

Scientific Program

Meeting of the COST Action BM0702

EuroKUP (Kidney and Urine Proteomics)



Clinical Research Center for Rare Diseases

Aldo e Cele Daccò

Mario Negri Institute

Bergamo, Italy

Local Host: Ariela Benigni Ph.D

Organizers of the scientific program:

Ariela Benigni Ph.D

Aris Charonis, Ph.D

October 1-2, 2008

Wednesday October 1st

9.00-9.15 Welcome Session

Welcome address by the Action Chair, Antonia Vlahou
Welcome address by the Meeting Organizers,
Ariela Benigni and Aris Charonis

9.15-13.00 Session 1- Urinary proteomics: Chairs: H. Mischak and H. Dihazi

9.15-9.30 H. Mischak

Urinary proteomics: current status and obstacles towards clinical implementation

9.30-9.45 C. Masselon, M. Court, Y. DeRycke, A. Savignoni, V. Brun, C. Bruley, Y. Allory, B. Asselain, J. Garin

Validation of the effect of pre-analytical treatment of urine before quantitative proteomic analysis

9.45-10.00 L. Molina, N. Salvetat, S. Peres, F. Molina, C. Granier

Urinary proteins from healthy individuals: quantitative variations observed by 2D-GE and implementation of the DUP database

10.00-10.15 H. Dihazi, GA Muller, S Lindner, M Meyer, AR Asif, M Oellerich, F Strutz

Identification of a processed ubiquitin form as differentially secreted protein in diabetic nephropathy patients by urinary proteomics

10.15-10.30 JP Schanstra, S. Decramer, JL Bascands, C. Lacroix, A. Gonzalez de Perodo, C. Froment, B. Monsarrat, H. Mischak

Urinary proteomics to study obstructive nephropathy in newborns

10.30-11.00 Break

11.00-11.15 I. Zoidakis, M. Makridakis, P. Dimitraki, A. Vlahou

Resolving urinary and secreted proteins by gel based approaches

11.15-11.30 M. Sanchez-Carbayo

Linking genomics, epigenomics and proteomics for disease diagnostics using urinary specimens

11.30-11.45 M. Vickers

Proteomic analysis of rat urine following sub total nephrectomy

11.45-12.00 Lewandowicz, M. Bakun, T. Rubel, J. Imiela, M. Dadlez
LC-LTQ FTICR MS based relative and absolute peptide quantification
for detection of markers for bladder cancer and other conditions in urine
proteome and peptidome

12.00-12.15 Ferreira L, Quiros Y, Gonzalez de Buitrago JM, Lopez-
Novoa JM, Lopez-Hernandez FJ

Towards a urinary fingerprint of gentamicin-induced acute renal failure

12.15-12.30 Drube J, Schiffer E, Mischak H, Kemper MJ, Neuhaus T,
Pape L, Lichtinghagen R, Ehrich JHH

Urinary proteome pattern in children with DeToni-Debre-Fanconi
syndrome.

12.30-13.00 Special Lecture on Imaging/Profiling mass spectrometry

M Marchetti-Deschmann, E. Pittenauer, G. Allmaier

MALDI mass spectrometry in biomedical sciences: from true high
energy CID to imaging/profiling mass spectrometry

13.00-15.00 Lunch Break

**15.00-16.45 Session 2 - Kidney Proteomics: Chairs: J. Schanstra
and R. Banks**

15.00-15.15 C. Mayrhofer, G. Allmaier, D. Kerjaschki

Proteomic analysis of podocyte injury

15.15-15.30 KP Kypreou, N. Prakoura, P Kavvadas, P Karamessinis,
M Peroulis, A. Alberti, P. Sideras, S. Psarras, Y. Capetanaki, PK. Politis,
AS. Charonis

Altered expression of calreticulin during the development of fibrosis

15.30-15.45 S. Skeledzic, M. Veitinger, KA. Ahrer, M. Zellner, D.
Cejka, C Reichl, V. Jordan, F. Muhlbacher, G. Boehmig, R. Oehler

Immunoproteomic analysis of allo-reactive non-HLA specific antibodies
in haemodialysis patients before renal transplantation

15.45-16.00 R. Banks

Renal Proteomic Research in Leeds

16.00-16.15 C. Deltas, K. Voskarides, A. Pierides
In search for genetic modifiers and early urine biomarkers that predispose Thin Basement Membrane Nephropathy patients to Focal Segmental Glomerulosclerosis and renal failure

16.15-16.30 DS Goumenos
Factors implicated in the development of tubulointerstitial injury and progression of human glomerular disease

16.30-16.45 D. Vlahakos, N. Arkadopoulos, S. Siasakou, G. Kostopanagiotou, I. Kaliakmanis, D. Degiannis, M. Demonakou, V. Smyrniotis
Dexferoxamine (DFO) attenuates lipid peroxidation, blocks IL-6 production and confers renoprotection after acute hepatic ischemia in pigs

16.45-17.15 Break

17.15-19.00 Session 3 – Bioinformatics: Chairs T. Atwood and Eric Bongam-Rudloff

17.15-17.30 SC Carpentier, R Swennen, B. Panis
Statistical analysis for proteome analysis: a necessary evil or a powerful tool?

17.30-17.45 E. Lagercrantz, J Oelrich, AM Barrio, U. Landegren, E. Bongam-Rudloff
MolMeth: The Molecular Methods Database

17.45-18.00 SR Pettifer, D. Thorne, P. McDermott, TK Attwood
Putting the fun back into bioinformatics: making biological databases and bioinformatics tools talk to each other!

18.00-18.15 Melanie Hilario, A. Kalousis, C. Roos, JP Schanstra
Presentation of EuroKUP/e-LICO Joint Activities

18.15- 19.30 General discussion on scientific aspects

20.00-22.00 Dinner

Thursday October 2nd

9.00-10.0 **Session 4 – Special Lectures: Chairs: M. El-Nahas and A. Charonis**

9.00-9.30 **Special Lecture on Proteomic Biomarker Discovery**
B. Domon

Paradigm shift in proteomic biomarker discovery and evaluation

9.30-10.00 **Special Lecture on Nephropathy**

A. Benigni

Regression of chronic renal disease and the case of kidney self repairing

10.00-10.15 **Special lecture on new proteomic devices**

Marc Baumann

Developing tools for future proteomics

10.15-10.30 Break

10.30- 13.45 **Session 5 – COST Action Business meeting: Chairs A. Vlahou and A. Benigni**

10.30-10.45 How to access our website (by E. Bongam-Rudloff)

10.45-11.30 Presentations from the Chairs of Work Packages

11.30-13.30 Management Committee meeting

13.30-13.45 Closing Remarks by A. Vlahou

Contributed Abstracts

Total of 25 abstracts

Abstracts appear in the order they will be
presented

Urinary proteomics: current status and obstacles towards clinical implementation

Harald Mischak

Chronic kidney disease (CKD) is accompanied by early changes in intra- and extracellular proteins, which can serve as specific indicators (biomarkers) of disease long before evident clinical symptoms. However, these biomarkers are largely unknown today. Owing to its availability, ease of collection and correlation with renal (patho)physiology, urine is an attractive source for such biomarkers. Several different technological approaches were recently initiated, aiming at deciphering the urinary proteome and identifying specific biomarkers for CKD. Unfortunately, these efforts, while enabling identification of several biomarkers, did not yet result in any change in clinical practice.

