

# REPORT

## Proteomics for everyday use: Activities of the HUPO Brain Proteome Project during the 5<sup>th</sup> HUPO World Congress

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Long Beach hosted this year's annual congress of the Human Proteome Organisation (HUPO). In addition to the numerous sessions, talks and poster presentations organized by HUPO itself, several events were arranged by the HUPO initiatives. The Brain Proteome Project (HUPO BPP) was very active, initiating three pre-congress workshops: (i) the kick-off meeting of the EU-funded ProDaC consortium (Proteomics Data Collection) that is aiming at the bioinformatics Standardization in the proteomics field; (ii) the workshop "Standardization Issues in Proteomics: Perspectives from Vendors" giving an overview about the lessons learned by proteomics industrial partners; (iii) the 6<sup>th</sup> HUPO BPP Workshop "New Proteomics Approaches for further HUPO BPP Studies" offering new concepts for brain-related proteomics studies.

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### Introduction

"Translating Proteomics from Bench to Bedside" – the 5<sup>th</sup> HUPO World Congress really lived up to its topic due to numerous workshops, talks and presentations dealing with clinical, biological and technical issues. From October 28 to

November 1, 2006 more than 1800 participants got together at the Convention Hall in Long Beach, CA, to discuss recent developments in proteomics and the related fields. Prior to the main congress, two days were organized dedicated to education and the work of the initiatives of HUPO. These initiatives are voluntarily driven by interested groups around the world and mostly focus on the study of a distinct human organ. The Brain Proteome Project (HUPO BPP) analyzes the brain and neurodegenerative diseases, working closely together with the Proteome Standards Initiative (HUPO PSI). The HUPO BPP initiated a pilot phase to assess state-of-the-art proteomics approaches and to elaborate a standardized as well as centralized data reprocessing pipeline. The results of the participating groups, the workflow of the central reprocessing and the continuing interpretation of the data sets were compiled in a PROTEOMICS special edition

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**Abbreviations:** BPP, Brain Proteome Project; HUPO, Human Proteome Organisation; ProDaC, Proteomics Data Collection; PSI, Proteome Standards Initiative

and presented at the 5<sup>th</sup> HUPO World Congress, offering new interesting facts about working in large proteomics consortia. The HUPO BPP aims at sharing this knowledge with the scientific community and therefore initiated a number of activities during the congress, namely the kick-off meeting of the EU-funded ProDaC consortium (Proteomics Data Collection), the workshop “Standardization Issues in Proteomics: Perspectives from Vendors” and the 6<sup>th</sup> HUPO BPP Workshop “New Proteomics Approaches for further HUPO BPP Studies”.

### ProDaC kick-off meeting

ProDaC is a Coordination Action project within the 6<sup>th</sup> EU Framework coordinating the development and implementation of international standards for the representation of high-performance proteomics data. Its focus is the standardized data collection, and furthermore the standardized data analysis of protein identification by mass spectrometry. The basis to develop an international data exchange network of proteomics data repositories will be established. This will include the implementation of prototypes of automated data submission pipelines in the leading European laboratories. In total, 12 core partners, all well-known European bioinformatics groups and proteomics laboratories, will participate over the whole 30 month runtime of this project. Enhancing these efforts, data providers, experienced European proteomics laboratories, will provide appropriate data derived from state-of-the-art proteomics technologies for proof-of-concept and utilize the newly developed software tools in the next stage. Additionally, international, non-EU laboratories, both academic and commercial, will utilize the tools as associated partners in the third phase of the planned activities, and thus benefit from the standardized data repositories developed, to

generally observe and guide the standards development. The whole process will be accompanied by a number of high-ranking scientific journals, which will be actively involved in the standards development, and will define mandatory supporting information for submitting articles.

