MPSA 2006

Lille, France, August 29th > September 2nd

16th meeting of Methods in Protein Structural Analysis

International Association for Protein Structure Analysis and Proteomics

Posttranslational Modifications : **Analysis and Funct**

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Welcome From The Chairman

Welcome to the MPSA 2006 Conference, and welcome to Lille, a Flemish city in Northern France, at the very heart of Europe!

MPSA is organized every two years and hosted alternatively in the USA and in Europe. MPSA 2006 will be take place at the University of Lille1, in Villeneuve d'Ascq, at a 10 minutes distance from the old medieval city core of Lille.

This Conference series is the premier meeting for presenting the latest results in protein structural analysis. This year's meeting will present a focused program around post-translational modifications, their analysis and the study of their structural and functional implications. The conference will cover the latest analytical techniques to detect and quantify phosphorylation, methylation/acetylation, ubiquitination/sumoylation, glycosylation and other modifications, and will pay special attention to the structural impact and function of these posttranslational modifications. The conference program will consist of invited and oral presentations and poster sessions. As was the case for MPSA2004, we expect to share the most innovative presentations with the wider community of scientist working in this field through the publication of a series of mini-reviews in the FASEB Journal. In addition, a special prize will be given to the best posters presented at the meeting.

For the duration of the conference, there will be exhibition stands of many industrial participants presenting their latest innovations in analytical and biochemical technology in the general field of protein structural analysis and proteomics.

We hope the strong Social Program will enable participants to sample the history and culture of this Flemish city, including a welcome reception, excursion and banquet. Partners of conference attendees will enjoy a memorable program, including an opportunity to explore Brughes, Ghent, Arras and other Flemish cities in the surrounding areas.

For the Organizing Committee,

Guy Lippens

M. Guy LIPPENS
MPSA 2006 chairman

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MPSA2006 program

29.08.06 / Tuesday

am

9:30 - 12:00 > Proteomics course 1st session

pm

2:00 - 5:00 > Proteomics course 2nd session

6:00 - 7:00 > **Opening lecture : CD Allis**

30.08.06 / Wednesday

am

9:00 - 9:40 > KP Lu
9:40 - 10:20 > TK Sixma

10:20 - 11:00 > Coffee break

11:00 - 11:40 > VG Wilson
11:40 - 11:55 > I Landrieu
11:55 - 12:10 > H Nika

12:00 - 14:00 > Lunch

13:30 - 14:00 > Presentation by Agilent

pm

2:00 - 2:40 > AL Burlingame
2:40 - 3:20 > P Roepstorff
3:20 - 3:40 > C K Gevaert

3:40 - 4:20 > Coffee break

4:20 - 5:00 > ME Linder
5:00 - 5:20 > Grangeasse
5:30 - 7:30 > **Poster Session I**

31.08.06 / Thursday

am.

9:00 - 9:40 > NL Kelleher
9:40 - 10:20 > DW Hunt

10:20 - 11:00 > Coffee break

11:00 - 11:40 > C Rochette-Egly
11:40 - 11:55 > J Dieker
11:55 - 12:10 > P Radivojac

12:00 - 14:00 > Lunch

13:30 - 14:00 > Presentation by Abgent

pm.

2:00 - 2:40 > D Kirkpatrick
2:40 - 2:55 > L Waanders
2:55 - 3:35 > F. McLafferty
3:35 - 4:00 > B.G. Davis

4:00 - 4:35 > Coffee break

4:35 - 5:00 > D. Pappin
5:00 - 7:00 > **Poster Session II**

20:00 > Gala Dinner in Lille

01.09.06 / Friday

am.

9:00 - 9:40 > A Dell
9:40 - 10:20 > J Peter-Katalinic

10:20 - 11:00 > Coffee break

11:00 - 11:40 > N Callewaert
11:40 - 12:20 > N Taniguchi

12:00 - 14:00 > Lunch

pm.

