International Research Journal of Natural and Applied Sciences
Volume-2, Issue-3 (March 2015)
ISSN: (2349-4077)

PROTEIN BIOCHIP- A REVIEW

DR. AJAY SHARMA, Senior Lecturer (Botany)
Govt. Bangur College, Deedwana, District – Nagaur (Raj.)

DR. ANTARYAMI KAUSHIK, Senior Lecturer (Botany)
Govt. College, Suratgarh, District – Sri Ganganagar (Raj.)

DR. SANJEEV KUMAR BANSAL, Senior Lecturer (ABST)
SBD Govt. College, Sardarshahar, District – Churu (Raj.)

Abstract

A Protein biochip is a collection of miniaturized testsites - also commonly referred to as microarray- arranged on a solid substrate that permits many Protein tests to be performed at the same time to achieve higher throughput and speed. The miniaturized test site can be placed on to a 2-diamentional surface, such as a glass slide, or a 3-diamentional surface such as a bead. In the case of 2-diamentional surface, multiple spots are arrayed, each containing a different immobilized protein or capture agent (or duplicates for control purposes), and are pooled into groups for parallel analysis.

Present Status

The rate of advance of drug discovery and development technologies in the past decade has been exponential. If we turn the clock back ten years. Gene Bank (http://www.nccbi.nlm.nih.gov/) only contained 217 million base pairs of sequence (compared with 42 billion base pairs today), DNA microarrays had not been commercialized, protein identification by mass sepctormetry had just become a reality [Mann and Wilm 1994, Yates et.al. 1993], and automation was just beginning to enable high-throughput screening. Now the whole human genome has been sequenced and can be studied on a single DNA microarray, thousands of proteins can be identified in a single mass spectrometry run, and entire compound libraries can be screened in a matter of days. When protein biochips [Templim et.al. 2004] began to emerge as the most probable candidate for the next great class of drug discovery and development technology at the beginning of this century, the common preception within the research community was that the rate of advances in protein biochip technology would match that DNA microarrays, mass spectrometry and/or gene sequencing. This, however, has not proven to be correct. The amount of data per experiment (defined as output) that could be obtained from the earliest protein biochips [Haab et.al. 2001, Clark et. al. 1999, Macbeath and Schreiber, 2000, Phizicky et. al. 2003] is still the standard several years later, with significant but not exponential increases in output expected within the next few years.

The primary reason for the slower than expected progress of the protein biochip industry has been the limited availability of content [Bodovitz 2003]. This problem is defined by a lack of high quality capture agents that can be immobilized on the surface of a biochip - defined as a capture protein biochip - to bind to proteins from complex mixtures for the purpose of detection and quantification. In contrast of DNA microarrays, in which cDNA capture sequences can be predicted by Watson-Crick base-pairing, protein capture agents have significantly more complex interactions with their ligands. Protein binding involves charge, hydrogen bonds and/or weak hydrophobic forces. Moreover, binding often requires specific 3-dimensional conformations, post-translational modifications and/or co-factors. Because of this complexity, each capture agent must be optimized empirically, presenting a significant challenge for large scale production. Furthermore, even in the cases where more than 100 high quality antibodies are available for capturing 100 different proteins, only approximately 30 to 40 of these can be placed together on the same biochip before the cross reactivity between sandwich pairs becomes overwhelming [Schweitzer et.al. 2002].

The analogy between DNA and protein biochips has fared better when applied to interaction protein biochips, where a limited number of proteins or peptides are immobilized on a surface to examine specific protein interactions. In this case, content is not a significant limitation because peptides can be synthesized and proteins can be produced using recombinant technology. The potential limitation is the question surrounding the physiological relevance of the detected interactions; in contrast to capture protein biochips. the interactions between immobilized protein or peptide and soluble protein are supposed to represent biochemical event that take place in a cell or biological fluid in vivo. All of these interactions take place on the surface of a biochip under a single set of conditions. which goes against the traditional dogma among biochemists that each biochemical reaction is unique and affected by a wide range of variables, including buffer conditions, protein conformation and co-factor. By contrast, Michael Snyder's research group at Yale (http://.yale.edu/synder/) and several companies, such as lnvitrogen Corporation (http.//www.invitrogen.com), Protagen AG (http:// www.protagen.com), Pepsan Systems BV (http://www pepscan.n1) and Jerini AG (http://www.jerini.com), have shown compelling data with interaction protein biochip, in which the relevance of interactions was at least partially validated through other experimental methods [Bodovitz et. al. 2004].

Near-term prospects

The content problem initially slowed down the emergence of capture protein biochips, but developers have forged ahead by using available content and focusing on specific research areas. According to our recent analysis, at least 49 companies offer commercial capture protein biochip products and/or services, including both planar and bead-based platforms; scores of additional platforms are also available through collaborative agreements or are still under development [Bodovitz et. al. 2004, Bodovitz et. al. 2005). The analytes measured include

adipokines, angiogenesis factors, apoliopoproteins, cardiovascular disease markers, cell signaling molecules, chemokines, coagulation proteins, common allergens, cytokines, drug of abuse, endocrine hormones, growth factors, HLA antigens matrix metalloproteinases, and serum cancer biomarkers. Alzhemier's disease, apoptosis, autoimmunity, cancer, cardiovascular disease, connective tissue disorders, immunoglobulin isotyping, inflammation, infectious disease, metabolic disease, oncology and sepsis. In addition to research and development tools, capture protein biochips are also currently used as diagnostic assays for medical conditions, such as allergies, autoimmune diseases, drug abuse, infectious diseases, heart failure and other coronary problems. In addition to diagnosing disease. capture protein biochips have the potential to identify biological warfare agents The National Institutes of Health (http://www.nih.gov/) have allocated in excess of $1 billion to fund research on bioterrorism agents and development of vaccines and diagnostic tests.

