The Promise of Protein Microarrays

Despite some formidable technical challenges, protein microarray and other biochip technologies are among the most important tools for the analysis of cell physiology as a set of linked networks.

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At this early stage of the 21st century, people seem to be feeling a sense of indifference to technological advances. After all, these advances seem to come at such a rapid pace and in so many different areas of life that we can barely keep up with them. When the person on the street considers technology, she or he probably thinks more about enhancements than breakthroughs. The familiarity of having a computer on the desktop, with continual upgrades that themselves dwarf the computing power of the systems of two or three decades ago, has only amplified that feeling.

Molecular and cell biologists, however, are like the explorers of old — more attuned to breakthroughs than to enhancements. Technology for these scientists often is about *frontiers.* And the frontiers they explore have major implications for the pharmaceutical component of healthcare.

One relatively new technology — protein microarrays — is an ideal example of a new frontier. Ken Howard, a reporter for the online GenomeWeb, calls it "the next big *little* thing" (1). Protein microarray technology is, in part, an outgrowth of the human genome project. Thanks to the successful sequencing of the human genome, as well as the sequencing of genomes of other organisms, scientists today have access to a catalog of all human genes and the genes of many plants, animals and microorganisms. This genetic information allows one to determine the protein makeup of cells, which is critical because the protein is the "worker" substance of a cell.

Development of Microarray-based Analytics

What's so special about this new technology? Protein microarray technology allows the simultaneous analysis of thousands of molecular parameters with a single experiment (2). In one example, researchers were able to print a very large number of protein spots on a single glass slide and then use the slide to identify protein-protein and drug-protein interactions (3). The implications for researchers are tremendous, as Ken Howard was told: "Instead of spending 12 hours in a cold room [analyzing one protein], you can analyze 10,000 proteins in a single chip in 10 hours. It allows you to do things much more quickly and with greater precision."

Microarrays are produced in one of two ways. In some cases, on-chip synthesis is used. In others, researchers employ an arrayer based on contact printing or ink jet technology. Proteins can be deposited as microspots in specified positions on the chip.

To be fair, while the application of the technology to protein analysis might be recent, the microarray as an idea is hardly new. Roger Ekins and his colleagues first espoused the fundamental theoretical concepts beginning in the late 1980s (4). They determined that microspot ligand-binding assays were sensitive from both a theoretical analysis point of view, as well as from practical experiments.

Ekins' studies during the early 1990s led to a sensitive microarray-based analytical technology. Analytes, such as thyroid stimulating hormone (TSH) or Hepatitis B surface antigen, were detected down to femtomolar concentrations. The key, Ekins and his colleagues found, was the ability to control the concentration of capture molecules in a small area, called a "microspot."

Researchers at the Natural and Medicinal Sciences Institute (NMI) at the University of Tübingen in Germany summarized two important principles for the high sensitivity obtainable in a microarray format: that the binding reaction occurs at the highest possible target concentration and that the capture-molecule-target complex localized in a microspot generates a higher signal density (in comparison to a macrospot) (5). The researchers provide multiple examples of the considerable utility of protein microarray technology in studying different kinds of

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protein interactions, and suggest that "further developments and optimization of array production and array performance combined with high-throughput generation of protein targets and ligands will extend the number of applications of protein microarrays dramatically."

The intellectual appeal of protein microarray technology is obvious: it affords researchers a way to address questions at various levels by studying the interactions between protein-protein, antigen-antibody, enzyme-substrate, protein-DNA and ligand-receptor.

Technical Hurdles Remain

Today, the transition of protein microarrays and other biochips from theory to actuality is evidence of just how quickly genomics-based medicine is emerging. Yet, some major technical hurdles must be overcome before protein microarrays will impact the drug discovery process significantly. That's where challenges and opportunities arise for the pharmaceutical and diagnostic industries.

The source of capture molecules and the difficulties of dealing with proteins represent the greatest obstacles to widespread use of protein microarrays. Proteins are diverse molecules with varying physical/chemical properties, including charge and post-translational modifications (glycosylation, acetylation or phosphorylation). Also, proteins that have been immobilized on a surface in the form of an array must be kept in a functional state. This hampers the ability of researchers to achieve high throughput with high-quality protein production techniques — the very achievement needed, according to the NMI researchers, to extend protein microarray applications. The solution might lie in the development of directed protein microarrays, which are predicated on the belief that some classes of proteins might be more amenable to manipulation than others.