2:00 - 2:40 > BM Olivera
2:40 - 3:20 > LW Ruddock
3:20 - 3:35 > S Serrano

3:35 - 4:20 > Coffee break

4:20 - 5:00 > C Locht
5:00 - 6:00 > **Closing lecture : T Pawson**

6:00 - 6:30 > Business/Announcements/Closing

02.09.06 / Saturday

Trip to Brughes (Belgium)

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16th meeting of Methods in Protein Structural Analysis

Oral communications

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Tuesday, the 29th of August – 06:00 pm

Opening lecture

Dr ALLIS C. David / alliscd@rockefeller.edu

Authors : Dr ALLIS C. David
Topic : Methylation / Acetylation

Content : C. David Allis, Chromatin Biochemistry and Epigenetics Chromatin, the repeating polymer of DNA and associated histone proteins, is the physiological template of our genome. As such, elaborate mechanisms have evolved to introduce meaningful variation into chromatin for purposes of altering gene expression and other important biological processes, including the repair of damaged DNA and chromosomal dynamics. Introduction of covalent histone modifications, chromatin remodeling by ATP-dependent complexes, and utilization of histone variants are three major mechanisms by which variation can be introduced into the chromatin fiber. Together, this variation might form a “histone code” that remains poorly understood. Two general types of mechanisms have been proposed to explain the function of covalent histone modifications: 1) “trans” mechanisms that involve the binding of effectors that engage specific covalent marks in a context-dependent fashion, and 2) “cis” mechanisms that involve structural alterations in the chromatin fiber by changing internucleosomal contacts. One long-range goal of our research program is to gain mechanistic insight into the function(s) of histone covalent modifications in cis and trans pathways. We favor the general view that histone proteins are major carriers of epigenetic information. The fundamental structure of chromatin suggests that all DNA-templated processes, including a wide range of epigenetic phenomena, are influenced by chromatin alterations with far-reaching implications for human biology and disease, notably cancer.

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Wednesday, the 30th of August – 9:00 am

Conformational regulation after phosphorylation: From atomic structure to human disease

Dr LU Kung Ping / klu@bidmc.harvard.edu

Authors : L. Pastorino, G. Wulf, F. Suizu, M. Balastik, G. Finn, J. Lim, T. H. Lee, X. Z. Zhou, L. Nicholson and K. P. Lu (Email : klu@bidmc.harvard.edu)

Topic : Phosphorylation

Content : Protein phosphorylation on certain serine or threonine residues preceding proline (Ser/Thr-Pro) is a central signaling mechanism in diverse cellular processes, and its deregulation can lead to human disease. Although these Ser/Thr phosphorylation events had long been proposed to function via induction of conformational changes, little was known about the nature of the conformational changes and whether they are regulated untile recently. We have recently identified a small new enzyme called Pin1 that not only binds to specific phosphorylated Ser/Thr-Pro motifs in certain proteins, but also greatly accelerates their cis/trans isomerization by over 1000 fold, thereby regulating protein structure between two distinct conformations, which can be directly visualized by NMR. Moreover, such conformational changes following phosphorylation can have profound effects on phosphorylation signaling by regulating a spectrum of target activities, including catalysis, protein dephosphorylation, protein interaction, subcellular location, and/or protein turnover. Therefore, Pin1-catalyzed phosphorylation dependent prolyl isomerization remains a unique mode for the modulation of signal transduction after phosphorylation.

We have demonstrated that this new post-phosphorylation regulatory mechanism is tightly regulated under physiological conditions via multiple mechanisms, including E2F mediated transcription regulation and protein phosphorylation. More importantly, deregulation of Pin1 plays a critical role in the pathogenesis of certain human diseases, notably cancer and Alzheimer's disease. In cancer, many oncogenes themselves are directly regulated by phosphorylation on Ser/Thr-Pro motifs and/or to trigger signaling pathways involving phosphorylation on these motifs. We have found that Pin1 is overexpressed in a wide range of human tumors and its overexpression correlates with poor clinical outcome in prostate cancer patients. Importantly, Pin1 functions as a novel catalyst essential for the activation of multiple oncogenic pathways, including Neu/Ras/c-Jun, Wnt/beta-catenin and cytokine/NF-kappaB. Consistent with this idea, Pin1 overexpression disrupts centrosome duplication checkpoint, inducing centrosome amplification, chromosome instability, cell transformation and tumorigenesis in vitro and in vivo. In contrast, Pin1 knockout mice are almost completely resistant to tumorigenesis induced by transgenic overexpression of certain oncogenes such as oncogenic Neu or Ras. Importantly, rapid inhibition of Pin1 in many different cancer cell lines via transient expression of antisense Pin1 RNA, RNAi or dominant-negative mutants triggers cancer cells to enter apoptosis, while long-term inhibition of Pin1 via stable expression suppresses cell transformation phenotype in vitro and tumor development in nude mice. These results indicate that Pin1 plays an essential role in oncogenesis and may be an attractive and novel molecular target for cancer diagnostics and therapeutics.

