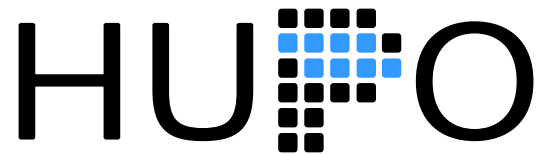


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Human Proteome Organisation



Human Liver Proteome Project (HLPP)

Welcome Back!

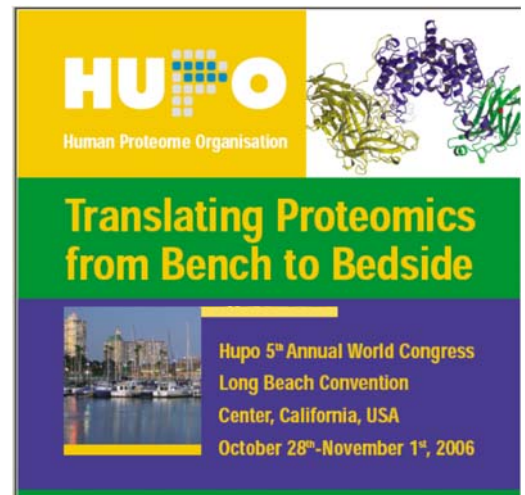
This is the second issue of the HLPP newsletter. Please accept our apologies for the delay with this issue. A heavy travel schedule and the winter holiday season combined to throw us off schedule.

We welcome your ideas, comments and suggestions. Please send all input to the HLPP Newsletter Contact shown at the bottom of the last page.

Fall 2006 HLPP Workshop Report

In October, immediately prior to the HUPO 5th Annual World Congress in Long Beach, a brief workshop was held to present achievements to date. The scientific objectives of HLPP are to characterize the proteome of the healthy human liver (expression profiling, cellular localization, post-translational modifications, protein-protein interactions) and to develop tools for its study (sample banking, ORFeome, antibodies). The information and tools that are generated by this international effort will be available to the scientific community with the hope that they will help understand, diagnose and treat liver diseases more effectively.

Summaries of the presentations appear on the following pages.



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- **Wantao Ying and Xiaohong Qian (Beijing Proteome Research Center): Construction of the Human Normal Liver Proteome Expression Profile**

The work finalized so far includes 1- the standardization of tissue acquisition, sample processing, protein extraction, protein/peptide separation and identification and data submission and management; 2- the semi-integration of data from multiple platforms, the semi-quantitative analysis of shotgun data, the comparison between SDS-PAGE, 2-DE, shotgun and LC-shotgun, preliminary annotation of key physiological and pathological pathways in the liver and GO annotation.

The next phase will focus on the following:

- 1 - Profiling of subcellular organelles including quantitative analysis using high accuracy mass spectrometry
- 2 - Isolation of hepatocytes and other liver cell types and their profiling analysis
- 3 - Characterization of post-translational modifications of liver proteins
- 4 - Standardization for data quality control at both the peptide and the protein levels
- 5 - Intra- and inter-organelle quantification of proteins

To date, the expression profiling of the human fetal liver has been finalized with 2,495 unique proteins identified and reported (see HLPP publications section). The data are also publicly available in the PRIDE database at the European Bioinformatics Institute (www.ebi.ac.uk/pride/startBrowse.do) by selecting the heading: "HUPO HLPP: Beijing Proteome Research Center" (accession #1779-1853).

The expression profiling of the first adult liver reference sample (referred to as the French liver sample) is also finalized with 4,998 unique proteins identified. The expression profiling of the second adult liver reference sample (referred to as the Chinese liver sample) is currently under analysis with 6,788 proteins identified to date.

- **Laura Beretta (Fred Hutchinson Cancer Research Center): Human Liver Expression Profiling – Analysis of the adult liver reference sample**

The work was described in four sections: methods used for protein extraction, separation and identification; comparison between the transcriptomic and proteomic profiles of the reference sample; comparisons with the human fetal liver and the HPPP human plasma proteomes; and applied strategies to the discovery of biomarkers for liver diseases.

Intact proteins extracted from the reference sample were extensively separated using a three-dimensional approach (a combination of 2D HPLC and SDS-PAGE). Following in-gel digestion, the resulting peptides were analyzed by LC coupled to tandem mass spectrometry. The generated data were submitted to CPAS (Computational Proteomics Analysis System) developed at the Fred Hutchinson Cancer Research Center, using the X!Tandem search engine and ProteinProphet. Using a cut-off of 0.9 ProteinProphet score, 8149 individual proteins and 472 protein groups were identified by at least 3 peptides. A relative protein abundance score was calculated for each identified protein. Annotation of the identified proteins with properties such as MW, Gene Ontology classification and post-translational

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modifications is underway.

