

## Neuroproteomics comes of age

Identifying changes in a patient's proteome—the set of proteins that are produced at a given time—should enable researchers to develop diagnostic tests and new therapies. However, progress in neuroproteomics has been slow. James Butcher investigates.

For the Human Brain Proteome Project see <http://www.hbpp.org/>

When the first draft of the human genome was completed in July 2000, many observers predicted that the next big challenge would be mapping the human proteome—a description of how the set of proteins produced during a lifetime changes with ageing and disease.

Unsurprisingly, given the complexity of the task, 7 years later the field of proteomics is still in its infancy. However, the publication in August, 2007, of the Minimum Information About A Proteomics Experiment (MIAPE)—analogous to the influential MIAME guidelines that describe how microarray experiments should be reported in scientific papers—heralds the beginning of the end for the harmonisation process.

In the not too distant future, researchers will at last be able to start to answer clinically important questions with these standardised proteomics techniques, which have the potential to aid the diagnosis of neurological disease and help to identify targets for therapeutic intervention.

The Human Proteome Project (HUPO), launched in February 2001,

is coordinating the effort to better understand the human proteome. One of its first decisions was to create three organ-specific initiatives: the Human Liver Proteome Project, the Plasma Proteome Project, and the Human Brain Proteome Project (HBPP). Since then, four additional projects have been initiated, each one based in one country but with collaborators from all over the world. The HBPP was initially chaired by Helmut Meyer and Joachim Klose and has a strong European slant.

Michael Hamacher, who works with Helmut Meyer at the Medizinisches Proteom-Center at the Ruhr-University Bochum, Germany, says that each of the organ-specific projects has a backbone of researchers from the same country as the chairpersons. For example, the HBPP has a strong German contingent, the plasma project is dominated by US researchers, and the liver project is heavily influenced by Chinese researchers. "It's a little bit hard to get American people inside our project", says Hamacher, "but we have some guys there who are now in close contact with HUPO. We hope we will get a more broad community soon."

Hamacher says that from the very beginning the problem the HBPP faced was how to start the project given the many known pitfalls of proteomic techniques: "You can't just do a global analysis and hope you find the proteins that are involved in Alzheimers", explains Hamacher. "If you do a proteome analysis with the methods that are available at the moment, you will have a snapshot only, because it is not sensitive enough and the samples are too complex."

The HBPP did two pilot studies to assess the validity of the techniques currently available; the preliminary results of these have now been

published and provide information on how data collection and analysis can be harmonised across independent sites. Similarly, HUPO's Proteomics Standards Initiative has been working hard to provide guidance on standardised collection, integration, storage, and dissemination of proteomics data. The MIAPE guidelines, which the Proteomics Standards Initiative recently published, set out the information that should be included in a proteomics paper.

"It is important to realise that the actual reproducibility of an experiment is not specifically addressed by the MIAPE guidelines", says Lennart Martens (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK), one of the authors of the MIAPE guidelines and a member of the HBPP. "Rather, MIAPE aims to provide the minimal information necessary to attempt a repeat experiment and to assess the way in which reproduction has been attempted and evaluated by the data authors. As such, the quality of the research should not be positively or negatively influenced by MIAPE, but adherence to these guidelines will provide reviewers and readers with the necessary information to come to a more informed judgement."

Danilo Tagle, Program Director for Neurogenetics at the National Institute of Neurological Disorders and Stroke, who was not involved in producing the guidelines, says that the MIAPE guidelines should achieve what the MIAME guidelines did for gene array expression data; that is, provide the research community with a minimum guideline for what constitutes the acquisition, analyses, and reporting of proteomic data. "It is the culmination of a lengthy

For MIAPE see *Nature Biotechnol* 2007; 25: 887–93.