We have employed capillary electrophoresis coupled mass spectrometry (CE-MS) as a high-resolution method for analysis of urinary peptides and proteins from 800 to 17,000 Da. To date, over 5000 individual samples were analyzed, all data were deposited in a database, currently containing 5,010 relevant unique urinary peptides. This abundance of data enabled the identification of biomarkers for CKD, and for the differential diagnosis of specific diseases, e.g.: focal segmental glomerulosclerosis, IgA nephropathy, vasculitis, and diabetic nephropathy. The biomarkers were validated in blinded studies with high sensitivities and specificities. Furthermore, specific biomarkers enabling prognosis or assessment of response to therapy were identified and validated. These results reveal that urine largely reflects (patho)physiological changes in the extracellular matrix and indicate that a single highly specific biomarker for any disease does not exist, high accuracy can be accomplished by combining several biomarkers to a specific panel.

A current problem in many of the urinary proteomics approaches appears to be the low number of datasets that are frequently reported and a lack of standards and comparability of reports, all in consequence result in lack of credibility especially in the clinical community. To cope with these issues, we propose the implementation of a standard protocol for sample collection, strict requirement to report the analytical platform performance, implementation of minimal requirements for a clinical proteomics study (e.g. ethical approval, clear clinical question, appropriate statistics). Further, a standard human urine sample which will be utilized as a reference, should greatly ease inter-platform comparison. Those measures should enable the comparison of data between laboratories, consequently will in many cases allow validation of results in larger and independent datasets.

Validation of the effect of pre-analytical treatment of urine before quantitative proteomic analysis

Christophe Masselon¹, Magali Court¹, Yann DeRycke², Alexia Savignoni², Virginie Brun¹, Christophe Bruley¹, Yves Allory³, Bernard Asselain², Jérôme Garin¹

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3. INSERM unité 841, IMRB, Equipe "Carcinogénèse et pathologie moléculaire des tumeurs urologiques" - Hôpital Henri Mondor - Créteil, France

Over the past few years, biomarkers discovery of renal or urinary tract diseases in human urine by proteomics methods has garnered tremendous interest. In spite of several limited scope studies of urine collection and storage procedures for proteomics, few guidelines have emerged from the scientific community, and they are subject to debate.

In a clinical setting, urine collection may involve spiking the sample with a protease inhibitor cocktail and cold storage (0-4°C) for an extended period of time (up to 6 hours) before a low speed centrifugation step to eliminate cellular debris. However, considering that proteases have ample time to operate at 37°C during urine retention in the bladder, it is questionable whether adding inhibitors after collection would result in significant changes in the urinary proteome. Furthermore, it is well known that many proteins (e.g. uromodulin) precipitate at cold temperature under salt conditions. Therefore, both the use of protease inhibitors and the storage temperature require careful investigations for quantitative proteomics studies.

Preliminary results obtained in our laboratory have suggested that the addition of protease inhibitors could have a beneficial effect on protein quantitation in urine samples. Likewise, the storage of urine at 0°C seemed to affect the detected protein amounts, pointing to a possible precipitation effect.

In order to confirm these results, we undertook a comparative analysis of the differences between urine samples from 12 healthy donors treated/untreated with a chemical protease inhibitor cocktail (Roche Complete EDTA-free[®]) and stored at 0°C or 20°C for 4 hours prior to centrifugation. For each sample, a control was taken at the time of collection and processed right away. All samples were processed immediately after centrifugation and not deep-frozen to minimize protein losses during storage and thawing. Proteins were isolated using ultrafiltration and deposited on SDS-PAGE gel. Following a short migration, gels were cut, proteins were digested with trypsin, and the resulting peptide mixtures were analyzed using HPLC and MS/MS on a LTQ-FT instrument (Thermo Fisher Scientific). Label-free quantitative analysis of the observed proteins was used to validate the influence of pre-analytical sample treatments.

Urinary proteins from healthy individuals: quantitative variations observed by 2D-GE and implementation of the DUP database.

Laurence Molina¹, Nicolas Salvetat¹, Sabine Peres, Franck Molina, Claude Granier²
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France

The normal urinary proteome is currently being deciphered. However, if proteomic studies tell us about the presence of proteins in the sample studied, the extent of inter-individual variability of the normal urinary proteome has rarely been appreciated. It is, however, important to know to which extent the urinary proteome is variable in the context of biomarker discovery. To address this question, we separated by 2D-gel electrophoresis proteins from the urine of ten male and ten female apparently healthy individuals and analyzed the variability of each spot throughout the 20 gels. The spots from the twenty gels were aligned and the proteome variability of normal urine was represented on a synthetic gel using the Sili2Dgel software developed in our laboratory. Using this approach, 974 spots were aligned. We found that a majority of spots were highly conserved, e.g., 62.9 % were present in all 20 samples analyzed and 85.8 % were found in 50% or more urines. A strong conservation of the normal urinary proteome is thus observed by this approach.

In parallel, we have constructed the Database of Urinary Proteins (DUP). It is a carefully curated and non-redundant database of all proteins that have been reported in 12 proteomic publications as being present in the urine of healthy individuals. A DUP entry contains the IPI identification number, the protein sequence and information about protein description; the link with publications having found the protein in the urine is available interactively. Several tools are available to query easily the database. The list of human normal urinary proteins (as yet, more than 1800) can be visualized using a user-friendly web interface and the whole collection of proteins is represented in a clickable “virtual 2D gel” using a graphical tool. The DUP database constitutes a solid reference dataset for protein in urinary biomarkers discovery and the study of renal diseases. We feel this database should become a useful tool in urinary proteome research.

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Identification of a processed ubiquitin form as differentially excreted protein in diabetic nephropathy patients by urinary proteomics

Hassan Dihazi, Gerhard A. Müller, Sandra Lindner, Markus Meyer, Abdul R. Asif, Michael Oellerich, and Frank Strutz

Identification of markers for prediction of the clinical course remains a major challenge in the management of diabetic nephropathy. We established a proteomics approach for identification of diabetic nephropathy related biomarkers in urine. We used SELDI-TOF mass spectrometry and SAX2 protein arrays to compare protein profiles from urine of four defined patient groups. Samples from patients with type 2 diabetes (DM) ($n = 45$) without nephropathy and without microalbuminuria (DM-WNP), DM patients with macro- or microalbuminuria (DM-NP) ($n = 38$), patients with proteinuria due to non-diabetic renal disease ($n = 34$), and healthy controls ($n = 45$) were analysed. Anionic exchange, reverse-phase fractionation, gel electrophoresis, and mass spectrometry were used to isolate and identify proteins with high discriminatory power.

A protein with m/z 6188 ($p < 0.0000004$) was strongly released in the urine of healthy controls, patients with proteinuria due to non-diabetic disease, and DM-WNP in contrast to DM-NP-patients. A m/z 14766 protein ($p < 0.00008$) was selectively excreted in the urine of DM-NP patients, whereas the protein with m/z 11774 ($p < 0.000004$) was significantly excreted by patients with proteinuria and DM-NP. The m/z 11774 and m/z 14766 mass peaks were identified as beta-2-microglobulin and UbA52, an ubiquitin ribosomal fusion protein respectively. The protein with m/z 6188 was identified as a processed form of ubiquitin. Moreover the ubiquitin degradation assay confirmed the potential role of a urinary protease whose absence was specific for diabetic nephropathy.