The following work packages have been defined (for more information, please visit [www.fp6-prodac.eu](http://www.fp6-prodac.eu)):

- (i) Standards for Proteomics Data Representation
- (ii) Standards Implementation
- (iii) Data Integration Tools
- (iv) Proteomics Repository Adaptation
- (v) Data Flow Management
- (vi) Proteomics Data Exploitation
- (vii) Consortium Management

These work packages are led by Christian Stephan (MPC), Lennart Martens (EBI) and Frederic Levander/Jari Häkkinen (Lund) taking care that the respective duties are delivered in time by the participants. In order to introduce the groups and to begin the practical work, a kick-off meeting of the core partners took place on Friday, October 27 (Fig. 1). The work packages were presented, discussed and general rules decided. Future ProDaC meetings will be associated to the HUPO PSI Spring meeting and the HUPO World Congresses, thus the next meeting will take place in Lyon, France, April 26, 2007, and in Seoul, South Korea, October 5–10, 2007. The attendees agreed to promote the ideas of ProDaC, starting in Long Beach where the project aroused much interest among the congress attendees.

### Perspectives from Vendors

The results of the HUPO BPP pilot studies as published in the PROTEOMICS special edition have shown that a critical evaluation of techniques and strategies in proteomics is



**Figure 1.** The attendees of the ProDaC kick-off meeting on October 27, 2006.

necessary and essential for the development of reliable technology in the future. In order to sum up the strategies the industrial partners in the proteomics field are developing, due to the standardization efforts within HUPO, the workshop “Standardization Issues in Proteomics: Perspectives from Vendors” was held on Saturday, October 28, 2006. In this session, chaired by Friedrich Herberg (Kassel, Germany), Howard B. Gutstein (Houston, Texas) and Helmut E. Meyer (MPC) company representatives and bioinformaticians were asked to present and discuss their point of view in regard to the need for standardization: What is the take-away message the companies concluded from the recent standardization efforts (*e.g.* HUPO PSI and HUPO BPP)? To provide a framework for the discussion, Christian Stephan (MPC) summarized the lessons that the HUPO BPP consortium has learned from the HUPO BPP Pilot Studies. During this pilot phase it became clear that standardization, the organization of data management and the synergistic effects of a consortium of collaborators are of outstanding importance to any big proteome analysis. The reprocessing workflow and especially the use of the same standards turned out to be highly valuable to assure that results are comparable. Lennart Martens introduced in his talk “Staying in Orbit: Why a repository needs standards” the solutions of the HUPO PSI. A short description of the PSI standards for mass spectrometry (mzData) and protein interaction (analysisXML) was completed by the scheme of the ontologies, of the document approval process and of the Proteomics Identification Database (PRIDE). Interested colleagues can have a closer look at <http://psidev.info>.

The representatives of the different vendors agreed in principal with this point of view, stressing different aspects and ideas, but also asking critical questions. In his talk “AB/MDS SCIEX Perspectives” Sean L. Seymour (Applied Biosystems) brought up the problem of how results can be compared, *e.g.* when thinking of isoforms and false positive rates. He proposed a “states model” from sample to MS data to detect proteins, where the common formats of the states should be defined, but not the prescription of how to get between the points. Detlev Suckau (Bruker Daltonics) discussed “Quantitation vs. Profiling: Find the right approach for your proteomic application”. He showed the low reproducibility within some proteomics studies, pointing out that shotgun LC-MS/MS analysis of entire proteomes might be largely irreproducible and non-quantitative. Pre-fractionation is one of the key issues increasing sensitivity and demanding a comprehensive statistics and a bioinformatics platform as elaborated in the HUPO BPP. The topic of reproducibility especially in view to quantification was discussed by James Langridge (Waters) in his talk “Future perspectives in proteomics and standardization”. He focused on a multiple search engine strategy that allows adoption of standards, comparison across research domains and verification of results. The “Standardization in Interaction Analysis” was the task of the next speaker Jos Buijs (Biacore). Focussing on the potential to standardize interaction analyses he also stressed the necessity of minimum requirements for quantitative proteome analysis.

