

Protein Research Calls for Advanced Instruments

FEATURE

by Ineke Malsch

Push to discover more about structure and function

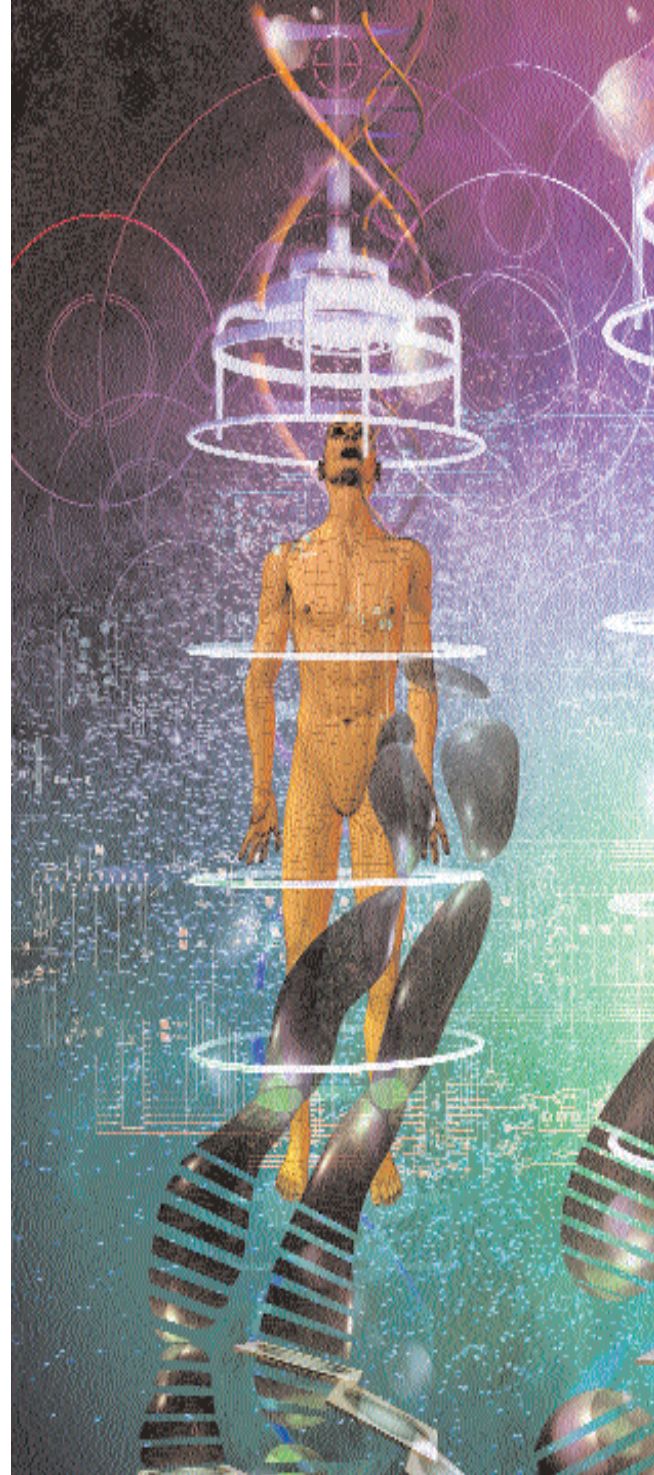
With the mapping of the human genome, proteomics has rapidly emerged as an exciting new field of research, one that complements rather than replaces genomics. The genome holds all of an organism's genes, which carry the codes for the proteins that it needs to function. Proteomics is the integrated study of proteins and their biological functions and processes, such as hormone production and the immune-system response. It allows the study of protein structure and protein-protein interactions, and today, the science is becoming a major tool in biomedical and drug development research. Carrying out and advancing such studies more efficiently and effectively, however, will require new, cutting-edge instrumentation.

Experts envision proteomics playing a key role in deciphering cancer, diabetes, heart disease, and other life-threatening ailments, and the scientific community has responded. Last year, the international Human Proteome Organization began promoting and coordinating a global effort to advance proteomics research and discovery. In the United States, the National Institutes of Health established a Proteome Special Interest Group in 2001 to help keep its scientists and other researchers current on advances in the rapidly changing field.

The surge in proteomics research is accompanied by a growing market value. According to a forecast by Front Line Strategic Consulting, Inc. (San Mateo, CA), published in February, the proteomics market will reach \$2.68 billion in 2008, up 76% from an estimated \$1.52 billion in 2003. The forecast projects a compound annual growth rate of 12% in a market that consists of four segments: protein separation, protein characterization, content and bioinformatics, and services.