The release of high amounts of UbA52 in urine of DM-NP patients could serve as a diagnostic marker. The identification of the protease and longitudinal studies in larger patient groups will determine the usefulness of the short form of ubiquitin as a marker for predicting the clinical course and the potential role of the protease in the pathophysiology of diabetic renal involvement.

Urinary proteomics to study obstructive nephropathy in newborns

JP Schanstra, S Decramer, JL Bascands, C Lacroix, A Gonzalez de Perodo, C Froment, B Monsarrat, H Mischak.

Obstructive nephropathy is a frequently encountered disease in newborns. Ureteropelvic junction (UPJ) obstruction represents about half of these obstructive nephropathies. Although not life threatening, UPJ obstruction necessitates for a number of patients close and invasive follow up to determine the evolution of the obstruction. Our research aims the selection of non-invasive biomarkers of UPJ obstruction that can be used in the clinic but that also lead to the identification of human urinary biomarkers of UPJ obstruction to better understand the pathophysiology.

Capillary electrophoresis coupled to mass spectrometry (CE-MS) has been shown to be a powerful tool for the selection of clinical useful urinary peptide biomarkers. We have used CE-MS for the selection of biomarkers of UPJ obstruction that allowed prediction of the evolution of the disease, several months in advance, in a prospective trail on 36 UPJ obstruction patients. We are currently performing a large scale validation of these CE-MS selected biomarkers in a prospective multicenter French trial involving 358 patients (2008-2011). Our follow-up projects include:

- 1) The use of CE-MS urinary proteome analysis to determine the change of the urinary peptidome with age.
- 2) The use of CE-MS urinary proteome analysis to determine whether UPJ obstruction induces permanent renal lesions after 5 year follow up.
- 3) CE-MS is focussing on the low molecular weight proteome. Currently we are preparing complementary studies aiming identification of higher molecular weight biomarkers in UPJ obstruction.

Taken together these different projects should yield clinical useful and non-invasive tools for UPJ obstruction and generate hypothesis on the pathophysiology of this frequently encountered disease in newborns.

Resolving urinary and secreted proteins by gel based approaches

I Zoidakis, M. Makridakis, P. Dimitraki, and A. Vlahou.

Division of Biotechnology, Biomedical Research Foundation of the Academy of Athens.

One of the main research interests of our group is the identification of biomarkers for bladder cancer aggressiveness. As potential biomarker sources we investigate a) urine from bladder cancer patients and b) secreted proteins from cell lines. In the former case, we currently employ a combinatorial approach involving preparative electrophoresis and two-dimensional electrophoresis (2DE) as a means to increase protein resolution. This technique is based on the separation of urinary proteins according to size by preparative electrophoresis and their subsequent separation based on charge and size by two-dimensional electrophoresis. Optimized gels systems for the resolution of different mass ranges, such as Tricine –16% acrylamide, Glycine-11% and Glycine -8% acrylamide are employed. This approach is currently being employed in the context of the FP7 program DECanBio for the identification of biomarkers for bladder cancer progression.

In parallel to the urine analysis, we also tackle the proteomic analysis of secreted proteins from cancer cells. We have recently developed an aggressive variant (T24M cells) of the commonly used T24 bladder cancer cell line. In order to get insight into the molecular changes associated with cell aggressiveness, we characterized these cell lines at the chromosomal and proteomic levels. Using *in vitro* transwell assays we observed that the motility of the parental T24 cells increases when cultured in the presence of conditioned medium from the more aggressive T24M cells. For the proteomic analysis of the secretome, we employ a modified protocol based on Rabilloud et al (Proteomics 2007, 7, 1757-1770) which involves separation of the secreted proteins by two-dimensional electrophoresis and their subsequent analysis by mass spectrometry.

Linking genomics, epigenomics and proteomics for disease diagnostics using urinary specimens

Marta Sanchez-Carbayo

Tumor Markers Group, Spanish National Cancer Center

The advent of high throughput technologies is allowing the comprehensive simultaneous measurement of disease specific molecular determinants at the DNA, RNA and protein level. The combined information provided by these technologies is critical for the discovery of molecular events associated with disease progression that could be translated into specific biomarkers and targeted therapies. The application of such technologies in matching tissue and body fluids for patients affected by neoplastic and non-malignant diseases and controls could potentially accelerate the development and validation of novel multiplexed strategies alternative to current diagnostic and clinical outcome stratification biomarkers. Easily attainable urinary specimens represent non-invasive samples with high potential for disease characterization and the identification of specific biomarkers for kidney and bladder related diseases. In the Tumor Markers Group at the CNIO, we are working on the optimization of protocols to apply several -omics techniques to urinary samples for disease progression characterization and biomarker discovery in bladder cancer. We will discuss the most relevant findings that we have obtained linking the information of genomic, epigenomic and proteomic approaches for disease diagnostics using urinary specimens.

Proteomic analysis of rat urine following sub total nephrectomy

Melissa Vickers, Sheffield Kidney Institute, The University of Sheffield

Introduction: Subtotal nephrectomy (SNx) is an established model of experimental chronic kidney disease (CKD). Following SNx, Wistar rats develop hypertension, proteinuria and renal fibrosis leading to a decline in kidney function. Proteomic analysis of urine from this model could lead to the identification of proteins involved in novel disease mechanisms or that have the potential to serve as markers of CKD. The aim of the present study was to identify proteins with a higher abundance in urine from rats with experimental CKD.

Methods: Urine was collected from male Wistar rats 90 days following SNx (n=8) or sham operation (n=4) which were performed under isoflurane anaesthesia. 400µg of urinary protein was precipitated with 3 volumes of 10% TCA/acetone, actively rehydrated and subjected to isoelectric focussing on a pH 3-10 IPG strip prior to SDS-PAGE size separation. Gels were stained with coomassie blue and analysed with PDQuest spot mapping software (Bio-rad). 17 spots with the largest increase in abundance (>60 fold) in SNx rat urine compared to sham were excised from the gel, digested with trypsin and sequenced by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or liquid chromatography quadropole ion trap tandem mass spectrometry (LC-Qtrap MS/MS). The National Center for Biotechnology Information database was then used to match peptide sequences to their protein of origin. Molecular weights were taken from 2D gels.

Results: By day 90, protein excretion from SNx rats increased by approximately 6 fold (19.2 ± 3.1 vs 112 ± 37.2 mg/24h, $p < 0.05$), glomerulosclerosis by 6 fold (28.7 ± 13.2 vs 4.8 ± 1.2 %, $p < 0.05$) and tubulointerstitial fibrosis by 11 fold (14.1 ± 1.2 vs 1.3 ± 0.3 %, $p < 0.05$) compared to sham. In addition to this, the creatinine clearance of SNx rats was more than 3 times lower than values for sham operated rats (2.2 ± 0.1 vs 0.6 ± 0.1 ml/min, $p < 0.05$). Approximately 300 protein spots were detected by PDQuest software on each gel. Of the 16 spots sequenced by mass spectrometry twelve of them were matched to acute phase proteins, including the β chain of fibrinogen (44kDa), complement factor b (36kDa) and haptoglobin (38kDa). Other protein matches of interest include retinol binding protein (18kDa) and its binding protein transthyretin (12kDa), superoxide dismutase 3 (31kDa), fetuin beta (40kDa) and a perlecan metabolite (25kDa) were also identified.

