Highlights from the Society for Medicines Research symposium held September 19, 2002, in London, United Kingdom.

Proteomics: New Developments in Target Discovery

by Judit M. Nagy and Katherine A. Brown

Summary

The Society for Medicines Research in collaboration with the Biological and Medicinal Chemistry sector of the Royal Society for Chemistry held a meeting on September 19, 2002, in London, United Kingdom to discuss proteomics in drug discovery. The meeting gave the most up-to-date overview of current progress in this new field, the challenges *in silico*, *in vitro* and *in vivo*, together with consideration of the increasing contribution of bioanalysis, bioinformatics and pharmacogenomics. Speakers from Celera Genomics, Oxford GlycoSciences and GlaxoSmithKline, among other companies and institutions, were present. © 2002 Prous Science. All rights reserved.

With the emergence of whole sequenced genomes and with the development of automated high-throughput technologies, it has become possible to analyze the entire expressed *prote*in product of a gen*ome* (the proteome) at a given time. Proteomics involves the application of a range of protein separation analytical and bioinformatic technologies to thousands of proteins simultaneously.

The science of proteomics applied to the process of drug discovery has undergone a revolution in recent years. The continuous introduction of new instrumentation and powerful bioinformatics techniques has given rise to the possibility of embarking on large-scale proteomics projects and has led to the formation of the Human Proteome Organisation to aid human drug development. Successful introduction of a new drug onto the market is an extremely costly and complicated process, mainly because of high failure rates in drug discovery. Target discovery, which was the focus of the September 19, 2002 meeting organized jointly by the Society for Medicines Research and the Biological and Medicinal Chemistry Sector (Industrial Affairs Division) of the Royal Society of Chemistry in London, is the first step of the drug discovery pipeline. The postgenomic era has substantially widened the selected number of possible targets that have opened up a new generation in the pharmaceutical industry. Proteomics has become an active part of drug discovery, particularly in the identification and validation of target discovery. The meeting gave the most up-to-date overview of current progress in this new field, the challenges *in silico*, *in vitro* and *in vivo*, together with the consideration of the increasing contribution of bioanalysis, bioinformatics and pharmacogenomics. The meeting attracted a large and enthusiastic audience to hear about the research efforts of leading international research teams whose immediate aim is to exploit proteomics in target discovery.

The meeting was opened by Dr. Scott D. Patterson (Celera Genomics, Rockville, Maryl-and, U.S.A.), who gave an excellent overview of present techniques used in proteomics, discussing their role in diagnostics and therapeutics. He established that the emphasis on true and reproducible quantitation of protein expression levels in a variety of samples will be an effective and highly efficient method of generating drug targets with a high degree of utility. To achieve this aim, Celera Genomics has set up an industrial-scale proteomics-based discovery platform consisting of cell biology,

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protein chemistry and mass spectrometry technical groups. The company has been able to establish processes for target discovery for small molecule drug targets as well as therapeutic antibody target differentiation for cell surface proteins. Dr. Patterson shared a successful example of this application, a potential marker for pancreatic cancer, with the audience. He emphasized the importance of candidate evaluation through genomic sequence matching and expression-level validation by cloning and expressing the potential target, checking its RNA expression level and following its presence by FACS (fluorescent-activated cell sorter) analysis.

The research strategy for target discovery and the reason that proteomics should be an integrated part of the research and development process was described by Dr. Christian Rohlff (Oxford GlycoSciences Ltd., Abingdon, U.K.). An atlas of the human genome is being built by Oxford GlycoSciences, who is using all the possible proteomics technologies including two-dimensional–based separation, isotope tagging (ICAT; isotope-coded affinity tag) and multi-dimensional HPLC (high-pressure liquid chromatography). The bioinformatics data output integrates both genomic and proteomic approaches for the molecular dissection of disease. Such platforms are likely to impact positively on the discovery of clinically relevant proteins. Several examples were mentioned that focused on the large protein isoforms found in biological samples. A case study in human lumbar cerebrospinal fluid identified a schizophrenia-associated protein with 29 isoforms. The question as to whether these isoforms are disease-related is under investigation. The company is also involved in development of new separation techniques. It further developed the ICAT technology, which is known as solid phase isotope tagging (Zhou, H. et al. Nat Biotechnol 2002, 20: 512–5). Finally, we heard about the company's progress in cancer membrane proteomics.

