbiased way." Dan Schroen, product manager for drug discovery products at **Nalge Nunc International** (NNI), offers a more expansive view of chips' promise. "Protein microarrays produce unsurpassed amounts of data in a shorter period of time, using less reagents and materials than a typical screen," he explains.

Despite those advantages, protein microarrays have so far gained only slow acceptance from the research community. "I don't feel that they have been fully appreciated yet," says Timothy Burland, president and CEO of **GWC Technologies**. "There are a lot of technical challenges, and a lot of people are not sure how to proceed." However, he adds, "Companies like GWC are helping researchers by providing specific suggestions on how to proceed."

Larry Gold, CEO and chief scientific officer of **SomaLogic**, argues that the industry bears some responsibility for that situation. "People want proteins chips; the demand is there," he explains. "But nobody has been able to deliver yet." Soleil Shams, founder and president of **BioDiscovery**, takes a similar view. "Certainly protein chips are not as mature as 2-D gels and other methods," he says. "But everyone is hopeful that we will get there." Gold shares that expectation. "Protein chips," he predicts, "will be as big for researchers as the yeast-2-hybrid approach."

Indeed, signs point to imminent expansion of the use of protein microarrays in and beyond the research laboratory. "They are used primarily in the academic area, but they are rapidly making inroads into the pharmaceutical area as well," says Santosh Arcot, product leader for array systems at **PerkinElmer Life and Analytical Sciences**. "Protein microarrays have begun to penetrate the clinical research area," adds William Rich, CEO of **Ciphergen Biosystems**. "We've seen an expansion in publications to about 40 in the last three months – mostly in the clinical research area and most focused on biomarker discovery and translations into types of diagnostic assays."

Three Basic Types

Protein biochips come in three basic types. Capture chips grab proteins of interest, in much the same way that DNA chips capture their counterpart DNA sequences. This type of protein biochip can use antibodies, antibody mimics such as affibodies, or aptamers – single stranded nucleic acids that, like antibodies, can bind to target molecules. Interaction chips use immobilized proteins, peptides, or other small molecules to study the interaction of proteins in a sample. These microarrays can provide insights into a protein's function, its interaction with other molecules, and the strength of those interactions. And the reverse screening approach uses tiny amounts of cell sample immobilized in a microarray format on a solid support.

The chips also come in a variety of shapes and sizes. The tools range from platforms much like the traditional ELISA systems, using 96-well microwell plates, to treated plastic and glass microscope slides that can provide thousands of spots or features with different molecules attached to each spot.

NNI's Schroen outlines the range of possible uses for protein microarrays. "For instance," he says, "protein-antibody interactions performed previously in ELISAs using the entire wells of a 96-well plate can now be miniaturized into multiplexed array features in the same wells or on a one-inch by three-inch slide. Microarray technology can also be employed to analyze protein-DNA interactions, which may have previously been assessed using time- and material-intensive approaches such as blotting or electrophoretic mobility shift assays. Clinical diagnostics represents perhaps one of the largest areas of potential impact and growth, as diagnostic products begin to enter the marketplace."

What factors should influence researchers' choice of the type of protein biochip for their work? "It often depends on the research project, commercial goals, or clinical/diagnostic needs," Schroen says. "The goal determines the choice of chip," adds Amy McCann, global product leader for array systems at PerkinElmer Life and Analytical Sciences.

Off-the-Shelf or Do-it-Yourself?

Whatever the application of their microarrays, users must choose between making their own and buying them off the shelf – although that decision is often made for them by the lack of appropriate off-the-shelf chips. "The decision has to do with the specific way they're performing their research and whether the chip exists commercially," McCann explains. Commercial availability is a significant roadblock. "A key differentiator between genomics and proteomics is that you can buy a whole genome chip off the shelf," her colleague, Arcot, points out. "We're not there yet with protein chips."

Commercial protein microarrays that have reached the market have several advantages. "If your institute has the equipment and the facilities, you can maybe make your own," RayBiotech's Huang advises. "But if a commercial product is available it's probably better to buy it, as it takes a lot of time, effort, and money to make your own protein chip. There's also the issue of reliability and reproducibility."

Do-it-yourselfers face a daunting task identifying the equipment and supplies necessary to produce a protein biochip. They need not only to acquire several different instruments but also to ensure compatibility among them. To simplify in-house production of protein biochips, several companies have designed integrated systems specifically for producing protein microarrays. They include **BioIntegrated Solutions**, **Genetix**, and **Genomic Solutions**.