Some different aspects were introduced by GE Healthcare’s Günter Theßeling. In his talk “HUPO and the Technology Providing Industry – Opportunities for Partnership” he referred to the HUPO mission statement to define promote proteomics through international cooperation and collaborations. He proposed a HUPO – Industry partnership including sponsoring, training, education, standards and recommended operation procedures. This also means that HUPO does not endorse selected techniques, but that industry partners have to show minimum requirements of their products. The talks were accompanied by vivid discussions in the plenum about standards, their definition and usefulness. All participants agreed that there is a strong need to use standardized probes also for calibration issues, collect protocols for sample preparation and develop algorithms for the analysis of MS data, which allow the comparison of quantitative proteome studies from different labs. The session ended with the common consensus that all speakers announced to implement and to use the standards developed by the HUPO PSI.

## New Proteomics Approaches for further HUPO BPP Studies

In this session, HUPO BPP colleagues presented new approaches and animal models for neurodegenerative diseases giving a critical evaluation of techniques and strategies in proteomics. This session was chaired by Helmut E. Meyer and Young Mok Park (KBSI Daejeon) welcoming the attendees.

The first speaker, Herbert Thiele from Bruker Daltonics, gave a comprehensive overview of the heterogeneity of today’s proteomics. He concluded that recent proteomics workflows have to be flexible, similar to the HUPO BPP Reprocessing Strategy, containing a composite decoy database concept, multiple search engines and an automatic data validation. A concept for increasing protein identification rates as well as for information integration in public data repositories was presented. The following part was dedicated to new and improved techniques in recent proteomics. Howard B. Gutstein introduced “The Pinnacle Method of 2-D Gel Analysis” giving an overview about the gel analysis software “pinnacle” developed by his group. Pinnacle is able to automatically detect and quantify spots in large series of 2-D gels with high reliability and precision. Helen Kim (Birmingham, AL) also presented 2-D gel approaches in her talk “Optimal 2-D gel proteomic analysis of brain proteins affected by dietary polyphenols: a multi-faceted approach”. Her group is identifying the proteomes of tissues affected by beneficial agents such as dietary anti-oxidants/polyphenols revealing subproteomes of proteins important for disease prevention. The analysis of 2-D gels and oxyblots clearly showed the positive neuroprotective effect of polyphenols using a combined experimental set-up of state-of-the-art and conventional approaches. New insights into the study of human cerebrospinal fluids (CSF) were given by Jing Zhang (Seattle, WA). He stressed the importance of quality control

starting with the CSF collection and the controversial discussed question of depletion of abundant proteins. Zhang presented data derived from his iTRAQ workflow and the characterization of glycoproteins promising interesting insights in neurodegenerative diseases. Christian Stephan closed the series of talks with a summary of the above mentioned EU project “ProDaC: Proteomics Data Collection” pointing out that the collaboration and cooperation with all interested groups is very welcome.

The final discussion was moderated by Helmut E. Meyer presenting the activities of HUPO BPP in 2005/2006. The series of numerous meetings and workshops (Table 1) will be continued in 2007, including the 7<sup>th</sup> HUPO BPP workshop at the EBI, Hinxton, UK from March 7 – 9, as well as the 7<sup>th</sup> HUPO BPP workshop during the 6<sup>th</sup> HUPO World Congress in Seoul, Korea, presumably on October 5. Helmut E. Meyer subsumed the overall feeling of the last meetings as follows: The two main problems in proteomics today are the huge complexity of the samples to be analyzed on the one hand and the limited resolution of the techniques available on the other hand. Most of the proteins that seem to be interesting in many diseases show low abundance differing between 8–10 magnitudes, while (differential) approaches, such as DIGE, are able to cover a dynamic range of two orders of magnitude. This difference in the orders of magnitude is the reason why every recent single strategy used in the laboratories worldwide “only” takes a snapshot of the whole that in addition might be contorted. This does not mean that the data obtained is wrong or not useful – contrarily the data is very valuable when different groups study the sample of interest with their very own approaches and combine the output using the same tight quality criteria. Subfractionation could be a second (complementary) way to reduce complexity and to increase reproducibility.