Conclusion: The use of 2D gels coupled with MALDI-TOF MS or LC-Qtrap MS/MS successfully identified peptides with a higher abundance in urine from rats with experimental CKD. In addition to acute phase proteins other proteins identified which may be of particular interest to the pathophysiology of CKD included superoxide dismutase 3 a free radical scavenger, fetuin beta, a vascular calcification inhibitor and a metabolite of perlecan, a heparan sulphate proteoglycan.

LC-LTQ FTICR MS based relative and absolute peptide quantitation for detection of markers of bladder cancer and other conditions in urine proteome and peptidome.

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High sensitivity nanoLC-ESI MS (nanoAcquity (Waters) LC coupled to LTQ FTICR (Thermo) MS) was applied for detection and quantification of peptides originating from two different fractions of urine collected from patients of Nephrology and Urology Wards of Miedzylesie Specialist Hospital and healthy volunteers. A pilot, small scale, study results will be shown in which hundreds of peptides in urine proteome and peptidome, representing numerous proteins, can be detected uniquely in bladder cancer samples, being absent in healthy volunteer's samples.

A pilot set consisted of 5 cancer cases and 5 healthy samples. After centrifugation of urine, the supernatant was fractionated by filtration through 5kDa filter into peptidome and proteome. Peptidome was analysed by LC-MS without further steps. Proteome was precipitated by methanol/chloroform, resolubilised and subjected to trypsin digestion and cysteine alkylation. 1 µg of tryptic digest was used for the LC-MS analysis. At the protein identification step in all cancer and healthy (separately pooled) samples >600 proteins were detected. Peptides from >300 proteins were repeatedly found either in cancer or both in cancer and healthy samples, with peptides of 142 proteins unique for cancer (24 of these proteins detected by 2 or more peptides). Several known marker proteins were also detected: Fibronectin, Kallikrein-1, Zinc-alpha-2-glycoprotein, Alpha-1-acid glycoprotein 1, Alpha-1B-glycoprotein (A1BG), Haptoglobin, Serotransferrin, Soluble E-cadherin, Tenascin C (Cytotactin), Clusterin (Apo-J, Complement cytolysis inhibitor). At present the series of samples is being expanded and after appropriate statistical analysis a set of best peptide markers will be selected. Subsequently their isotopically labeled versions will be synthesized and serve as internal standards allowing for absolute peptide quantitation at marker panel verification step. Also other pathologies (autosomal dominant polycystic kidney disease, diabetic nephropathy, renal cell carcinoma) will be included into the study for extraction of condition-specific marker sets.

Towards a urinary fingerprint of gentamicin-induced acute renal failure

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Acute renal failure (ARF) is a serious condition with high human and socioeconomic repercussion. A key aspect for the clinical handling of ARF is an early diagnosis, for which a new generation of urine markers is currently under development, including kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) and others. A further refinement of diagnosis is necessary for the identification of the specific cause of ARF. This gains especial importance under those circumstances where several potentially nephrotoxic insults converge, such as in multidrug therapeutic courses. In this sense, the identification of a specific urinary fingerprint of every relevant nephrotoxicant will afford a great clinical advantage. In this work we have identified a total of 54 proteins differentially expressed (either augmented or diminished) in the urine of rats with gentamicin-induced renal damage. Many of these markers are common to the renal damage induced by other insults, but a few others might serve as differential markers of gentamicin's nephrotoxicity. For this purpose, we have applied different differential proteomic approaches, including 1-D and 2-D electrophoresis combined with ESI-QUAD-TOF and MALDi-TOF, and 2D-CF electrophoresis. These methods have proved to be partially overlapping and partially non redundant at protein identification. This study provides a first step towards the generation of a urinary fingerprint of gentamicin's nephrotoxicity, as well as new marker candidates whose early diagnostic capacity must be tested.

Urinary proteome pattern in children with DeToni-Debré-Fanconi syndrome

Jens Drube^{1*}, Eric Schiffer^{2*}, Harald Mischak², Markus J. Kemper³, Thomas Neuhaus⁴, Lars Pape¹, Ralf Lichtinghagen⁵, Jochen H.H. Ehrich¹,

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Background:

The DeToni-Debré-Fanconi-Syndrome (FS) is characterized by renal glucosuria, loss of electrolytes, bicarbonate and lactate, generalized hyperaminoaciduria and low molecular weight proteinuria. We studied the urinary proteome of proteins with a molecular weight below 20 kDa to study the hypothesis that smaller proteins excreted in the urine may be indicative of pathogenetic mechanisms leading to dysfunction of the proximal tubule. Proteins with higher molecular weight excreted in FS are well known and have been investigated intensively without revealing the cause of progressive renal failure in FS.

Methods:

First we established a urinary proteome pattern using capillary electrophoresis mass spectrometry (CE-MS) of 7 paediatric patients with cystinosis and 6 patients with ifosfamide induced FS. We conducted a blinded prospective study consisting of 11 patients suffering from FS and of 9 patients with renal disease other than FS. Amino acid sequence of the marker proteins were obtained by CE- or LC-MS/MS analysis.

Results:

We established and validated a urinary protein pattern to identify patients with FS. Specificity was 89%, sensitivity was 82% in the blinded control group. The marker peptides constituting the proteome pattern derived from osteopontin, uromodulin and several fragments of collagen alpha-1.

Conclusion:

CE-MS can be used to diagnose FS in paediatric patients and might be a future tool for non-invasive diagnosis of FS. The marker proteins for FS included reduced amounts of fragments of osteopontin and uromodulin indicating a loss of function of tubular excretion in FS regardless to the underlying cause. Six different fragments of the collagen alpha-1 (I) chain were either elevated or reduced in the urine. This indicates a change of proteases in collagen degradation as the underlying cause for the development of renal insufficiency in FS patients.

MALDI mass spectrometry in biomedical sciences: from true high energy CID to imaging/profiling mass spectrometry

Martina Marchetti-Deschmann, Ernst Pittenauer and Günter Allmaier

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The possibilities of vacuum matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) in combination with different mass spectrometric analyzer as well as in tandem and/or multi stage configuration will be illustrated for structural characterization of complex biopolymers [E. Pittenauer, M. Zehl, O. Belgacem, E. Raptakis, R. Mistrik and G. Allmaier, *J. Mass Spectrom.* **41**, 421 - 447 (2006). E. Pittenauer and G. Allmaier, *Combinatorial Chemistry & High Throughput Screening*, *in press* (2008).] and for the molecular imaging [M. Marchetti and G. Allmaier, *J. Mass Spectrom.* **43**, *in press* (2008).] and profiling of biological surfaces.