Proteomics labs

A protein is a biological molecule consisting of a linear chain of amino acids. At normal ambient temperatures, the amino acid chain is folded into a complex three-dimensional structure, which for decades has presented a major challenge to researchers trying to decipher the make-up and function of proteins. The European Molecular Biology



Laboratory's (EMBL's) Proteomics Visitor Facility (Heidelberg, Germany) exemplifies a well-equipped proteomics laboratory. It includes instruments for protein separation, imaging, and robotics sample preparation, and mass spectrometers, computers, and advanced software. Most of the equipment was made by Micromass-Waters, Ltd. (Manchester, England), and Bio-Rad Laboratories, Inc. (Hercules, CA), which together market ProteomeWorks, a complete set of laboratory instruments for proteomics research. Other major producers of proteomics systems include Amersham Biosciences (Piscataway, NJ), Applied Biosystems Group (Foster City, CA), and Proteome Systems, Ltd. (Sydney, Australia).



Anthony Robinson

Analysis

A proteomics analysis usually involves complex mixtures of different biological molecules, such as a blood or tissue sample from a patient. The analyst uses electrophoresis or chromatography to separate the molecules into fractions. In a second step, he or she concentrates the interesting proteins as spots on two-dimensional gels for two-dimensional electrophoresis. The protein fractions then are physically separated in one dimension by charge using isoelectric focusing, and in the perpendicular dimension by molecular weight. The result is a slide covered by a gel that contains an irregular pattern of spots, which are the separated proteins.

The analyst then stains the gels and creates images of the spots. The images are analyzed with automated image-analysis software. The protein spots of interest are cut from the slide and digested using enzymes to process the sample for further study. The amino acids of the selected proteins are finally identified using a mass spectrometer, and they are characterized using protein arrays, confocal microscopes, chromatography, and two mass spectrometers working in tandem. Handling the large amounts of data generated by these experiments requires bioinformatics software and large-scale data storage.

Companies worldwide are researching new high-throughput alternatives to two-dimensional electrophoresis to speed up the process. To date, however, there is no suitable replacement technique for two-dimensional electrophoresis on the horizon that can compare with it. Companies are also developing new analytical instruments to study the physical and chemical properties of proteins and how they function.

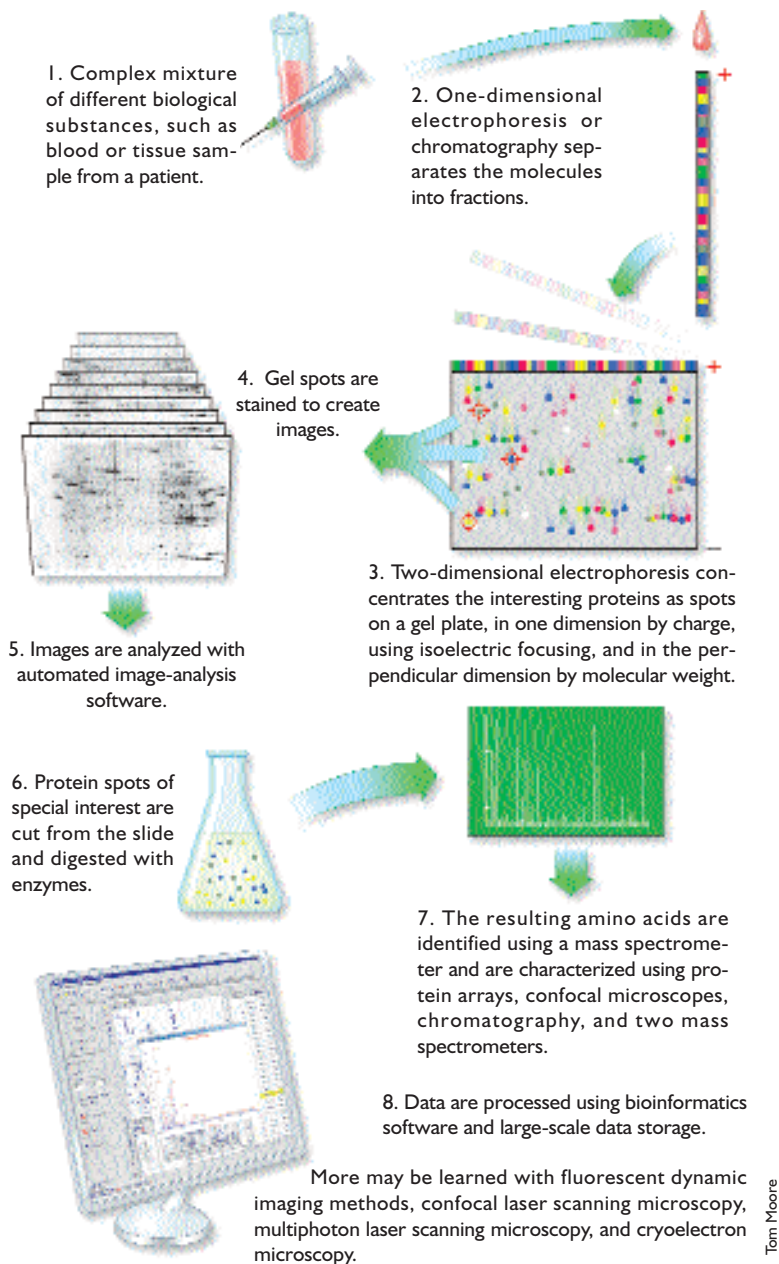
One major trend in the market for proteomics laboratory equipment is the formation of joint ventures that allow companies that produce complementary instruments to offer a fully equipped laboratory. For example, ProteomeWorks, marketed jointly by Bio-Rad Laboratories and Micromass-Waters, is an integrated suite of instruments whose components are made separately by the two companies. Similarly, Amersham Biosciences and Thermo Electron Corp. (Waltham, MA) jointly market mass spectrometry-based proteomics research instruments. Initially, the two companies will co-market Amersham Biosciences' Ettan MALDI-ToF Pro mass spectrometer and Thermo Electron's Finnigan line of ion-trap mass spectrometry systems, including the ProteomeX proteomics workstation. The two companies also plan to collaborate in developing next-generation systems specifically designed for proteomics applications.

