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Innovations

Zyomyx

Protein Chips: Protein Chemistry Comes to the Surface

High-throughput approaches have had an important impact on biological research over the past decade, particularly in drug discovery. Pharmaceutical companies routinely employ massively parallel formats in their attempts to screen large libraries of synthetic or natural small molecules for new drug leads—the impetus being both efficiency and economy of scale. The advent of DNA chip technology in the mid-90s represented an extension of ultra-high-throughput science to gene expression profiling and coincided nicely with the sequencing of the human and many other genomes. Not only were the sequences available, but the solid-state technology was there, borrowed from the semiconductor and high-tech industries—from silicon chips to ink-jet printing to photolithography. Big pharma was one of the early adopters of the nascent technology, as the new tools promised to facilitate the search for expression markers and drug targets associated with cancer and other disease.

High-throughput science paired up with protein science in the late 90s, and the field of "proteomics" was born. Falling under the proteomics banner were protein expression profiling, functional genomics, and the study of protein-protein interactions. The new field needed new tools, and according to Zyomyx (www.zyomyx.com), a small biotech company in the Bay area, protein chips represent the next logical step in the evolution of massively parallel screening technology. Proteins are generally the key players in disease, they reason—so why not measure them directly? DNA chips might tell you which genes are on and which are off, but mRNA levels do not necessarily correlate with protein levels in a cell. Moreover, DNA chips cannot tell you whether the corresponding proteins are active, scheduled for degradation, or even in the right place in the cell. In theory at least, the case for the protein chip is compelling; the question on the minds of many, however, is whether this theory can be made a practical reality. According to Zyomyx chief executive Larry Cohen, the answer is an unequivocal yes.

The reality is that arraying proteins to a solid surface in an active form is neither a simple nor straightforward task. In fact, there really is no standard chemistry for affixing proteins to a solid surface, and there will likely never be one. Proteins have much greater variation in structure and function than nucleic acids and are also much less stable than DNA. The delicate three-dimensional structure of proteins is directly linked to their function and can be severely affected by the nature of the surface during the immobilization process and thereafter. Denaturation is a particular problem when proteins find themselves at a solid-liquid interface, as on the surface of a silicon chip. As Zyomyx founder and current CTO Peter Wagner explains, "the fundamental challenge in developing protein chips derives from the interplay between protein structure and the molecular complexities of surface chemistry. When developing surface-based analytical tools, it is important to look at the materials through 'biomolecular glasses'."

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Some of the earlier work on engineering DNA microarrays took place at Stanford University, with labs such as Pat Brown's and Ron Davis' at the forefront. It was there that Wagner met Zyomyx cofounder Steffen Nock, at the time a post doc in the laboratory of Jim Spudich in the Department of Biochemistry. Wagner was a biophysicist specialized in surface chemistry and was a Humboldt fellow also working with Spudich. Nock was trained as a traditional biochemist and molecular biologist and knew how to produce high-quality recombinant protein. They were part of a multidisciplinary group studying ways of affixing, arranging, and imaging active protein molecules on solid surfaces. The goal of their project was to study how molecular motor proteins moved on surfaces. "Needless to say, surface chemistry and how these motor proteins attached to the solid interface were essential to the outcome of these experiments," adds Wagner.

The year was 1998, and Silicon Valley was in the grips of the high tech and dot-com craze. DNA chip technology was at its peak, and the industrialization of biology was in vogue. According to Nock, who, like Wagner, hailed from Europe, the atmosphere at Stanford was dramatically different from the stodgy, academic nature of European science at that time. Many of the faculty at Stanford had either started biotech companies or were advising them. "Venture capitalists were literally roaming the halls looking for investment opportunities," says Nock, "we saw the buzz over DNA chips and it occurred to us that our work could be of interest to both the investor and pharmaceutical communities."

Although the business would be born out of Nock and Wagner's collaboration at Stanford, much of the technology underlying Zymoyx's vision had its origins at the Swiss Fed-

eral Institute of Technology in Zurich twelve years earlier. There, Wagner had put together a scientifically diverse, multidisciplinary research program that merged nanotechnology with biology. The group was dedicated to the study of protein function at the molecular level, and surface-based analytical tools and methods of protein immobilization were instrumental for these studies. "Few biologists appreciated the complexity of surface chemistries, and few surface chemists appreciated the complexity of protein structures. The key was to get these groups working with each other," explains Wagner. The outcome of this work was key to the new tools Nock and Wagner wanted to create at Zyomyx.

Those new tools were chip-based arrays of recombinant protein to facilitate the study of protein expression and function. The original idea was to create functional assays for high-throughput drug discovery—to facilitate screening of a panel of kinases, for example, against different substrates. Armed with a PowerPoint presentation and design specs for a prototype chip, Nock and Wagner went on the road to sell their idea to the community. They spent about a year talking to investors and big pharma, gathering intelligence on the potential interest in their "protein chip." What they learned was that what people really wanted to do was expression profiling, similar to what DNA chips were doing for mRNA expression.

They secured their first million from Skyline Ventures in late '98, followed by an additional eight million the next year. The cash allowed them to incorporate and recruit staff, many drawn from the same Zurich group of which Wagner had been a part years earlier. Zyomyx now has about $64 million venture funding in the bank and receives payments from a strategic collaboration with the Japanese biotech firm Fujirebio, Inc. Importantly, they hope to have their chips in tests with six groups worldwide by the end of 2002.