Particular the early incorporation of MALDI MS in analytical strategies will be emphasized and the focus will be on peptide/proteins and their posttranslational modifications as well as lipids [G. Stübiger, E. Pittenauer and G. Allmaier, *Anal. Chem.* **80**, 1664 - 1678 (2008).]. The possibilities and also limitations [C. Mayrhofer, S. Krieger, E. Raptakis, and G. Allmaier, *J. Proteom. Res.* **5**, 1967 - 1978 (2006).] of the MALDI technique in connection with biopolymers will be discussed. Some preliminary data of the application of this technique to perform molecular imaging (distribution of known compounds of interest) and molecular profiling (distribution of unknown compounds, which might become of interest to the scientific investigation, and identification of these) will be presented.

Proteomic analysis of podocyte injury

C. Mayrhofer^{1,2}, G. Allmaier² and D. Kerjaschki¹

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Podocyte injury/dysfunction is a common and determining factor in diseases affecting the glomerular filtration barrier. Our group is particularly interested in the functional basis of podocyte dysfunction commonly observed in association with loss of glomerular filtration function in idiopathic NS. Therefore we developed and applied different proteomic techniques, amongst others a technique to selectively target cell surface molecules of glomerular cells *in vivo* and podocytes *in vitro*.

In the present study we explored changes on the proteome level that parallel the disturbances of podocyte architecture in the early stages of a well established experimental disease. Morphological and functional changes closely resembling the human idiopathic minimal change disease that is the most common cause of idiopathic NS in childhood were induced in rats by administration of puromycin aminonucleoside (PA). Expression differences between extracted proteins from isolated normal and nephrotic glomeruli were analysed by a quantitative proteomic approach including two-dimensional fluorescence difference gel electrophoresis (2-D DIGE) and vacuum matrix-assisted laser desorption/ionisation (vMALDI) mass spectrometry.

The data clearly indicated that different functional groups of proteins are intimately connected with early stages of puromycin aminonucleoside nephrosis (PAN) *in vivo*. The majority of these proteins were primarily related to fatty acid metabolism and redox regulation. Key enzymes of the mitochondrial β -oxidation pathway and antioxidant enzymes were consistently down regulated in PAN. These changes were paralleled by increased expression of CD36. Divergent expression of fatty acid utilizing proteins could be shown in cultured podocytes exposed to PA *in vitro* as well. Moreover, we demonstrated not only the coordinated down regulation of enzymes of the cellular antioxidant defense mechanisms in PAN *in vivo* but the modulatory effects of albumin bound fatty acids on the expression of Mn-superoxide dismutase, a major antioxidant enzyme, in PA induced podocyte lesions *in vitro*. Taken together, the data indicated that a disturbed handling of fatty acids paralleled with an impaired antioxidant defense mechanism in podocytes may contribute to PA induced podocyte lesions.

In summary, the proteomic analysis offered novel illumination of some cellular events leading to podocyte injury causing glomerular dysfunction.

Altered expression of calreticulin during the development of fibrosis

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Tissue damage following injurious stimuli leads to inflammation and fibrosis. To understand the molecular mechanisms and the proteins involved in the fibrotic process, we have used a well established animal model of renal fibrosis, the unilateral ureteric obstruction rat model and analyzed the alterations at early (2 days) and late (8 days) time intervals using a classical proteomic approach. Renal cortex samples were examined by 2-D gel electrophoresis and spots consistently showing quantitative differences by PD Quest software analysis were further characterized. Data analysis demonstrated a correlation between calreticulin up-regulation and progression of fibrosis. Calreticulin, is involved in Ca^{++} homeostasis but has not been previously implicated in animal models of fibrosis. Proteomic analysis consistently revealed up-regulation of calreticulin in both early and late time intervals. These findings were further confirmed by biochemical and morphological approaches. Next, animal models of lung fibrosis (bleomycin-induced) and heart fibrosis (desmin-null) were examined. In the lung model, calreticulin expression was up-regulated from early time intervals, whereas in the heart model no change in the expression of calreticulin was observed.

In cultured HK2 cells, a renal proximal tubule well differentiated and immortalized epithelial cell line, TGF- β led to up-regulation of calreticulin expression. Bioinformatic analysis of the proximal promoter sequence for calreticulin revealed several motifs corresponding to binding sites for transcription factors that are downstream effectors of the TGF- β signaling pathway. Experimental investigation of these mechanistic interactions is currently under way.

In cultured A549 cells, a lung alveolar epithelia cell line, treatment with TGF- β was sufficient to significantly up-regulate the expression of calreticulin, whereas under the same experimental conditions E-cadherin was dramatically reduced.

Based on our observations we propose that calreticulin may be a key molecule in the fibrotic process, playing a crucial role in early steps of epithelial to mesenchymal transition, a process involved in the animal models used for renal and lung fibrosis but absent in the animal model used for myocardial fibrosis.

Immunoproteomic analysis of allo-reactive non-HLA specific antibodies in haemodialysis patients before renal transplantation

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Background: The presence of allo-reactive antibodies in renal transplant recipients is one of the main determinants of organ rejection. Antibodies against donor's HLA-proteins lead to severe rejection events. However, good HLA-Matching does not guarantee rejection-free long-term graft survival. Recent data showed that also non-HLA antigens are targets for antibody-mediated rejection.

Objective of the study: This proteomics study aims at investigating the frequency and specificity of allo-reactive non-HLA-specific antibodies in haemodialysis patients.

Methods: Serum-samples were taken before graft-transplantation from 29 haemodialysis patients. Lymphocytes from 10 patients as well as from 20 healthy volunteers were used as a source of antigen. Allo-reactive anti-lymphocytic antibodies were investigated by one & two dimensional gel electrophoresis and Western blotting. Antigenic proteins were identified by mass spectrometry.

Results and conclusion: Presence of antibodies against a variety of lymphocytic antigens was observed in high and low PRA haemodialysis patients. This includes always also antibodies against proteins with a molecular weight other than HLA. The specificity of these non-HLA targeting antibodies differs between patients. In addition, antibodies from each single patient diversify between lymphocytes from different healthy volunteers. The antigenic proteins were identified as the cytoskeletal proteins Vimentin, alpha and beta Tubulin, and Lamin B1 and the cytoplasmatic protein Rho GDP-dissociation inhibitor 2. Since the presence of allo-reactive non-HLA specific antibodies have been already associated with impaired long term graft survival, the specificity of these antibodies -as identified here - could be used to improve donor-recipient matching in future.

Renal Proteomic Research in Leeds

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The group at Leeds comprises an integrated multidisciplinary team including postdoctoral scientists with expertise in mass spectrometry, biochemistry, cell biology, chemistry, biostatistics and bioinformatics, technicians, data managers, clinicians, research nurses, clinical chemists and research students. It is based in labs in the Cancer Research UK Clinical Centre within the Leeds Institute of Molecular Medicine on the St James's University Hospital campus. The proteomic approaches being used include 2D-DIGE, 1 and 2D LC-MS/MS (shotgun, SILAC and iTRAQ), SELDI/MALDI profiling together with supporting technologies such as laser-based microdissection and plans for imaging mass spectrometry in the near future. Associated with the technological approaches are the development of new purpose-designed bioinformatic tools and investigation of statistical models for clinical proteomics. Funding sources include the University, NHS R & D, a Programme award and project grants from Cancer Research UK, MRC, Kidney Research UK, DIUS (via a collaboration with AstraZeneca and Cardiff University) and the Department of Health.