Separation technologies

Two-dimensional electrophoresis remains the most important technology, albeit an imperfect one, for separating proteins from cells or tissues. It is a low-throughput technique capable of characterizing only 20 to 30% of the proteins in a sample. DNA microarrays, a competing technology, measure the expression, or activity, of genes and allow scientists to predict the proteins for which the turned-on DNA carries the code. It is a high-throughput technique, but it is hindered by the fact that one DNA sequence can code for many modified proteins.

A microarray consists of a microchip on which biological molecules are immobilized. Several start-up companies are competing to develop novel protein-microarray technologies such as a specific microarray of antibodies against a set of known pathogenic proteins. In genetics work, the molecules on a microarray usually consist of DNA strands. For proteomics research, however, other chips are more suitable, such as those containing antibodies or other proteins. In these microarrays, a prede-

PROTEIN ANALYSIS



terminated pattern of known proteins or antibodies is immobilized in standardized microwell plates with 96 or 384 wells on a plate. A sample of proteins being studied can be tested for reactions with a known antibody or other protein in each well. A major advantage of this technique is that a robot can analyze the wells to identify a reaction between known antibodies or proteins and the protein under study.

Proteome Systems is developing protein chips based on the piezoelectric dispensing of antibodies and chemicals onto a two-dimensional gel-electrophoretic array of immobilized proteins that are to be characterized. This technique offers a cost advantage because it allows the continued use of gel electrophoresis, which is standard equipment in most proteomics laboratories.

Integration

Leading instrument manufacturers are also rapidly improving their two-dimensional gel electrophoresis and other gel-based systems. Amersham Biosciences, for example, recently introduced the Ettan Spot Handling Workstation, a robotic sample-preparation system that integrates bioinformatics, data storage, and processing. The workstation automates the excision and digestion of proteins that might serve as drugs from two-dimensional gels. Once the proteins are isolated from the gel, they can be identified by matrix-assisted laser desorption-ionization time-of-flight (MALDI-ToF) mass spectrometry. Bio-Rad and Micromass also offer robotic instruments that enable excision and digestion of proteins from two-dimensional gels. Similarly, Proteome Systems provides this capability in a proteomics system in which all analytic components, from sample preparation to mass spectrometry, are integrated into a sample-management and data-analysis bioinformatics platform.

Other trends in instrumentation research include developing porous silicon biosensors for identifying proteins, antigens, and DNA; applying ultraviolet resonance Raman spectroscopy to investigate protein folding; and using holographic images of proteins and other organic structures to detect changes caused by biochemical reactions.

Identification and characterization

To identify and characterize an unknown protein, researchers use a combination of chromatography and mass spectrometry to break it down into its amino acids and identify them by their mass spectrum. A liquid-chromatography system can separate the fractions of a protein sample before analysis. A mass spectrometer forms ions from a sample and separates these ions by their mass-to-charge ratio.

Making sense of the huge amounts of data generated by characterizing all of the proteins involved in human diseases will require large-scale data storage and bioinformatics software. IBM is one company investing heavily in the development of computational biology, and it has formed a strategic alliance with Proteome Systems to provide bioinformatics systems for proteomics researchers that include enormous storage capacity and Internet access.

