Proteomics: new technologies and their applications

Kewal K. Jain, Bläsiring 7, CH-4057 Basel, Switzerland. tel/fax: +41 61 692 4461, e-mail: jain@pharmabiotech.ch, URL: http://pharmabiotech.ch

The increasing interest and activity in proteomics is evidenced by a recent conference, which attracted a large attendance and was devoted entirely to proteomic technologies. The 4th IBC conference on *Proteomics* was held in Basel, Switzerland on 1–2 March 2001 and covered important recent developments in this field.

In the opening keynote address, Matthias Mann (MDS Proteomics, Odense, Denmark) defined proteomics as the large-scale analysis of the function of gene products not including genomics, bioinformatics and mRNA studies, even though these are integrated as tools into proteomics. A wider definition includes methods with genetic readout such as two-hybrid systems and structural genomics; a narrower definition includes only large-scale approaches where actual proteins are studied[1].

Mann described techniques for protein identification using MS in an integrated system, including innovations of this technique such as MALDI (matrix-assisted laser desorption/ionization) MS, nanoelectrospray MS/MS, robotic spot excision, quadropole–TOF (time of flight), microcapillary HPLC–MS/MS, and data management. Technology is now maturing to identify all human proteins at high sensitivity and in a reasonable throughput and more than 250 proteins have been identified so far. Several proteomic technologies can now be used for investigating cellular functions and to enhance classical biochemical approaches as a part of functional proteomics.

Twenty-five presentations were made at this conference. Only highlights of the articles are presented here and other new developments have been described in a special report on this topic[2].

Mass spectrometry

Mass spectrometry remains the workhorse for identification of proteins but the standard techniques have limitations when it comes to accurate quantification of proteins and peptides in complex mixtures. Isotope-coded affinity tag (ICAT) peptide labelling is an approach that combines accurate quantification and concurrent sequence identification of individual proteins in complex mixtures[3].

Tim Nadler (Applied Biosystems, Foster City, CA, USA) presented the optimized ICAT and validated the technique by demonstrating data using real biological samples. The advantages of this technique are the potential for automation and the relative quantification by MS. A limitation of this approach is that it does not give information about post-translational modification. Randall Nelson (Intrinsic Bioprobes, Tempe, AZ, USA) described high-throughput protein characterization using MS immunoassay (MSIA) combined with bioreactive probes and applied to several body fluids. Examples presented included human cystatin C and β_2 microglobulin screening of urine samples, which has clinical relevance as biomarkers of inflammatory ailments. Throughput rates of ~100 samples per hour were achieved. This technique is easy, fast and amenable to robotics and protein profiling by MSAI can detect protein–protein and protein–small-molecule interactions.

Functional proteomics

Walter Blackstock (GlaxoSmithKline, Stevenage, UK) gave an excellent overview of proteomic technologies within the broad field of genomics. The focus was mainly on two areas:

- The use of proteomic technologies to characterize protein complexes and pathways to create maps of spatial and temporal expression of proteins in a 'virtual' cell; and
- The quantitative study of global changes in protein expression in tissues to look for disease markers.

These are usually protein–protein interactions and add value to the target validation process.

The MDS Proteomics (Odense, Denmark) approach to protein–protein interactions was described by Ron Hendrickson and involves a fully integrated programme. The three interacting components of this platform are:

- PathMap, an automated pathway mapping system for identification of crucial interactions and targets;
- BIND, a database for storage and interpretation of information as well as for target selection; and
- LeadFinder for prioritization and quantification of hits whereby information is generated for drug discovery.

Other methods of examining protein–protein interactions were discussed by Terry McCann (Perkin Elmer Life Sciences, Boston, MA, USA). McCann and colleagues have designed a technology that uses green fluorescent protein (GFP) and multibeam confocal imaging systems for analysing the functions of proteins in living cells. The combination

1359-6446/01/$ – see front matter PII: S1359-6446(01)01785-8

of technologies enables 2-D PAGE (polyacrylamide gel electrophoresis)/MS approach in which the target protein is tagged with GFP enabling the imaging of protein–protein interactions. This approach offers the advantage that complex pathways of cell biology can be viewed without destroying the cell.