For Human Proteome Organisation (HUPO) see <http://www.hupo.org/>

For a summary of the HBPP pilot studies see *Proteomics* 2006; 6: 4890–98



Newcastle University/Simon Fraser Science Photo Library

A 2D gel used in proteomics research

process getting community buy-in and feedback in coming up with these guidelines and I applaud HUPO for spearheading such an effort", he says.

This extensive and ongoing validation and harmonisation process is important because it is difficult to reproduce a proteomics experiment, both within and between laboratories. Many different experimental setups can be used to study proteins; by contrast, the human genome project exclusively used gene sequencers. In addition, although each human being has one genome, the proteome varies throughout life and within different tissues, which adds many orders of magnitude to the complexity. Finally, because proteins degrade quickly, the methods used to prepare the tissue samples can have a huge effect on the results of the experiment.

Because of the complexity of the brain, these problems are amplified in neuroproteomics. "The major problem is signal-to-noise", says Howard Gutstein (UT-MD Anderson Cancer Center, Texas, USA). "Specific functions are mediated by small areas in the brain, and these areas are very heterogeneous. Thus, if you were to see a 5-fold change in protein expression in a cell type that constituted 10% of the cells in a small region, the signal would be diluted to 0.5-fold."

Gutstein also says that it is difficult to obtain specific subtypes of cells from small regions of brain and analyse them with proteomics because of the limits of sensitivity, specificity, and reproducibility of the analytical techniques. "Another problem is that looking for biomarkers of neurological diseases in plasma is not likely to be very useful due to the presence of the blood-brain barrier", says Gutstein. "Changes in molecules involved in pathophysiology in the brain may not be reflected in the circulation."

The field of neuroproteomics faces special challenges given the complex cellular and subcellular architecture of the CNS. "Much of proteomics

work dealing with the human nervous system has dealt with post-mortem material or CSF, although the availability of well-validated animal models for a number of neurological conditions, ranging from brain injury and trauma to neurodegeneration, will certainly help the field to be at par with the liver or plasma proteome projects", says Tagle.

However, Martens believes the success or failure of neuroproteomics will depend not only on the researchers' ability to detect subtle changes in the protein composition of particular cells or tissues but also on the extent to which scientists can trace the modifications on these proteins through time and space, as well as the way the different protein forms interact. "This certainly represents a challenging goal, even with the advanced techniques available today", says Martens.

Martens predicts that the clinical effect of neuroproteomics will occur in three phases. The first phase will focus on improving and extending diagnostics. Many neurological diagnoses are currently made on the basis of secondary evidence (for example, cognitive tests and questionnaires, indirect reflex tests for neuronal signal transduction, or the presence of inflammatory markers in the CSF). "This makes diagnosis difficult and sometimes subjective", says Martens. "A better knowledge of the proteome of the brain in health and disease should first lead to the development of better diagnostic tests that can either complement or replace existing methods. Ideally, we should ultimately find highly sensitive 'early onset' biomarkers for the many neurodegenerative diseases, which would allow us to diagnose and intervene in these conditions before irreversible damage is done."

During the second phase, Martens predicts that the new diagnostic techniques will enable researchers



Instrumentation for mass spectrometry

to re-evaluate the classification of diseases of the nervous system. "It is quite possible that certain conditions that share similar symptoms are derived from different underlying molecular mechanisms", he says. "Conversely, afflictions currently considered separate and unrelated could, in fact, share such molecular mechanisms."

"The third phase will obviously consist of leveraging the improved understanding of the molecular basis of many afflictions to remedy or counteract them", predicts Martens, who believes that this step can only be successfully realised once the diseases are classified on the basis of their underlying molecular mechanisms. "Incomplete understanding of the underlying mechanisms might result in the testing of potential therapies on highly mixed populations, purely based on the similarity of their symptoms", he says.

For this vision to be realised, the HBPP needs long-term funding. The pilot studies were done with existing grants, and Hamacher is hopeful that the project will be able to attract funding from the European Union's framework programme and from national research bodies.

James Butcher  
james@two-cultures.com