These considerations are the basis of a master phase draft that should lead to “HUPO BPP affiliated projects”. In order to reach this goal, the HUPO BPP will:

- (i) coordinate subprojects
- (ii) help elaborating study design/strategies and help during work flow (need for a scientific study committee)
- (iii) continue the work of the Data Collection Center, including MS-data reprocessing for approved studies, open access of data (PRIDE) and tool development as well as dissemination (work will be accompanied by the EU-funded project ProDaC)
- (iv) offer workshops and training courses, advance proteomics technologies, write reviews, support networking (including HUPO, EuPA, brain tissue banks) and identification of suitable mouse models, affected families and, human samples
- (v) improve cooperation with proteomics journals

HUPO BPP subprojects concentrate on:

- human body fluids with a focus on Parkinson’s Disease, Alzheimer’s Disease, (Huntington’s Disease) and accompanied by the EU funded project cNEURO
- mouse models
- brain cancer and stroke
- systems biology of the brain

All activities will be realized under the aspects of standardization and in close collaboration with the HUPO PSI.

The 5<sup>th</sup> HUPO World Congress clearly showed that the HUPO initiatives are very active in defining standards, in networking, in performing studies as well as in promoting the newest insights gained on proteomics. As can be seen from the outlook and plans of all initiatives these efforts will be broadened further – promising a wide acceptance of the standards and strategies elaborated by HUPO.

**Table 1.** Workshops and Meetings 2005–2006

| Timeline             | Meeting                                                                                                                      |
|----------------------|------------------------------------------------------------------------------------------------------------------------------|
| End of 2004          | Implementation of Data Collection Center at the MPC, Bochum, Germany                                                         |
| January 28, 2005     | 3 <sup>rd</sup> HUPO BPP Bioinformatics Meeting at Protagen, Dortmund, Germany (15 attendees)                                |
| April 8, 2005        | 4 <sup>th</sup> HUPO BPP Bioinformatics Meeting at EBI, Hinxton, UK (15 attendees)                                           |
| June 1, 2005         | “HUPO BPP International Workshop on Mouse Models for Neurodegeneration” in Doorwerth, The Netherlands (30 attendees)         |
| July 7, 2005         | 5 <sup>th</sup> HUPO BPP Bioinformatics Meeting at EBI, Hinxton, UK (15 attendees)                                           |
| August 27, 2005      | 4 <sup>th</sup> HUPO BPP Workshop in Munich, Germany during the 4 <sup>th</sup> HUPO World Congress (70 attendees)           |
| September 2, 2005    | Chinese-German Workshop “Proteomics & Systems Biology”, joint HUPO LPP and BPP workshop, MPC, Bochum, Germany (40 attendees) |
| January 9 – 11, 2006 | Jamboree of the HUPO BPP Bioinformatics Committee at EBI, Hinxton, U.K. (18 attendees)                                       |
| February 15–16, 2006 | 5 <sup>th</sup> HUPO BPP Workshop “Bridging Proteomics and Medical Science” at UCD, Dublin (75 attendees)                    |
| March 29, 2006       | Korean-German Workshop “Profound Proteomics Strategies” in Daejeon, Korea (20 attendees)                                     |
| June 29, 2006        | ProteinScape training course at Bruker Daltonik GmbH, Bremen, Germany                                                        |
| September 01, 2006   | Steering Committee Meeting during the 7 <sup>th</sup> Siena Proteomics Meeting, planning of the Long Beach workshops etc.    |
| September 2006       | Proteomics Special Issue - “The HUPO BPP Concerted Proteome Analysis of the Brain”, <i>Proteomics</i> 2006, 6 (18)           |