Our main research focus is in renal cancer (renal cell carcinoma – RCC) but we are also pursuing other renal research and other clinical proteomics in other areas for biomarker discovery as a result of collaborations in Leeds, for example in various clinical trials, ovarian cancer, multiple sclerosis and cholangiocarcinoma. Our renal-related research falls into 3 areas.

- Renal cancer. Principal aims are biomarker/therapeutic target discovery but with a programme also investigating the findings of our studies in relationship to the underlying pathogenesis. Projects involve diagnostic and prognostic marker discovery, metastatic markers, predictive markers for TKI therapies and using von Hippel Lindau (VHL) -based model systems to characterise the clinical importance of the changes at the genetic and protein levels and understand the underlying pathology.
- Renal transplant rejection. A new project focussed on discovery and validation of markers of acute rejection has just commenced.
- Nephrotoxicity. In collaboration with AstraZeneca and Cardiff University we are currently evaluating and validating potential new markers of nephrotoxicity in animal and human model systems and additionally recently have begun marker discovery in a human model system based on cisplatin-based treatments.

Our research is underpinned by banks of frozen samples of various types for the disease areas under study which include serum, plasma, urine, CSF, bile and, in the case of renal cancer, normal and malignant tissue. For RCC we also have access to formalin fixed paraffin-embedded tissue and proteomic analysis of this is an area we are currently investigating. All of these samples have accompanying clinical data.

We are also now exploring how best to complete the biomarker pipeline from the lab to the clinic as a major challenge in clinical proteomics is defining and implementing the optimum route to take emerging new markers further. Ultimately, markers emerging from the discovery and validation processes will warrant a full clinical trial at multiple centres with full assessment of clinical utility and health economic evaluation. The process needs to be rapid, accessible and robust – however developing the most appropriate model and infrastructure for this is not straightforward or cheap and we are developing and exploring various possible structures to take this forward with expertise from the relevant disciplines.

In search for genetic modifiers and early urine biomarkers that predispose Thin Basement Membrane Nephropathy patients to Focal Segmental Glomerulosclerosis and renal failure

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The recent description of multiple gene defects in hereditary podocytopathies and in hereditary glomerular basement membrane diseases has dramatically improved the current state of our knowledge on the renal glomerular filtration barrier. Recently described mutations in glomerular basement membrane (GBM) genes, such as collagen IV and laminin in patients with hematuria and severe nephrotic syndrome, add to other experimental data supporting the hypothesis that the GBM may also have a significant role in protein filtration, a function, previously attributed exclusively to the podocytes.

Autosomal collagen IV genes, *COL4A3/COL4A4*, on chromosome 2q36-q37, when mutated in homozygous state, cause the classical full blown Alport Syndrome, which accounts for about 15% of Alport cases, the rest being the X-linked form. Heterozygous mutations in these same genes were thought to cause only a mild form of renal disease (thin basement membrane nephropathy – TBMN). This is usually accompanied by recurrent or persistent microscopic hematuria with no progression, and considered of excellent prognosis. However, recent data from our laboratory show that many patients who carry such mutations may later on in life develop focal and segmental glomerulosclerosis, on top of the TBMN and the microscopic hematuria, a situation that frequently progresses to chronic renal failure or even end-stage renal disease. More specifically, among 59 patients older than 51 years, 2/3 develop chronic kidney disease and nearly 1/3 progress to ESKD.

The role of unknown modifier genes may explain the heterogeneity of symptoms in TBMN and other glomerular diseases and in particular the selected development of chronic kidney disease. The molecular communication between GBM and podocytes may also be a key factor in the search for these major genetic modifiers while their understanding may improve novel drug design for glomerular diseases.

While we are interested in identifying putative genetic modifiers that predispose TBMN patients to chronic or end-stage kidney disease, we are also hypothesizing that there might be useful early biomarkers in urine, that identify these patients, well before the onset of recognizable renal impairment. To this end, we would like to search for protein markers using a proteomics approach and mass spectrometry, in the urine of two groups of subjects, ages 30-40 years. Group A will be healthy people of the general population. Group B will be patients who are heterozygous carriers of a *COL4A3* or a *COL4A4* mutation and will be followed prospectively for 3-5 years in order to identify who will remain with isolated microscopic hematuria and who will progress to chronic kidney disease. This will hopefully allow us to associate certain urine biomarkers with the predisposition to disease progression.

We hope that discussions during the Bergamo meeting will help us to better design this proteomics project, by selecting the most appropriate patients and collect the correct samples.

FACTORS IMPLICATED IN THE DEVELOPMENT OF TUBULOINTERSTITIAL INJURY AND PROGRESSION OF HUMAN GLOMERULAR DISEASE

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Background/Aim: Glomerular diseases are followed by histological changes in the tubulointerstitial area. The degree of tubulointerstitial injury is better correlated to the clinical outcome than glomerular changes. Transforming Growth Factor (TGF- β_1), myofibroblasts (α -SMA⁽⁺⁾ cells), apoptosis and reduced number of peritubular capillaries have been implicated in the development of renal fibrosis. The purpose of this study is to identify relation of these factors with the severity of tubulointerstitial injury and clinical outcome of patients with various types of glomerular disease.

Patients/Methods: Forty four patients were included in the study. The original diagnoses were membranous nephropathy (n=20), IgA nephropathy (n=12), minimal changes disease (n=8), focal segmental glomerulosclerosis (n=4). Normal renal tissue from 10 patients who underwent nephrectomy for hypernephroma was also examined. The expression of TGF- β_1 , α -SMA⁽⁺⁾ cells, bax and bcl-2 proteins in the tubulointerstitial area and the number of peritubular capillaries (CD34⁺ cells) were investigated by immunohistochemistry in kidney biopsy sections using specific antibodies. The extent of immunostaining was estimated by morphometric analysis.

Results: TGF- β_1 , α -SMA⁽⁺⁾ and bax, bcl-2 proteins were identified in the tubulointerstitial area in kidney biopsies from patients with various types of glomerular disease and their expression was significantly increased compared to controls. Significant correlation of the expression of TGF- β_1 , and α -SMA⁽⁺⁾ with the degree of interstitial fibrosis, tubular atrophy and renal function impairment were observed. The extent of TGF- β_1 immunostaining was related to the presence of α -SMA⁽⁺⁾ cells and to the expression of bax and bcl-2 in the tubulointerstitial area. The number of peritubular capillaries was significantly reduced in patients with intense interstitial expression of α -SMA⁽⁺⁾ cells and in those with impaired renal function.

Conclusion: Increased expression of TGF- β_1 , α -SMA⁽⁺⁾ cells and markers of apoptotic process as well as reduced number of peritubular capillaries were identified in the tubulointerstitial area of patients with various types of glomerular disease. These factors are probably involved in the pathogenesis of tubulointerstitial scarring and should be targets of specific treatment in the future.