Dr. Patrick Camilleri (GlaxoSmithKline, Harlow, U.K.) discussed the role of proteomics in studies of drug safety. The group set up a database for biomarkers that are relevant to toxicology studies and called the database Toxicoproteomics. These markers have been identified by comparing protein profile differences before and after drug challenge. Although proteomics is lagging behind microarray technology, the U.S. FDA is encouraging the application of both microarray and proteomic methodologies to mechanism-based risk assessment. In conclusion, proteomics is expected to create new therapeutic opportunities by pushing the current frontiers of innovation in biomedical discovery and development.

The subject of protein arrays for the assessment of target discovery was presented by Prof. Ian Humphery-Smith (Glaucus Proteomics, Odijk, The Netherlands). The first message of the presentation was that instead of identifying more new targets, selectivity and specificity is more important and can be achieved by screening protein and antibody biochips. The immobilized proteins are recombinant proteins that are expressed after vigorous quality-controlled cloning, purified through dual affinity purification, prepared to a standard concentration and assessed for quality using mass spectrometry. The company, after many years of work, has set up an infrastructure for the automated production of these proteins, developed the specific surface chemistry for immobilization and set up high-performance computing solutions for process-wide data analysis.

The next presentation, by Dr. John E. Walker (MRC Dunn Human Nutrition Unit, Cambridge, U.K.), focused on the mitochondrial proteome and the identification of potential drug targets for diseases caused by dysfunction of this organelle. The 15 kilobase genome of mitochondria changes with time and can reduce energy production. These genomic changes are most likely due to damage caused by free radicals generated during oxidative phosphorylation in the mitochondria and are natural products of electron transfer but damaging to DNA. Out of the 2,000 mitochondrial proteins 100–150 are involved in energy conversion. Many of these large complexes have been identified, and detailed structural information is available. This information facilitates drug target discovery of diseases associated with mitochondrial malfunction, such as Parkinson's disease, Alzheimer's disease and aging.

In a complimentary presentation, Prof. Julio E. Celis (Institute of Cancer Biology and Danish Centre for Human Genome Research, Copenhagen, Denmark) focused on proteomic strategies in cancer. The lecture started with a review of the technology that the group is using in the challenging field of early identification of cancer markers. Tissue biopsies are used first of all to establish a normal control. The tumor section is further characterized using a range of methods including two-dimensional electrophoresis, microarray analysis, cell culture experiments and immunohistochemistry staining. The results are integrated into a database of signal pathways. Approximately 400 tumors per year are investigated in this manner. In addition, signature patterns are now being devised to analyze the effects of hormonal therapy and chemotherapy.

Dr. Walter Blackstock (Cellzome AG, Elstree, U.K.) opened his presentation with the mission statement of Cellzome: successful target selection is the result of linking potential drugs to entire protein pathways. As he pointed out, cells are densely packed with proteins, 300 g/L, so most proteins in a cell will not function in isolation. The technology used for the mapping of protein interactions was developed by the company. Proteins are isolated through tandem affinity purification, then identified by mass spectrometry. Results are validated using the data in combination with other experimental observations. The most extensively studied organism through the use of this method is yeast, *Saccharomyces cerevisiae*, and its established protein function pathways are integrated into the company's database (http://yeast cellzome.com). An interaction map involving around 6,000 proteins has now been established.

The next presentation, by Prof. David Eisenberg, (UCLA-DOE Center for Genomics and Proteomics, Los Angeles, California , U.S.A.), was the perfect follow-up to the previous one. The first slide showed a densely packed *Escherichia coli* (Fig.1), emphasizing the point heard in the previous talk, that is, that a cell is a network of protein interactions. He introduced the two approaches his group has taken to studying protein networks. The first one combines four computer-based methods (Rosetta Stone, Phylogenetic Profiles, Gene Neighbour and DNA Microarray Coexpression Analysis) to establish functional interactions between protein pairs. The second approach reconstructs physically interacting proteins based on published data in the scientific literature and integrates it into a database called Database of Interacting Proteins (http://www.dip.doe-mbi.ucla.edu). A new feature in this database is the addition of various covalent modification states of proteins, since these modifications will influence protein–protein interactions.