In parallel, the transcriptomic profile of the same sample was established using the Affymetrix chip containing 54,675 probe sets. This analysis identified 10,982 genes expressed in the reference liver sample. Comparative analysis of the transcriptomic and the proteomic data sets was presented. The results indicated that protein products for over 50% of the genes expressed were identified using this proteomic approach, including protein products of low-abundance genes.

Comparison with the human fetal liver proteome recently published (see HLPP publications section) identified 945 (out of 1807) proteins commonly expressed in both fetal and adult livers. Comparison with the HPPP human plasma proteome identified 792 (out of 3017) proteins detected in both plasma and liver tissue.

Discovery of biomarkers for liver diseases: Three main strategies are being used in Dr. Beretta's group for discovery: tissue-based (from human samples), serum-based (from human samples), and animal model-based (including both tissue and serum). For tissue-based analysis, a similar strategy as described above is used. For serum-based analysis, immunodepletion is used first followed by the same proteomic approach. Candidate markers are compared with existing markers (e.g., AFP) to determine whether sensitivity and specificity can be improved; combinations of candidate markers may give better results than individual markers used alone; a number of examples were presented.

Future directions: To refine the criteria for selection of candidate markers, to prioritize proteins for production of antibodies, to further compare liver and plasma proteomes, to evaluate and validate methods for label-free protein quantification.

- **Pengyuan Yang (Institute of Biomedical Sciences, Fudan University): ORFeome and Protein-protein Interactions**

Dr. Yang reported that approximately 5,500 open reading frames (ORFs) of genes expressed in the human liver have been cloned. Of these 5,500 ORFs, approximately 4,000 have been constructed into vectors for use in yeast two-hybrid assays. The majority of these ORF clones are in the range of 500-1500 base pairs in length. A web interface has been created in order to access the current ORF bank data set (<http://202.127.18.238/hlpp/>)

Dr. Yang also presented the undergoing effort to establish a liver protein-protein interaction (PPI) map. To date, 1249 baits have been used for PPI studies. Approximately 30 baits per day can be processed in yeast two-hybrid assays. Antibody chips containing over 1000 probes are also used for this subproject. The resulting data are further validated by co-immunoprecipitation or by affinity purification. The largest network identified to date includes 1035 PPIs between 810 proteins.

- **John Bergeron and Alexander Bell (McGill University): Quantitative Proteomics of Detoxification in the Hepatic ER**

The endoplasmic reticulum (ER) organelles in the liver play an essential role not only in protein synthesis and folding but also in liver detoxification. The proteomes of highly enriched organellar endoplasmic reticulum (ER) rough and

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smooth membranes and Golgi fractions from rat liver were characterized. Organellar proteins were resolved by 1D-SDS PAGE, the gels were sliced and the proteins were in-gel digested with trypsin. Peptides were extracted and subjected to LC-QToF MS analysis. Identified proteins were annotated into 23 functional categories and the detoxification category was further curated into 13 sub-functions.

The Cytochrome P450, Glucuronosyltransferase and Carboxylesterase families of proteins, with 34, 11 and 9 isoforms identified, respectively, represented about 67% of the total ER protein as determined by redundant peptide counting although these highly sequence related families represented only 54 of the 122 detoxification proteins. Peptide heatmaps, revealing frequency, identification and location of peptides (assigned at >95% confidence) in these families were emphasized. KDEL- and KKxx/KxKxx-related trafficking motifs were identified in the Carboxylesterase and Glucuronosyltransferase proteins whereas several Cytochrome P450s revealed an exposed DxD motif (as deduced from their x-ray crystallography) that may be involved in trafficking. Peptide sequencing information was presented that distinguishes Cytochrome P450 allelic isoforms and database DNA sequencing errors were identified. Proteomics readily distinguished multiple members of the Cytochrome P450, Glucuronosyltransferase and Carboxylesterase families of proteins, distinguished allelic isoforms differing by a single amino acid and corrected DNA-related sequencing errors in the protein database.