Deferoxamine (DFO) attenuates lipid peroxidation, blocks IL-6 production and confers renoprotection after acute hepatic ischemia in pigs

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Background: Acute renal failure (ARF) is often encountered in patients with Systemic Inflammatory Response Syndrome (SIRS) and Multiple Organ Failure (MOF) and contributes substantially to their grave prognosis. Inflammation triggered by cytokines, chemokines and reactive oxygen species released by necrotic tissue is an important early event in the cascade of apoptosis and necrosis. In an attempt to bring additional clarity into these issues we tried to address two key questions: (a) are we able to inhibit lipid peroxidation and diminish inflammation after acute hepatic ischemia in pigs by the use of the iron chelator deferoxamine? (b) are these biochemical findings clinically useful by moderating SIRS and protecting organs including kidneys?

Methods: Acute hepatic ischemia and SIRS were induced in 14 female anesthetized and mechanically ventilated pigs (22 + 1.5 Kg) after complete liver devascularization, as previously described. Then, animals were randomly allocated to Group DFO (n=7) which received constant infusion of 150 mg/kg deferoxamine (Desferal, Novartis) in saline, and the control Group C (n=7) which received only saline infusion at the same rate. In addition, a 6:3:1 mixture of Lactated Ringer's, Hydroxyethyl-starch 6% and dextrose 5% was infused at 5 mL/kg/hr. Cardiovascular monitoring, balance studies, hematologic and biochemical analysis, measurement of TBARS, as an index of lipid peroxidation and of TNF- α and IL-6, as indices of inflammation were all carried out by the proper techniques. Renal and hepatic biopsies for histopathologic examination were obtained after euthanasia at 24h.

Results: Animals in Group DFO had higher mean arterial pressure than controls at 18h (DFO;95+/-8 vs. C;67+/-8 mmHg, P<0.01) and 24h (DFO;84+/-10 vs. C;49+/-8 mmHg, P<0.01) postoperatively. Temperature, heart rate, the volume and composition of infused fluids, urine output, CVP and weight gain did not differ between the two groups. However, both serum protein (DFO;3.7+/-0.15 vs C;2.1+/-0.34 g/dL) and sodium concentrations (DFO;133+/-3.7 vs C;117+/-12.8 meq/L) were dramatically lower (P<0.01) in the control relative to DFO treated pigs at the end of the experiment. Serum TBARS increased in Group C (3.2+/-0.09 at baseline; 5.1+/-0.9 at 12h; and 5.54+/-0.9 nmol/ml at 24h, P<0.05), but remained unchanged in Group DFO (3.3+/-0.19 at baseline; 4.1+/-0.6 at 12h; and 3.8+/-0.5 nmol/ml at 24h). Furthermore, elevation of IL-6 was dramatically blocked by DFO and serum IL-6 levels remained lower both at 12 (DFO; 33+/-12 vs. C; 520+/-121 pg/ml, p<0.01) and 24 hrs (DFO;13+/-9 vs. C; 463+/-152 pg/ml, p<0.01) postoperatively. No evidence of tubular necrosis or TUNEL-positive cells were observed in kidney biopsies from DFO treated animals. In contrast, both TUNEL-positive cells and stripes of tubular necrosis were both found in the control pigs.

Conclusions: DFO attenuates lipid peroxidation, blocks IL-6 production, ameliorates SIRS and confers renoprotection after acute hepatic ischemia in pigs. Proteomic analysis of urine samples in the future, will help in the discovery of appropriate early markers for the development of acute renal failure.

Statistical analysis for proteome analysis: a necessary evil or a powerful tool?

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Protein separation via two-dimensional gel electrophoresis (2DE) is not the most userfriendly technique and has not the highest throughput but it is still unrivalled in resolution and indispensable for the characterisation of protein isoforms. After separation through 2DE several hundreds of individual protein abundances can be quantified. The datasets contain hundreds of different proteins that are correlated. Proteins fit within the larger entity of networks and interact with each other. Both, a good experimental set-up and a valid statistical approach are essential to get insight into the data and to draw correct conclusions. Our understanding of a biological system is usually rather limited and data may be very heterogeneous and complex. Exploratory multivariate data analysis approaches a biological problem from a different angle and tries to describe patterns, relationships, trends, outlying data, etc. The field of multivariate analysis consists of those statistical techniques that consider multiple related random variables as a single entity and attempts to produce an overall result taking the relationship among the variables into account. It displays the interrelationships between the large number of variables and is able to correlate multiple proteins to a specific experimental group. Principal Component Analysis (PCA) is one of the multivariate possibilities to perform explorative data analysis. PCA condenses the information contained in a huge data set into a smaller number of artificial factors, which explain most of the variance observed. Using the DIGE approach we started from one significant time point and analyzed the kinetics in further detail. PCA gives a first insight in the complex dataset. It confirms the reproducibility of the replicates, identifies multiple proteins that are correlated to a treatment and shows the correlations among several proteins. The individual differences between the treatment and the sample time points are subsequently validated via confirmatory ANOVA. This is a nice illustration of how exploratory data analysis is performing, indicating correlations but also bringing up candidate markers that would have been missed when using only the routinely used confirmatory data analysis. Statistics are indispensable for biomedicine and are undoubtedly a powerful tool.

MolMeth: The Molecular Methods Database

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Motivation

MolMeth, short for molecular methods, is a database system that catalogs laboratory protocols and methods for the life sciences. It is of particular value for large-scale applications in biobanks and systems biology, but also provides value in scientific communication about molecular procedure in general. It is designed to meet a growing need for structure in protocol specifications while offering convenience for contributors and easy access for end users. Structured protocols offer several advantages over current "flat file" protocol databases, such as allowing protocol presentation be adapted for different purposes. It also provides a foundation for automated reasoning regarding protocols.

Methods

The system presents itself as a web site for searching and viewing protocols. Registered users can also submit and modify their protocols. Modifications result in a new versions with a distinct, permanent URL:s. There is also a web service which allows third party applications to retrieve structured versions of protocols, identified by unique accession numbers. The database stores various properties related to each protocol, including information about materials and available suppliers, versioning information, user comments, references and related entries. The submitter of a protocol is allowed to specify a publication date and rudimentary access rights. The basic structure of a protocol is modular, meaning that it can be built as a hierarchy of (sub-) protocols, combining steps into different protocols without duplicating common parts. Each protocol is also viewed as a function, which transforms an input to some output. The goal is to specify these using well defined ontologies.

Results

The modularity saves effort for authors when protocols have steps in common, or when a protocol is part of another, more extensive protocol. Protocols that are split into modules are still presented with contiguous instructions in a hierarchical list of steps, adapted for a specific setting if desired. The MolMeth team hopes that that the computational abilities arising from structured protocols will allow the system to automatically suggest steps for protocol authors or, given a start condition and a goal, even suggest entire protocols by combining smaller protocols from the database. It is already clear that structured protocols will play a role in the development of harmonised standards in several pan-European research infrastructures.

Read more: beta.molmeth.org

Putting the fun back into bioinformatics: making biological databases and bioinformatics tools talk to each other!