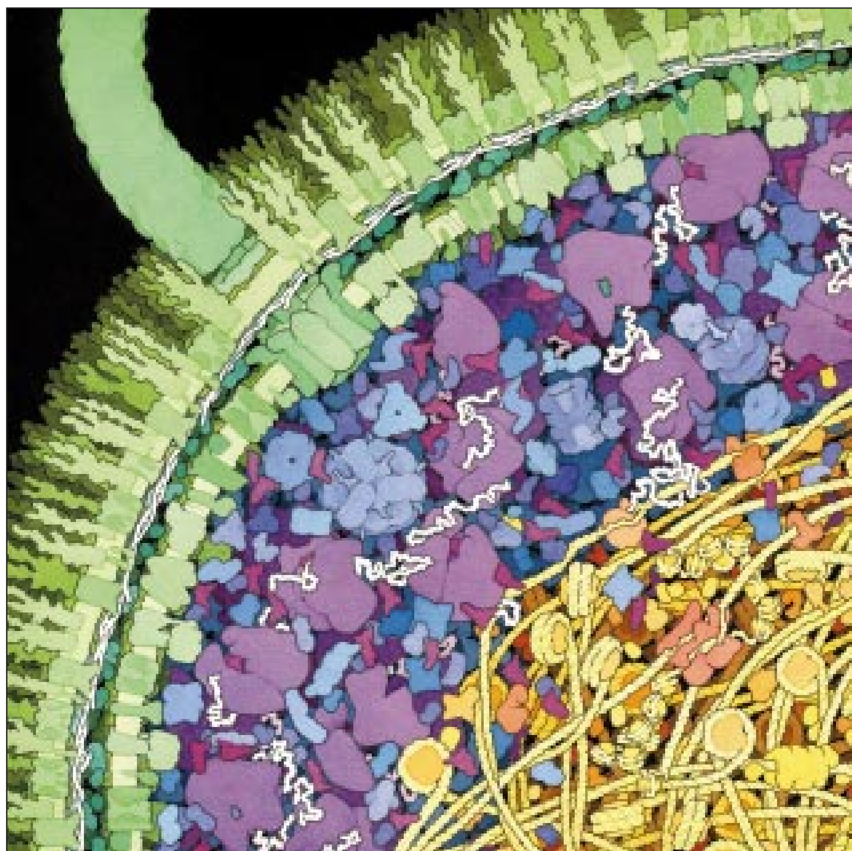

Fig. 1. *Escherichia coli*. This illustration shows a cross section of a small portion of an *Escherichia coli* cell. The cell wall, with two concentric membranes studded with transmembrane proteins, is shown in green. A large flagellar motor crosses the entire wall, turning the flagellum that extends upward from the surface. The cytoplasmic area is colored blue and purple. The large purple molecules are ribosomes, the small, L-shaped maroon molecules are tRNA and the white strands are mRNA. Enzymes are shown in blue. The nucleoid region is shown in yellow and orange, with the long DNA circle shown in yellow, wrapped around Hu protein (bacterial nucleosomes). In the center of the nucleoid region shown here, you might find a replication fork, with DNA polymerase (in red-orange) replicating new DNA (Reproduced with permission from David Goodsell, Scripps Research Institute, La Jolla, California, U.S.A.).

The final speaker of the meeting, Dr. Gareth Roberts (Proteom Ltd., Cambridge, U.K.), gave an evocative talk titled *"In Silico Discovery—The New Frontier*." Why do we need *in silico* approaches? Drug discovery is increasingly expensive and has been decreasing in productivity. We need to change to smart solutions utilizing the experimental information that genomics and proteomics provide. The company has applied advanced data mining and artificial intelligence solutions to identify rules and principles, limited by nature, that characterize protein–protein interactions. It has established an informatics platform called ProtoPrep, which is a protein interaction data warehouse for protein contact mapping and ligand learning to aid the identification of novel binding sites. All theoretical prediction is validated experimentally through company collaborations with academic and biotechnology laboratories. The great advantage of ProtoPrep is that it is quick, cheap and based on rational design, with guaranteed results each time. The talk was finished with successful examples of this approach.

The meeting was closed by Dr. Derek Buckle (Chair of the BCMS section of the Royal Society of Chemistry, U.K.). He expressed thanks to all the distinguished speakers of the day who traveled from far to share their latest results in the field. In summary, it is clear that proteomics is emerging to be an important tool in the area of target discovery. This meeting also demonstrated that proteomic-based research takes many guises and is thus poised to play a significant role in both fundamental and applied research efforts aimed at unraveling the underlying cellular functions that distinguish "normal" states from disease states.

Dr. Judit M. Nagy and Dr. Katherine A. Brown are members of The SMR. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are of a multidisciplinary nature, therapeutically focused and normally staged in or around London. Details about forthcoming meetings can be obtained from SMR, Secretariat, Triangle House, Broomhill Road, London SW18 4HX, U.K. Tel: +44 (0)20 8875-2431, Fax: +44 (0)20 8875-2424; http: //www.socmr.org; E-mail: secretariat @socmr.org