The list also includes PerkinElmer. "We're the tools provider for protein array fabrication, slides, scanning, and automated processing," McCann says. "Our Piezoarray is a noncontact printing system. We

have a hydrogel-coated slide with a three-dimensional substrate that's superior for proteins as it's a protein-friendly environment. When the chips are ready to go, our ProteinArray Workstation processes them. And then we take the arrays to the ProScanArray whose analysis capability will quantitate your protein arrays and overcome the data bottlenecks."

Issues of Attachment

Whatever type of molecules they use in their protein microarrays, researchers must ensure that the molecules attach to the arrays' surfaces. **BD Biosciences Clontech**, Genetix, **Greiner Bio-One**, and **Schleicher & Schuell** offer glass slides with treated surfaces that allow attachment of proteins. Treatment methods range from coating the glass surfaces with aldehydes to applying a matrix that creates a three-dimensional surface.

Proteomics researchers must also decide on the amount of reagent they need on their chips. Protein microarrays come in several varieties, from low-volume platforms to several types of high-volume platform.

NNI offers several surface treatments, such as Maxisorb, aminosilane, aldehyde, epoxy, and lysine for low-volume microarrays. Each treatment offers different binding mechanisms that researchers can tailor to specific applications. The company also offers both glass and polymer microscope slides as well as treated ArrayCote multiwell plates and slides. Spotting a single well of such plates with multiple features enables scientists to multiplex their assays. This permits the analysis of multiple targets in each well and studies of dose response. It also facilitates assay optimization. Schroen explains the rationale for this low-volume approach. "By fitting multiple thousands of features on a slide or plate," he says, "more data points can be gathered in a shorter amount of time using fewer resources."

Greiner Bio-One, meanwhile, has recently introduced a low-cost arrayer that scientists can use to spot their glass slides manually. The benchtop device can put down up to 768 spots per slide. Each spot has a diameter of about 500 microns and uses between three and five nanoliters of protein material. The microarrayer contains two printers – one 8-pin and the other 32-pin – to match 96-well and 384-well plates.

Plexigen offers the geneCube, a three-dimensional array made from stacked geneCards, for assaying hundreds of proteins with up to a thousand samples. The system's stacked layers allow flow-through parallel processing under conditions determined by the substrate applied to each card or layer. It allows for flexible experimental design and a wide variety of applications.

Capture-Based Chips

Several companies, including BD Biosciences Clontech, RayBiotech, and **Zyomyx**, have developed high-volume capture-based chips. Designed to isolate proteins of interest from a sample, these protein microarrays detect specific cytokines, identify which standard proteins exist in a sample, and even determine whether a protein has been activated by phosphorylation. "We developed the first cytokine antibody array," recalls Rani Huang, RayBiotech's marketing director. "Our general cytokine array can detect up to 180 different proteins in one experiment. We also have angiogenesis arrays, inflammation arrays, chemokine arrays, and disease-related antibody arrays." Adds Ray Huang: "We offer low price, so that any lab is capable and equipped for routine use."

Antibodies provide the obvious choice of molecules for use with capture chips. However, scientists know of only a few thousand different antibodies, and often experience problems finding an antibody with

sufficient specificity for use in microarrays. Companies such as **Cambridge Antibody Technology** and **Dyax** are creating large libraries of antibodies that can be used for protein arrays. But even with these focused efforts, creating new antibodies involves plenty of time and labor.

One alternative is to use antibody mimics. Swedish firm **Affibody** has engineered highly specific affinity proteins that bind to virtually any target protein. Called affibodies, they mimic monoclonal antibodies in many ways. Applying combinatorial protein engineering technology on a proprietary scaffold, the company can engineer these antibody mimics to possess such properties as specificity and affinity, while also possessing the robustness necessary to withstand a broad range of analytical conditions, including extreme pH and elevated temperature.

Aptamers provide another alternative. SomaLogic has decided to develop its protein microarrays using photoaptamers, which form specific covalent cross-links with target proteins when exposed to ultraviolet light. “Our business is spotting capture agents that catch proteins,” Gold explains. “The cross-linking allows stringent washing, lower background, and enhanced specificity. In fact our good photoaptamers bind to and cross-link one protein only in serum – the target analyte.”

The company focuses on clinical diagnostics, and particularly discovery of biomarkers and protein signatures. “However,” says Todd Gander, senior director of corporate development and strategic planning, “we’ll be available to clinical researchers through collaborations, and we will ultimately introduce research products, possibly through a strategic partner.”

Studying Interactions

Scientists can also use protein microarrays to study interactions among proteins or peptides. Although relatively few companies have moved into this business, **Biacore** and **Jerini Peptide Technologies** have released effective protein microarrays for interaction studies.