The reduction of sample volume is of great importance for applications in which very small amounts of analyte are available, such as in the analysis of tumor biopsiebiopsies. Multiple measurements on the same biochip (multiplexing) increase the amount of information obtained from a single experiment and enables researchers to examine patterns. This is crucial in areas such as immunology, where proteins, such as cytokines, function in concert. Multiplexing can increase the sensitivity and specificity and biomarkers by combining ones that only have limited predictive power as singlets, but high predictive power as panels [Bodovitz and Patterson 2003,] Moreover, multiplexing using interaction protein biochips offers the possibility to identify biochemical pathways and characterize the binding selectivity of antibodies and small compound across a representation of a cellular proteome in a single experiment. To take full advantage of its potential and to continue to grow in the near term, the protein biochip industry needs to bring robust product to the market with ever expanding content.

Long-term prospects

Once the protein biochip has established the reliability, utility and capabilities of its platforms, it will be ready to escape from under the shadow of the other drug discovery and development technologies that aim to study complete systems, from genome to proteome to compound library. The current process is based on the model of one target-one drug, but in reality, biological systems are complex and the majority of diseases are the result of multiple molecular changes. In this context, selecting just one target represents an arbitrary and excessive simplification. All of the molecular changes are important and potential therapeutics should be evaluated for their abilities to reverse as many of these changes as possible. Todd Golub's group at Harvard University (http.//wmv.broad.mit.edu/ broad/toddgolub.html) has demonstrated such a multiple targets-one drug paradigm using DNA microarrays (Stegmaier et.al. 2004). His approach, termed gene expression based high throughput screening, was used to identify compounds that induce the differentiation of acute myeloid leukemia cells. Dr. Golub's group

screened 1,739 compounds and identified eight that reliably induced the differentiation signature and furthermore, yielded functional evidence of bona fide differentiation. Furthermore, the ability to obtain efficacy and toxicity information from the same protein biochip assay might enable the screening process to become efficient enough to evaluate potential therapeutics in combination. Reversing a significant percentage of disease-related changes could require more than one therapeutic. The one target - one drug mode might fully evolve into the multiple targets-multiple drugs mode. Changing the drug discovery and development process at the molecular level might be the best medicine of all.

Summary

Even though protein biochips have fared poorly in comparisons with other high-output drug discovery and development technologies, such as DNA microarrays, the industry is now introducing a large number of protein biochip-based products for a range of applications. Based on the current level of success, we have projected that the market will grow from an estimated $122 million in 2002 to more than $500 million in 2013. This growth rate is based on the recent successes of the industry in providing new products, but might, in fact, be understated. Once protein biochips have established their reliability and versatility, they could be the most likely of the high-output technologies to truly revolutionize the drug discovery and development process.

Mann, M. and Wilm, M.S. (1994) Error tolerant identification of peptides in sequence databases by peptide sequence tags. Anal. Chem. 66, 4390-4399.

Yates, J.R. 3rd et al. (1993) Peptide mass maps: a highly informative approach to protein identification. Anal. Biochem. 214, 397-408.

Templim, M.F. et. al. (2004) Protein microarrays and multiplexed sandwich immunoassays : what beats the beads Comb. Chem. High Throughput Screen. 7, 223-229.

Haab. B.B. et. al. (2001) Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. Genome Bol. 2, RESEARCHCH0004 DOI: 10.1186/gb-2001-2-2-research0004 (http://genomebiology.com/2001/2/2/RESEARCH/0004)

Clark, M.D. et. al. (1999) Construction and analysis of arrayed cDNA libraries. Methods Enzymol. 303, 205-233.

MacBeath, G. and Schreiber, S.L. (2000) Printing proteins as microarrays for high-throughput function determination. Science 289, 1760-1763.

Phizicky, E. et. al. (2003) Protein analysis on a protemic scale. Nature 422, 208-215.

Bodovitz, S. (2003) The Protein Biochip Content Problem, Drug Discovery World, Fall, 50-55.

Schweitzer, B. et. al. (2002) Multiplexed protein profiling on microarrays by rolling-circle amplification. Nat. Biotechnol. 20. 359-365.

Bodovitz, S. et. al. (2004) Protein biochips parallelized screening for high output biology, BioPerspectives and Bachmann Consulting.

Bodovitz, S. and Patterson, S. (2003) Protein biomarker strategies. Drug Discovery World Fall, 67-78.

Stegmaier. K. et. al. (2004) Gene expression based high - throughput screening (GE-HTS) and application to leukemia differentiation. Nat. Gent. 36, 257-263.

Bodovitz, S. et. al. (2005) Protein Biochips: the calm before the strom Drug Discovery Today (DDT). Vol 10:283-287.