Donny Strosberg described the ongoing work at Hybrigenics (Paris, France) where functional signalling and metabolic pathways are being reconstructed on the basis of interacting proteins. Protein Interaction Maps (PIMs) and corresponding annotated databases are then created using HTS procedures and proprietary bioinformatic tools. In fact, the PIM of *Helicobacter pylori* has already been published[4].

Protein arrays and high-throughput techniques

Ian Humphrey-Smith (Universiteit Utrecht, Utrecht, The Netherlands) described peptide, protein and antibody arrays as an integrated approach to following human tissues in health and disease. The greater part of the protein mass found within living cells is encoded by approximately 10% of genes. Most of the proteins, therefore, occur with a low intracellular abundance and are beyond the reach of traditional proteomic technologies such as 2-D gels and MS. Yet, knowledge of these proteins is required for an understanding of the pathogenesis of diseases. Antibody microarrays/biochips were proposed as a solution because they enable coupling to sensitive detection systems, parallel analysis and miniaturization. Differential display enables antibodies with varying affinities to be employed on the same array. The following presentation by Lucy Holt (MRC Laboratory of Molecular Biology, Cambridge, UK) described the use of recombinant antibodies in proteomics[5]. Use of high-speed robotics is enabling millions of antibody–antigen interactions to be screened simultaneously. This approach obviates the need for phage selection and enables the identification of differentially expressed proteins in natural extracts as well as protein–protein interactions. The group is now exploring miniaturization of these arrays.

Since the post-genomic era, a need has been created for a large-scale method to purify, identify and characterize novel and pharmacologically relevant proteins. Stefan Schmidt (GPC Biotech, Martinsried, Germany) presented technologies for high-throughput protein expression and array-based characterization. Image analysis of arrays is done with its Biochip Explorer technology. This method has been optimized to process samples at a rate of 150 sec per sample with an average yield of at least 1 μg and a purity of over 80%, which is adequate for many assays in which thousands of proteins can be assessed for different purposes.

ProteinChip System (Ciphergen, Fremont, CA, USA), based on SELDI (surface-enhanced laser desorption/ionization) is an alternative system that can rapidly perform separation, detection and analysis of proteins at femtomole levels directly from biological samples. William Rich of Ciphergen presented both the ProteinChip 'benchtop' system and Tandem MS system, which have several advantages over the 2-D gel method. These include speed of detection (hours versus days), coverage of a broader region of the proteome, small sample requirement (1 ml or 500 cells), and combination of discovery of biomarkers and diagnostic assays in a single system whereas MS is limited to the discovery of biomarkers only. This has been exemplified by its use in the discovery of prostate cancer biomarkers. With ProteinChip technology, it was possible to discriminate between benign prostatic hypertrophy with bound PSA (prostate-specific antigen) and cancer of the prostate with free PSA. Advantages of this method over affinity LC–MS are similar to those over 2-D gels. Additional features of the new system include the fact that it is a non-expert, versatile benchtop system (in contrast to other expert, dedicated affinity LC–MS systems) and can be automated for high-throughput compatibility.

Although the basic technologies of proteomics are well established, key to success in their application to drug discovery is their ability to handle a large number of protein targets in parallel. Malcolm Pluskal (Proteome Systems, Woburn, MA, USA) discussed the Excise platform for integrated spot cutting, digestion and spotting for MALDI–MS as well as the applications of ProteoChip based on Chemical Printer, which employs the piezo-electric microvolume liquid dispensing for chemical analysis of the proteins resolved in a 2-D gel. After comparing with antigen and antibody arrays, he pointed out these 2-D gels (GelChip) are authentic protein chips although they are not predetermined arrays and the location of the proteins in the array results from knowledge of the charge and size of the proteins.

Applications

The combination of DNA microarrays with proteomics has revolutionalized molecular medicine, as these technologies reveal not only gene regulation events involved in disease progression but also targets for drug discovery and diagnostics[6]. Julio Celis (Danish Centre for Human Genome Research, Aarhus, Denmark) discussed the clinical applications of proteomics with emphasis on premalignant lesions. Proteomics combined with immunohistochemistry can be used for the study of squamous cell carcinoma of the bladder. Protein markers specific for epithelial cells of the tumour have been identified and antibodies developed against these markers. Analysis by means of immunofluorescence of cryostat sections of the tumour of patients with invasive disease is based on proteomic data.