Biacore provides biosensor chips with a range of immobilization chemistries. Those allow researchers to choose among various methods of coupling ligands to a sensors’ surface, to enable study of the ligands’ interaction with the analyte in the most appropriate manner. Biacore is also developing a protein array, based on its proprietary surface plasmon resonance technology, that should significantly increase the number of detailed biological evaluations that drug discovery teams can perform. Jerini offers several types of protein microarrays, including ProteaseSpots for studying protease activities and substrate specificities; PepSpots for mapping protein interactions and characterizing protein-protein contact sites; and PhosphoSite-Detector arrays for detecting potential phosphorylation sites in kinase substrate proteins.

ZEPTOSENS, a division of **Bayer AG Technology Services**, uses several different proprietary chemical treatments to coat its microarrays in such a way that they immobilize peptides, protein receptor molecules, or even cell lysates. The surface chemistry increases the ligands’ stability and decreases their nonspecific adsorption of unwanted molecules. The company’s surface-confined evanescent field detection approach requires neither amplification of enzymatic signals nor extensive sample preparation. And its ZeptoMARK high performance protein microarrays allow for homogeneous mix and measure assays.

Ciphergen Biosystems has taken a very different approach to protein microarrays. Its technology avoids the use of antibodies or other ligands altogether. Instead the company’s ProteinChip arrays use chromatographic surfaces, prepared by surface enhanced laser desorption/ionization technology, that enable the reproducible capture and study of unknown proteins from crude samples. “If you have different surfaces, such as anionic, cationic, and some kind of metal affinity, each one captures the proteins in a broad class,” Rich explains. “If you adjust the pH, you can catch large subclasses. When you don’t know

what proteins you're looking for, you can run this technology broadly as a differential profiling method. And you can tune the selectivity to your taste. Of course, you can also directly create antibody, protein, and DNA capture versions of our chips using our reactive surface chemistry chips and your own bioaffinity molecules."

Labeling and Amplification

Detecting the contents of protein microarrays involves a variety of labeling and amplification chemistries, including fluorescent, luminescent, and radioactive methods. **Evrogen**, **Invitrogen** and **Sigma-Aldrich**, among other companies, provide tags for labeling proteins. And **Molecular Devices** (Axon), PerkinElmer, **Tecan**, and **TeleChem International** offer microarray scanners and imagers.

Labeling a protein can interfere with its function. Since most drug targets are proteins, a label-free method has obvious interest for drug discovery. GWC Technologies uses surface plasmon resonance (SPR) imaging to detect molecular interactions on protein biochips and other types of microarrays. The method detects the presence of a molecule on a gold surface by the change in the local index of refraction that occurs on adsorption. "It fits in as a label-free method that enables you to analyze proteins' interactions with other proteins, different ligands, and nucleic acids," Burland says. "Our SPR imager can analyze anything. It doesn't care about the chemistry, but the mass on the surface makes it very versatile. Even if your needs change, your analytical instrument need not."

The imager also offers the advantage of real-time analysis. "You're looking at the computer screen and seeing what's happening on the chip without distorting what's going on," Burland continues. "If something happens very fast on one probe and very slowly on another, you can see it, change your experimental conditions, and otherwise adjust to the situation."

Managing the Data

After detection comes interpretation, which means bioinformatics. "The need for bioinformatics in particular and software in general in proteomics ranges from image analysis to managing all the data that are generated and performing quality control on the data," BioDiscovery's Shams says.

Companies such as **Accelrys**, BioDiscovery, and **MDL Information Systems** have developed suites of bioinformatic software to manage the storage and retrieval of data from protein microarray experiments, to mine those data sets, and to explore relationships among the data. BioDiscovery, meanwhile, has partnered with **Prolinx** to develop integrated software for analyzing protein microarrays. Why did they link up? "BioDiscovery is a leading software provider for gene expression and imaging analysis. Prolinx made protein chips," Shams explains. "It made a lot of sense to bring our experiences together."

To interpret their protein microarray data most effectively, researchers need meaningful access to the hundreds of databases worldwide that house textual and other information on proteins, DNA, and other biomolecules. To facilitate those efforts, **MiraiBio** has developed an Internet-based data mining system that it calls DNASIS GeneIndex. It enables researchers to query more than 20 public and commercial databases for DNA and protein sequences; by doing so they can narrow their searches and complete them as thoroughly as possible. And by integrating text mining technology and data related to life sciences, DNASIS GeneIndex provides a new approach for data searches that permits life scientists to discover previously untapped knowledge.