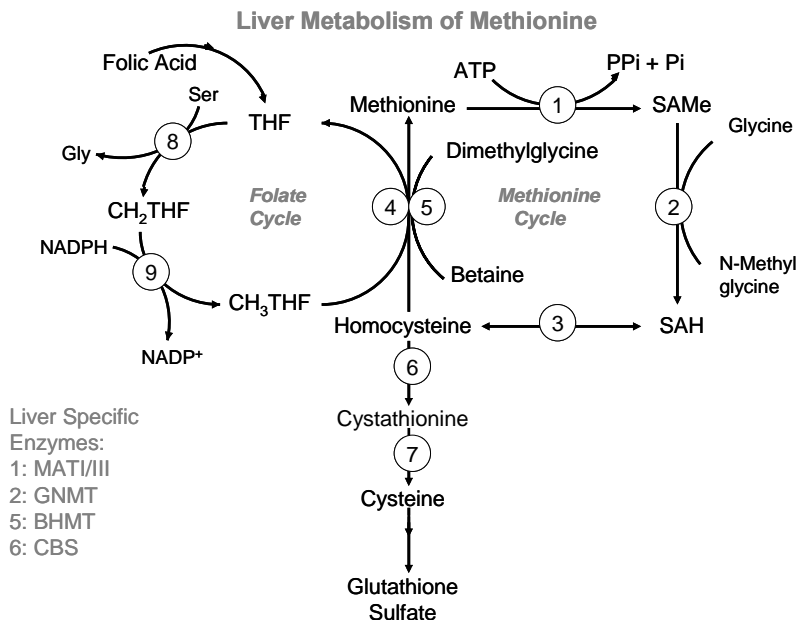
• José Mato (CIC bioGUNE): Liver Proteomics of Fatty Liver Disease

Dr. Mato introduced mouse models of non-alcoholic fatty liver disease (NAFLD) leading to hepatocellular carcinoma. These models target the metabolism of methionine and include MAT1A knockout mice. The MAT1A KO mice spontaneously develop steatohepatitis and later hepatocellular carcinoma.

Another key player in the metabolism of methionine is GNMT. Interestingly, a GNMT polymorphism has been reported to be associated with HCC in humans. GNMT KO mice

have been generated. These mice spontaneously develop steatohepatitis and fibrosis. Liver disease progression in older mice is under investigation.

The integrative gene expression profiling of liver biopsies of NASH patients with liver samples of MAT1A KO mice identified genes and gene pathways that are associated with NASH. Based on this information, Dr. Mato's group generated a



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novel mouse model and, remarkably, the mice spontaneously develop steatohepatitis. This mouse model will be reported in the literature in the near future.

Proteomic analysis of liver tissues from patients with steatosis or with NASH was performed using 2D gel electrophoresis (DIGE) to identify proteins differentially-expressed in NASH and in steatosis. Proteomics and metabolomics studies of sera from controls, patients with steatosis and patients with NASH were also performed. The generated data were presented.

- **Qihong Sun (Beijing Proteome Research Center): Antibody Bank**

Dr. Sun and colleagues have reported a strategy to establish the antibody bank of murine mAbs for human liver proteins using fractionated native liver proteins as immunogens. Synthesized peptides from liver-specific proteins and purified recombinant protein antigens have been used as well. To date, 1907 hybridoma cell lines have been established. Of these cell lines, 1400 have been characterized and produced monoclonal antibodies for approximately 150 different proteins. Rabbit polyclonal antibodies have been produced for 56 different proteins. Approximately 44% of the hybridoma cell lines have been assigned to immune response functions and approximately 41% of the monoclonal antibodies have been assigned to metabolic functions. The produced antibodies are currently used for depletion of highly abundant proteins, for immunohistochemistry and for isolation of protein complexes.

Additional Notes from Long Beach

At the HUPO 5th Annual World Congress in Long Beach, other talks were related to the liver proteome:

- Organellar Proteomics as a Paradigm for the Systems Biology Study of Disease. JJ Bergeron, McGill University, Montreal, Canada
- Integrative Analysis of the Liver Proteome as a Strategy for Liver Disease Biomarker Discovery. L. Beretta, Fred Hutchinson Cancer Research Center, Seattle, WA
- Distinguishing Progressive Fatty Liver Disease from the Non-Progressive Form Using Reverse Phase Phosphoproteomic Array Analysis of Intracellular Signaling Pathways. VS Calvert, et al., George Mason University, Manassas, VA
- Proteomics of Metabolic Staging of Hepatocellular Carcinoma. Wang R, et al., Mount Sinai School of Medicine, New York, NY
- Proteomic Analysis for Liver Mitochondria of db/db Mouse. Li X, et al., Beijing Genomics Institute, Beijing, China

HLPP-related Publications

Highlight: Publication by John Bergeron and colleagues in the journal *Cell*:



Resource

Quantitative Proteomics Analysis of the Secretory Pathway

Annalyn Gilchrist,^{1,6} Catherine E. Au,^{1,6} Johan Hiding,^{4,6} Alexander W. Bell,¹ Julia Fernandez-Rodriguez,⁴ Souad Lesimple,¹ Hisao Nagaya,¹ Line Roy,¹ Sara J.C. Gosline,² Michael Hallett,² Jacques Paiement,⁵ Robert E. Kearney,³ Tommy Nilsson,^{4,7} and John J.M. Bergeron^{1,7,*}