Christian Rohlff (Oxford Glycosciences, Oxford, UK) described his company's

strategy for molecular dissection of disease with applications to target discovery in human neurological disorders and cancer. A region-specific CNS tissue expression database has been constructed as a measure of disease specific to disease-relevant brain regions. This links novel target mechanisms identified in CNS tissues to database information relating to clinical, biological, image and annotation data. An integrated analysis of human cerebrospinal fluid and CNS tissue, which is being performed currently, could yield novel therapeutics or support the rationale of existing drugs.

Proteomics has also proved a valuable tool for the evaluation of heart disease. John Weekes (Imperial College School of Medicine, Heart Science Centre, Harefield, UK) described alterations in protein expression in dilated cardiomyopathy. Specific proteins are hyperubiquitinated in diseased hearts, and these proteins can be purified by affinity chromatography and then identified using 2-D PAGE/MALDI-TOF. The ubiquitin–proteosome pathway is therefore a possible target for therapeutic intervention in heart disease.

Michael Schrader (BioVision, Hannover, Germany) concluded the programme by discussing peptidomics, i.e. the analysis of peptides from biological sources and their application for the discovery of biomarkers and drug candidates. Peptides are particularly suitable for diagnostics because they result from regulatory/disease processes and soluble molecules facilitate robust sample preparation, while peptide fingerprints can be generated from a variety of different body fluids. Therapeutic advantages of using peptides include convenience of production and drug delivery.

Conclusions

This conference reflected the considerable progress that has been made in proteomics during the past few years. The technologies presented are having a considerable impact on biomarker identification and drug discovery. Although proteomics is being promoted as a separate industry, it is in fact a set of technologies, which are being increasingly used in combination with genomic technologies in the post-genomic era. The conference was an excellent overview of the state-of-art proteomic technologies and their applications, and provided a good forum for discussion between the academia and the industry.

References

1 Pandey, A. and Mann, M. (2000) Proteomics to study genes and genomes. *Nature* 405, 837-846

2 Jain, K.K. (2001) *Proteomics: Technologies and Commercial Opportunities* (4th edn), pp. 1–300, Jain PharmaBiotech

3 Gygi, S.P. *et al.* (1999) Quantitative analysis of protein mixtures using isotope coded affinity tags. *Nat. Biotechnol.* 17, 994–999

4 Rain, J-C. *et al.* (2001) The protein–protein interaction map of *Helicobacter pylori*. *Nature* 409, 211–215

5 Holt, L.J. *et al.* (2000) The use of recombinant antibodies in proteomics. *Curr. Opin. Biotechnol.* 11, 445–449

6 Celis, J.E. *et al.* (2000) Gene expression profiling: monitoring transcription and translation products using DNA microarrays and proteomics. *FEBS Lett.* 480, 2–16

Current progress on new therapies for Alzheimer's disease

Patrick C. May, Neuroscience Division, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN, USA. tel: +1 317 276 9093, fax: +1 317 433 7741, e-mail: may_patrick_c@lilly.com

The IBC's *9th Annual Conference on Alzheimer's Disease* held in Atlanta, GA, USA on 8–9 February was chaired by Sue Griffin (University of Arkansas VAMC, Little Rock, AR, USA) and featured speakers from academia, industry and government. The sessions succeeded in touching on the range of topics promised by the conference theme, namely *Gene discovery to therapeutic applications*.

The therapeutic applications discussed included most of those currently of interest for commercial development such as γ-secretase inhibitors, immunization approaches, cholesterol-modifying agents, anti-inflammatory agents and steroids. Given the publicity around the recent novel amyloid precursor protein β-secretase (BACE) discoveries, therapeutic approaches targeting β-secretase were clearly underrepresented with only one poster by Xiao Ping Shi (Merck Research Laboratories, West Point, PA, USA) devoted to the topic.

Most of the approximately 60 attendees were from pharmaceutical or biotechnology companies and they should have come away with a good understanding of the status of the early-phase clinical trials currently under way for Bristol-Myers Squibb's (BMS) γ-secretase inhibitor and Elan/AHP's β-blocker, as

1359-6446/01/$ – see front matter PII: S1359-6446(01)01786-X