In fact, Zyomyx delivered its first Protein Profiling Biochip in May of 2002 to its first "early access" collaborator, Specialty Laboratories, Inc., a world leader in the development and validation of clinical diagnostics for medical and pharmaceutical applications. These first chips are designed to measure chemokines, the molecular messengers of the immune system. Part of the logic behind creating a chemokine chip was to exploit the advantages that the protein chip format provides over a DNA chip for certain applications. Chemokines are secreted proteins that localize to very specific regions of the immune system, often quite distant to the cell type in which their mRNA was produced. DNA chips are simply not suited to such soluble analytes.

As with DNA chips, the starting point of the BioChip is a silicon wafer. Unlike DNA arrays, the surface is rendered hydrophilic rather than hydrophobic. For DNA arrays, hydrophobicity helps limit feature size so as to produce chips of the highest density. For protein chips, sensitivity is the key, rather than density. "With protein chips, there has been a paradigm shift from density to quality and accuracy of the individual data point," explains Nock, "and this creates a whole different set of analytical and process tools." The wafer is subjected to photolithography to create raised pillars on the surface of the chip that are coated with an inorganic material, such as titanium oxide. This creates a negatively charged surface onto which a layer of organic polymer is laid to create an interface suitable for the binding and orientation of the capture molecule of choice.

Although the detailed specifics of the manufacture of the chemokine chips are proprietary, Zyomyx did publish a paper last year (Ruiz-Talyor et al., 2001, Proc. Natl. Acad. Sci. USA *98*, 852–857) describing a prototype chip design. In that work, the authors used the organic polymer poly(L-lysine)-grafted-poly(ethylene glycol), PLL-g-PEG, with the PEG moiety derivatized with a biotin molecule. The positively charged PLL forms an ordered monolayer on the negatively charged surface of the chip, such that the PEG moiety points upward into the aqueous solution on the chip. PEG compounds resist nonspecific protein binding, significantly reducing the adsorption of proteins from the protein mixture being assayed. The biotin molecule provides a specific attachment point for the capture molecules of choice, creating a very specific bioreactivity for the cognate analyte. These capture molecules could be either streptavidin-linked antibodies or Fab fragments. Importantly, Zyomyx has inked supply and collaboration agreements giving them access to vast antibody libraries from three of the larger players in the area, BD Biosciences, Cambridge Antibody Technology, and Dyax Corp.

The raised pillars are only 50 microns in diameter, fitting about a million antibody molecules in each chip feature. According to Cohen, who joined Zyomyx in 1999, 90% of those molecules must be active and oriented properly to allow the necessary sensitivity for the assay. Getting almost a million protein molecules onto the surface without denaturing them is no easy task. To achieve this, Zymoyx has developed a proprietary technique for parallel printing. The design of the chip ensures that the capture molecules remain in solution, preventing drying out. A mere ten microliters of blood is used for the chemokine assay, which is "processed" by exposure to a second set of anti-chemokine antibodies and a fluorescence-based detection system.

The logic behind the collaboration with Specialty was to get the chips into the hands of those with experience in evaluating assays for reference laboratory uses. Clinical diagnostics is a key market the company is targeting, and optimization and standardization of the chips will be crucial for the eventual adaptation of the chips for use in clinical trials and preclinical studies. In fact, as this article went to press, Zyomyx announced a strategic alliance with Partners HealthCare Boston to provide the tools for a core protein profiling facility at Harvard University's teaching hospitals. "Comparing proteins that are expressed in diseased versus normal human samples allows researchers to identify and validate patterns of biomarkers that define specific diseases," explains Cohen, "Diagnostic tests based on these biomarkers will be important tools to enhance clinical trials design and ultimately to identify the most effective therapy for the patient."

A key selling point to their chips,

according to Cohen, is the fact that Zyomyx is not just providing chips, but a complete system. "This makes us unique in the protein chip marketplace at present," he adds. The fluorescence-based chip reader was codeveloped with Axon Instruments, a well-established producer of DNA chip readers. A fluidics workstation can process 12 chips at a time, in about 1–2 hours. Software is also provided for data capture and analysis. Then, of course, there are the chips themselves and the necessary reagents.

Although much of their future hinges upon the success of the Protein Profiling Biochip, Zyomyx is actively working on several related but distinct products with several partners. Canadian testing company MDS is working with them on a protein interaction assay—a sort of solid-state two-hybrid assay. Another product under development is a chip to measure the activity of arrayed ion channels—interesting drug targets, but particularly difficult to work with due to their membrane localization. Unlike the DNA chip market, which is dominated by a couple of formats and major manufacturers, the diversity of protein structure seems likely to give rise to a diversity of protein chip formats and a more diverse marketplace. Although the number of assays on the chemokine chip is only 25, higher-density formats—perhaps into the 1000s—are under development. Alternative detection systems, such as time-of-flight mass spectrometry, are also in the works. As the protein chip market has been estimated by some to eventually hit a billion dollars annually, the pay-off could be dramatic. As the efforts of Zyomyx have shown, understanding that market and the potential demand will be key to realizing these pay-offs.

***Chemistry & Biology* invites your comments on this topic. Please write to the editors at chembiol@cell.com.**

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