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Abstract:

We report more than 1400 proteins of the secretory-pathway proteome and provide spatial information on the relative presence of each protein in the rough and smooth ER Golgi cisternae and Golgi-derived COPI vesicles. The data support a role for COPI vesicles in recycling and cisternal maturation, showing that Golgi-resident proteins are present at a higher concentration than secretory cargo. Of the 1400 proteins, 345 were identified as previously uncharacterized. Of these, 230 had their subcellular location deduced by proteomics. This study provides a comprehensive catalog of the ER and Golgi proteomes with insight into their identity and function.

This article was featured in the Research Highlights section of the February 2007 issue of the journal *Nature Reviews. Molecular Cell Biology*: The building blocks of the secretory pathway. Pickett J. *Nat Rev Mol Cell Biol.* 2007; 8:94.

HLPP-related Publications continued

- HUPO HLPP takes on a herculean task. Cottingham K. *J Proteome Res.* 2006; 5:2077.
- A dataset of human fetal liver proteome identified by subcellular fractionation and multiple protein separation and identification technology. Ying W, Jiang Y, Guo L, Hao Y, Zhang Y, Wu S, Zhong F, Wang J, Shi R, Li D, Wan P, Li X, Wei H, Li J, Wang Z, Xue X, Cai Y, Zhu Y, Qian X, He F. *Mol Cell Proteomics.* 2006; 5:1703-7.
- Toxicoproteomics in liver injury and inflammation. Merrick BA. *Ann N Y Acad Sci.* 2006; 1076:707-17.

HLPP-related Publications continued

- Proteomic profiles of induced hepatotoxicity at the subcellular level. Zgoda V, Tikhonova O, Viglinskaya A, Serebriakova M, Lisitsa A, Archakov A. *Proteomics*. 2006; 6:4662-70.
- Proteomic analysis of autoantibodies in patients with hepatocellular carcinoma. Takashima M, Kuramitsu Y, Yokoyama Y, Iizuka N, Harada T, Fujimoto M, Sakaida I, Okita K, Oka M, Nakamura K. *Proteomics*. 2006; 6:3894-900.
- Molecular profiling of hepatocellular carcinoma in mice with a chronic deficiency of hepatic s-adenosylmethionine: relevance in human liver diseases. Santamaría E, Muñoz J, Fernández-Irigoyen J, Sesma L, Mora MI, Berasain C, Lu SC, Mato JM, Prieto J, Avila MA, Corrales FJ. *J Proteome Res*. 2006; 5:944-53.
- Modification of host lipid raft proteome upon hepatitis C virus replication. Mannová P, Fang R, Wang H, Deng B, McIntosh MW, Hanash SM, Beretta L. *Mol Cell Proteomics*. 2006; 5:2319-25.
- Cleavage of endoplasmic reticulum proteins in hepatocellular carcinoma: Detection of generated fragments in patient sera. Chignard N, Shang S, Wang H, Marrero J, Bréchet C, Hanash S, Beretta L. *Gastroenterology*. 2006;130(7):2010-22.

Liver History Note

"Haruspicy" is a method of divination or fortune-telling that was practiced by the ancient Etruscans, a people who established an advanced civilization in what is today the country of Italy before the rise of the Roman Empire.

The principles and rules of haruspicy were contained in the "Libri Tagetici," a collection of Etruscan books. The priest or "haruspex" carefully interpreted the will of the Gods by inspecting the internal organs of a sacrificial animal, often a sheep.

The priest examined the liver's shape, color, size, and markings to determine the messages sent from the Gods. Each section of the liver imparted specific information to the priest and any liver abnormalities served as an important basis for prediction of the future.

Pictured above is a bronze model of a sheep's liver, dated to approximately the third century B.C.E. This artifact is currently in the collections of the Musei Civici di Palazzo Farnese in Piacenza, Italy.

This bronze artifact is divided into sections which correspond to various stars and planets which in turn correspond to specific sections of the liver of the sacrificial animal. Etruscan inscriptions were carved into the surface to aid in the interpretation of the messages in the liver.



Calendar

01/05/07 – 01/11/07

3rd International Barbados Proteomics Conference: HUPO Initiatives
Bellairs Institute, Bridgetown, Barbados

03/05/07 – 03/08/07

US HUPO 3rd Annual Conference (www.ushupo.org/Default.aspx?tabid=31)
Seattle, WA



Organizational Structure of HLPP

HLPP Executive Committee (terms expire 12/31/07)

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Co-chairs

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