Protein microarrays may have made slow progress toward acceptance by proteomics researchers until now, but continuing improvements make them increasingly compelling. "Protein chip surfaces, formats,

materials, methods, and reagents are still emerging,” NNI’s Schroen says. “So it’s an exciting time to be involved in this field.”



Peter Gwynne (pgwynne767@aol.com) is a freelance science writer based on Cape Cod, Massachusetts, U.S.A. Gary Heebner

(gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

WEBLINKS	
ADVERTISERS	FEATURED COMPANIES
American Type Culture Collection (ATCC) diverse materials (such as microorganisms, cell lines, and recombinant DNA materials) to media and other reagents, as well as bioscience laboratory services. 703-365-2700 http://www.atcc.org/ Greiner Bio-One International [Germany] plasticware, microarrays, and microarray fabrication products +49 7022-948-0 http://www.gbo.com/bioscience Greiner Bio-One International [USA] 407-333-2800	Accelrys – a subsidiary of Pharmacopeia bioinformatics software http://www.accelrys.com/ Affibody protein microarrays based on antibody mimics http://www.affibody.com/ BD Biosciences Clontech treated microarray slides http://www.clontech.com/ Biacore International AB systems to study molecular binding http://www.biacore.com/ BioDiscovery bioinformatics software http://www.biodiscovery.com/ BioIntegrated Solutions automated liquid handling systems http://www.biointsol.com/ Cambridge Antibody Technology libraries of antibodies http://www.cambridgeantibody.com/

Ciphergen Biosystems, Inc.

instruments and arrays for
proteomics research

<http://www.ciphergen.com/>

Dyax Corporation

libraries of antibodies

<http://www.dyax.com/>

Evrogen

protein labeling kits and reagents

<http://www.evrogen.com/>

Genetix

instruments and supplies for array
fabrication

<http://www.genetix.com/>

Genomic Solutions

protein microarrays, fabrication
systems

<http://www.genomicsolutions.com/>

Greiner Bio-One International

microarray fabrication products

<http://www.gbo.com/bioscience>

GWC Technologies, Inc.

label-free detection systems

<http://www.gwctechnologies.com/>

Hitachi Genetic Systems/MiraiBio

bioinformatics software

<http://www.miraibio.com/>

Invitrogen Corporation

protein labeling and detection
systems

<http://www.invitrogen.com/>

JPT Peptide Technologies GmbH

peptides and protein arrays

<http://www.jerini.com/>

MDL Information Systems, Inc.

bioinformatics software

<http://www.mdl.com/>

Molecular Devices

image detection systems

<http://www.moldev.com/>

Nalge Nunc International

protein microarrays and supplies

<http://www.nalgenunc.com/>

PerkinElmer Life and Analytical Sciences

protein microarrays and proteomics workstations

<http://las.perkinelmer.com/>

Plexigen, Inc.

microarray/microfluidic systems

<http://www.plexigen.com/>

Prolinx, Inc.

protein microarrays

<http://www.prolinx.com/>

RayBiotech, Inc.

cytokine and other microarrays

<http://www.raybiotech.com/>

Schleicher & Schuell, Inc.

treated microarray slides

<http://www.s-and-s.com/>

Sigma-Aldrich Corporation

protein labeling kits and reagents

<http://www.sigma-aldrich.com/>

SomaLogic, Inc.

protein capture photoaptamers

<http://www.somallogic.com/>

Tecan

image detection systems

<http://www.tecan.com/>

TeleChem International, Inc.

image detection systems

<http://www.arrayit.com/>

	Zeptosens, a Division of Bayer (Schweiz) AG Technology Services protein microarrays and systems http://www.zeptosens.com/ Zyomyx, Inc. microarrays and protein analysis platforms http://www.zyomyx.com/
--	---

Note: Readers can find out more about the companies and organizations listed by accessing their sites on the World Wide Web (WWW). If the listed organization does not have a site on the WWW or if it is under construction, we have substituted its main telephone number. Every effort has been made to ensure the accuracy of this information. Inclusion of companies in this article does not indicate endorsement by either AAAS or *Science*, nor is it meant to imply that their products or services are superior to those of other companies.

**This article was published
as a special advertising section
in the 06 May 2005 issue of *Science***

**GetInfo – Improved online
reader service!**

**Search more easily for *Science* advertisers
and their products. Do all your product research at -
science.labvelocity.com**

**Visit <http://www.science-benchtop.org/>
to find this article as well as past special advertising
sections.**