What else is needed? For starters, rapid, high-quality isolation techniques are critical, the specific assay questions have to be designed and scientists need reliable ways to measure formation, as well as dissociation, of protein-protein interactions. Then, also, are the fundamental biology questions, such as how protein domains interact to transmit and control biological information. In addition, cell signaling pathways, in principle, can be characterized rapidly from the initial stages of receptor-ligand binding to the cascade of biochemical activities resulting from the first step. An exciting possibility is the ability to unravel the complex interactions that occur on a cell when more than one receptor-ligand binding occurs. This will bring greater ease to unraveling feedback, as well as feed forward loops. Finally, many cell types can be analyzed simultaneously, increasing the ability to generate knowledge in a broader biological base.

Post-translational modification and cellular compartmentalization of proteins also present challenges, as they affect activity, specificity and stability. Proteins have variable half-lives, meaning strategies for analyzing multi-component proteins are anything but straightforward. However, solutions to these challenges will lead to molecular portraits of normal versus diseased states of cells, allowing for more effective intervention into the complex cellular networks.

Despite all these technical challenges, protein microarrays hold the promise of becoming one of the most important tools for the analysis of cell physiology as a set of linked networks. The technology has the potential to lead to a systematic approach that is capable of assessing rapidly and in parallel the state of each component in the network. Then, perturbations via gene replacement, antisense technology or — more consequentially — small molecules with protein microarrays from diseased versus normal cells will lead to new small-molecule drug candidates. That is why the technology will be so central to the future of healthcare.

The NMI researchers offer an outstanding example of what the future holds in medical research, where, "protein microarrays will accelerate immune diagnostics significantly by analyzing in parallel all relevant diagnostic parameters of interest." They hypothesize the "analysis of multiple tumor markers from a minimum amount of biopsy material" and suggest that "new possibilities for patient monitoring during disease treatment and therapy will be developed based on this emerging technology" (6).

The Biochip Business

All this speaks to a burgeoning market for protein microarrays. Frost & Sullivan, a New York-based consulting firm specializing in emerging high-technology markets, projected in June 2001 that the biochip industry will surpass the $3.3 billion revenue mark by 2004, with the largest share garnered by the microarray segment (7). The firm "anticipates that diagnostic applications will

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begin to appear over the forecast period, first in clinical labs and physician offices and, gradually, beyond the forecast period, in private homes," with the market for biochip products reaching tens of billions of dollars annually by 2010.

According to another report, in 2000, more than 100 companies participated in the microarray and protein chip markets, and that number is growing by more than 35% each year (8).

For the pharmaceutical industry, this new technology is emerging at a critical juncture. In a period of great increase in the number of top pharmaceutical products scheduled to go off patent, tremendous growth in the demand for drugs, especially new drugs, and slowing growth and declining R&D productivity, the industry needs an increase in potential drug targets. The industry today is spending more money to develop fewer products. Protein microarray technology is one of several biochip technologies that can help shift the trend back to one of continuing innovation.

In today's pharmaceutical lab, business challenges are reducing drug development time, speeding up the identification of new and validated drug targets and developing high-quality information early on in the discovery process. Protein microarray technology measures up to all three of these needs. Its integration with other technology platforms will be critical to maximizing the value of protein microarrays. The technology also should be applied as part of emerging strategies for personalized medicine — one of the major promises of pharmacogenomics.

References

1. Ken Howard, "Protein Microarrays May Just Be the Next Big (Little) Thing," Feb. 21, 2002, at www.genomeweb.com.
2. H. Zlu et al., *Science* **293,** 2101-2105 (2001).
3. G. MacBeath and S.L. Schreiber, *Science* **289(5485),** 1760-1763 (2000).
4. See, for example, R.P. Ekins, *J. Pharm. Biomed. Anal.* **7,** 155-168 (1989); R. Ekins, et al., *Ann. Biol. Clin.* (Paris) **48,** 655-666 (1990); R. Ekins and F. Chu, *Ann. Biol. Clin.* (Paris) **50,** 337-353 (1992).
5. M.F. Templin, D. Stoll, M. Schrenk et al., *TRENDS in Biotechnology* **20:4,** 160-166 (April 2002).
6. Ibid.
7. Frost & Sullivan, June 13, 2001.
8. *Genetic Engineering News* (December 2000), at www.genengnews.com. PG