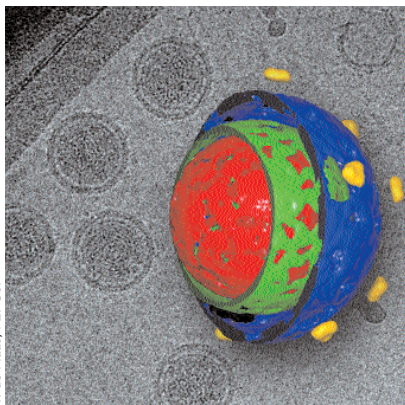
Scientists worldwide have placed their experimental results in online-accessible databases and on Web sites, but in a nonstandardized and difficult-to-compare fashion, which hampers the comparison and verification of research findings. In 2002, the Human Proteome Organization started the Proteomics Standards Initiative to develop data representation standards to facilitate comparisons, exchanges, and verification. The two areas initially targeted for standards are mass spectrometry and protein-protein interactions.

TABLE I. SOME KEY PLAYERS IN PROTEOMICS INSTRUMENTATION

COMPANY	LOCATION	PRODUCT	WEB SITE
Amersham Biosciences	Piscataway, NJ	Integrated system of laboratory instruments, together with Thermo Electron Corp.	www.amershambiosciences.com
Applied Biosystems Group	Foster City, CA	Laboratory instruments.	www.appliedbiosystems.com
Bio-Rad Laboratories, Inc	Hercules, CA	A complete system of laboratory instruments, together with Micromass-Waters.	www.bio-rad.com
FEI Co.	Hillsboro, OR	Scanning electron microscopy, including cryoelectron microscopy, which can be used to image frozen proteins and cells.	www.feic.com
Micromass-Waters, Ltd.	Manchester, England	A complete system of laboratory instruments, together with Bio-Rad.	www.waters.com
Proteome Systems, Ltd.	Sydney, Australia	Developing protein chips, and entered a strategic alliance with IBM to develop bioinformatics systems.	www.proteomesystems.com
Thermo Electron Corp.	Waltham, MA	Integrated system of laboratory instruments, together with Amersham Biosciences.	www.thermo.com

Learning more

Once researchers identify and characterize a protein, there remains a lot to learn about it. Fluorescent dynamic-imaging methods that use confocal laser-scanning microscopy and multiphoton laser-scanning microscopy are particularly useful and powerful tools for studying proteins. In these methods, a laser beam is focused onto a small point inside a cell, and, with the help of a scanning mirror or other hardware, the point is scanned through a focal plane. A photon-multiplier tube detects the light of fluorescent-tagged proteins coming from the focal plane, from which an image of the cell is constructed. Interactions between tagged proteins can be monitored



F. de Haas, FEI Co.

Figure 3. A three-dimensional tomographic representation of the bacteriophage $\phi 6$ virus is obtained with cryoelectron microscopy by plunge-freezing the sample in liquid ethane and scanning with a transmission electron microscope from several different directions up to a 140° span.

with this methodology. Loading cells with the appropriate fluorescent probes enables researchers to measure the effects of proteins on such phenomena as membrane potential, the generation of intracellular message molecules, and gene expression.

Companies and researchers continue to develop more powerful microscopes and sample preparation tools for use in areas such as the emerging field of cryoelectron microscopy. FEI Co. (Hillsboro, OR) sells such instruments developed by its subsidiary, Electron Optics (Eindhoven, The Netherlands). “We want to offer the customer a total solution for studying the real structure and macromolecular state of proteins, chemical compounds, polymers, and nanoparticles,” explains Marc Storms, FEI’s product manager in The Netherlands. “Vitrobot, our sample-preparation robot, can prepare the sample in an almost fully automatic way. It plunge-freezes any aqueous

suspension at optimal freezing conditions in liquid ethane or propane. The next step is to investigate the frozen sample in a transmission electron microscope at preferably helium temperatures.”

Because organic materials are vulnerable to radiation when cooled to cryogenic temperatures, researchers want to freeze samples above the freezing point of helium to avoid cooking them with the electrons from the beam. A frozen sample can be screened from several different angles spanning a 140° maximum range. The images from all these angles are combined to generate a three-dimensional tomographic representation of the sample, such as a complete protein or cell organelle. “This procedure results in tantalizing, colorful graphics,” Storms says.

Proteomics is still emerging as a well-defined field of research. There is ample room for established and start-up instrument manufacturers to position themselves in a growing world market. There are also many opportunities for interesting physics research in developing the proteomics laboratory instruments of tomorrow.

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Further reading

A description of the Proteomics Standards Initiative is available at <http://psidev.sourceforge.net>.


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Information on the Human Proteome Organization and its programs is available at <http://www.hupo.org>.

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B I O G R A P H Y

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