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Motivation: In the biological sciences, the need to analyse vast amounts of information has become commonplace. Such large-scale analyses often involve drawing together data from a variety of different databases, held on the Internet or on in-house servers. Supporting these tasks are *ad hoc* collections of software tools, which are often cumbersome and non-intuitive to use. Many such systems have been customised for a particular purpose or user-group, and are consequently not readily adaptable to other uses. Further, many routine bioinformatics tasks, despite increasing levels of automation, still require the unique experience and intuition of human researchers to interpret results in a meaningful biological way; yet many of the tools to help do this have poor interfaces, most are isolated from other tools and databases owing to incompatible data formats, and many have limited real-time performance when applied to realistically large data-sets – users consequently waste a lot of time trying to control the software and manipulate esoteric file formats rather than getting on with their research!

Methods: To confront these issues, harnessing expertise in human computer interaction, high-performance rendering and distributed systems, and guided by bioinformaticians and end-user biologists, we are building re-usable software components that, together, create a software environment that is architecturally sound from a computing point of view, and addresses both user and developer requirements. Key to the system's usability is its direct exploitation of semantics, which, crucially, gives individual components knowledge of their own functionality and allows them to interoperate seamlessly, removing many of the existing barriers and bottlenecks from standard bioinformatics analyses.

Results: The software, UTOPIA (User-friendly Tools for OPERating Informatics Applications), currently includes three main components: Find-o-Matic, which provides an iTunes-like interface for finding Web services and data objects – this allows simple keyword queries to be submitted to multiple databases and tools, and returns results in a unified format that can be sorted, arranged into 'playlists', and moved interactively to the other components; CINEMA, a flexible editor that allows alignments to be visualised at different scales, to be manually edited, and to be 'annotated' with structural and functional features (*e.g.*, locations of transmembrane domains, glycosylation sites, ligand-binding sites, and so on); and Ambrosia, a 3D structure viewer that represents molecules in various styles, and is able to overlay annotations and features depicted in CINEMA (UTOPIA is freely available from <http://utopia.cs.man.ac.uk>). The most recent component, Papyro, is a graphical tool for finding, reading and managing documents, and ultimately for making the literature comprehensible! It has access to the same underlying semantic model as all the other UTOPIAN tools; but, as it works with generic documents rather than with specific biological concepts, its application is much broader. We will demonstrate UTOPIA and Papyro, and suggest that these tools are poised to make a significant contribution to EuroKUP.

Presentation of EuroKUP/e-LICO Joint Activities

M. Hilario, A. Kalousis, C. Roos, JP Schanstra

The goal of the e-LICO project is to build an e-Laboratory for Interdisciplinary Collaborative Research in Data Mining and Data-Intensive Sciences. The proposed virtual lab will comprise three layers: the e-science and data mining layers will form a generic research environment that can be adapted to different scientific domains by customizing the application layer. The *e-science layer*, built on an open-source e-science infrastructure developed by one of the partners, will support collaborative authoring of knowledge and information resources such as ontologies and data bases. The *data mining layer* will be the distinctive core of e-LICO; it will provide comprehensive multimedia (structured records, text, images, signals) data mining tools. Methodologically sound use of these tools will be ensured by a knowledge-driven data mining assistant, which will examine the input data and user goals and interests to propose ranked workflows for a given application problem. Extensive e-lab monitoring facilities will automate the accumulation of experimental meta-data to support replication and comparison of data mining experiments. These meta-data will be used by a meta-miner to incrementally improve the assistant's workflow recommendations as it gains experience in a given application area. While the first two layers are domain-independent, the *application layer* is domain-specific. It will house the knowledge sources, input data, and data mining experiment records related to a particular application or domain.

While e-LICO was designed to be generic, it will be showcased on a systems biology task: biomarker discovery and molecular pathway modelling for diseases affecting the kidney and urinary pathways. This pilot application was selected since the inception of e-LICO in early 2007, based on previous collaboration between the coordinators of EuroKUP (FBRAA) and e-LICO (UNIGE). EuroKUP/e-LICO collaboration will revolve around three activities related to the kidney and urinary pathways: (1) development of a specialized ontology, (2) design and maintenance of a data base, and (3) use of the e-LICO environment to mine EuroKUP members' experimental data and build diagnostic or prognostic models in support of their research goals. In a preliminary poll conducted last April, a number of EuroKUP members have already signified their commitment to these activities. The goal of this presentation is to give a brief overview of the e-LICO project and, more importantly, to explore with EuroKUP members concrete ways of implementing these joint activities.

Paradigm Shift in Proteomic Biomarker Discovery and Evaluation

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Proteomic biomarker discovery and evaluation in plasma has to overcome two major challenges: i) the vast sample complexity due to a large number of proteins and their structural diversity, and ii) the wide dynamic range of protein concentrations spanning over ten orders of magnitude. In spite of the recent technology developments, alternative strategies are still needed as classical shotgun proteomics does not provide sufficient penetration into the proteome due to under-sampling issues.

We are proposing a two stage strategy, similar to the one successfully used in genomic screens: i.e. first establish a catalog of all elements (genes / proteins), and in a second stage use specific probes (proteotypic peptides) to screen for each element in multiple sample using a high throughput platform. Effective methods to reduce sample complexity together with high-performance LC-MS/MS platforms are essential.

In the initial discovery stage the isolation of a sub-proteome (N-glycosites), and its further fractionation using electro-focusing techniques is performed prior to the directed LC-MS and LC-MS/MS analyses using inclusion lists to detect, quantify, and identify biomarker candidates isolated from tissues, proximal bodily fluids, or plasma. In the second stage the candidates derived from previous proteomic experiments, transcriptomic, genomics studies, and literature knowledge are used to design a mass spectrometry screen leveraging the high-throughput selected reaction monitoring (SRM) approach. The technique offers unique selectivity and sensitivity over a wide dynamic range. In such experiments the peptides of interest isotopically labeled are added to the samples prior to their MRM analysis to ensure precise quantification, and use chromatographic (elution time) and mass spectrometric (ion intensities) properties to corroborate the identity of the analytes.

The recent scheduling capabilities of triple quadrupole instruments, combined with the exquisite sensitivity, selectivity and dynamic range of the SRM/MRM technique, has enable analysis of multiple analytes (several hundreds) present in plasma at in sub ng/ml range in a high-throughput manner. The approach was applied to several diseases, including cancers and diabetes.

Developing tools for future proteomics

Marc Baumann

The basic goal of a proteomic microchip is to achieve efficient and sensitive high throughput protein analyses, carrying out several measurements in parallel. A protein chip would either detect a single protein or a large set of proteins for diagnostic purposes, basic proteome - or functional analyses. Such analyses would include e.g. interactomics, general protein expression studies or detecting structural alterations or secondary modifications. Visualization of the results may occur by simple immunoreactions, general - or specific labeling or mass spectrometry (MS).

A key issue in the development of proteomic microchips is to produce materials and surfaces with targeted functionalities. We have used nano - and micro technologies to create surfaces which would have the capacity for fast liquid transfer and ultra sensitive molecule trapping and at the same time provide a high surface area in 3-D. Our future goal is to include the ability to use multiple features on the same chip e.g. immunodetection, concentration of low abundant proteins above their detection limit, trapping proteins with specific secondary modifications (e.g. phosphorylations) and finally, MS detection. Our preliminary results indicate the feasibility to produce such multifunctional chips, although currently tested on separate chips each.