We have also demonstrated that Pin1 is pivotal for protecting against age-dependent neurodegeneration in Alzheimer's disease. Neuropathological hallmarks of Alzheimer's disease are neurofibrillary tangles composed of the microtubule-binding protein tau and neuritic plaques comprising amyloid-beta peptides (Abeta) derived from amyloid precursor protein (APP), but their exact relationship remains elusive. Increased phosphorylation of tau and APP on Ser/Thr-Pro motifs is an important feature of Alzheimer's disease. We have shown that by accelerating cis to trans isomerization of specific phosphorylated Thr-Pro motifs in tau and APP, Pin1 restores the conformation of phosphorylated tau and APP, preventing the formation of plaques and tangles. As a result, Pin1 deletion in the mouse is sufficient to cause age-dependent tau hyperphosphorylation,

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tau filament formation and neurodegeneration resembling Alzheimer's disease. Moreover, Pin1 knockout alone or in combination with amyloid precursor protein mutant overexpression in mice increases APP amyloidogenic processing and elevates levels of Abeta42 in an age-dependent manner, similarly to those caused by a familiar Alzheimer mutant presenilin 1 transgene. In addition, Pin1 expression inversely correlates with the predicted neuronal vulnerability and actual neurofibrillary degeneration in AD, and Pin1 activity is inhibited by oxidative modifications in Alzheimer's brains. These results indicate that Pin1-deficient mice are the only gene knockout mouse model displaying both tau and Abeta-related phenotypes associated with Alzheimer's disease and demonstrate that Pin1 may be an elusive link between plaque and tangle pathologies of Alzheimer's disease. Consistent with this idea are recent independent genetic findings that a new genetic locus associated with late-onset AD has been identified to be on the chromosome 19p13.2 where the Pin1 gene is located and that the Pin1 promoter polymorphisms are associated with reduced Pin1 levels and increased risk for late-onset AD in the certain population. These results suggest that the lack of sufficient Pin1 may allow phosphorylated tau and APP to be accumulated in the pathological cis conformations, which may promote tau hyperphosphorylation and tangle pathology as well as amyloidogenic APP processing and plaque pathology in AD, respectively. Therefore, phosphorylation-specific prolyl isomerization plays an important role in certain human diseases and is an attractive new drug target.

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Wednesday, the 30th of August – 9:40 am

Structural and enzymatic effects of SUMO modification of ubiquitin and SUMO E2-conjugating enzymes

Dr SIXMA Titia / t.sixma@nki.nl

Authors : Puck Knipscheer, Andrea Pichler[1], Pim van Dijk, Alex Fish, Jesper V. Olsen[2], Frauke Melchior[3] and Titia K. Sixma
Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, The Netherlands, [1]Max F. Perutz Laboratories, Medical University Vienna, Vienna, Austria, [2]Max Planck Institute for Biochemie, Martinsried, Germany, [3]Georg August University of Göttingen, Göttingen, Germany.

Topic : Structural Methods

Content : SUMO and ubiquitin conjugation processes have emerged as critical signalling systems that are essential for cell stability, by controlling e.g. protein degradation and signalling processes. Here we discuss the way in which SUMO itself can regulate both ubiquitin and SUMO pathways by modification of the E2 enzymes themselves.

We have shown specific SUMO modification of E2-25K and Ubc9. In both cases the SUMO modification affects the catalytic efficiency of the E2 enzyme. In the case of E2-25K, a ubiquitin E2 enzyme, the SUMO modification interferes with formation of thioesters, by interfering with E1 interaction. This results in an impaired ability to form poly-ubiquitin chains.

In the case of the SUMO E2, Ubc9, the effect of SUMO modification of the E2 is very different. We show that despite the fact that the modification is on the same site, Ubc9*SUMO has normal thioester formation and E1 interaction. Comparison of the crystal structures of the sumoylated E2 enzymes shows that the SUMO is oriented differently, due to the presence of a Ubc9 specific region, which could explain the functional differences.
SUMO modification of Ubc9 strongly affects the efficiency of sumoylation in a target specific manner. We could show that it impairs modification of RanGAP, has no effect on Hdac4 or E2-25K, but strongly activates the sumoylation of Sp100. We show that this activation against Sp100 is because the covalent SUMO on Ubc9 acts as an E3-ligase, by increasing target affinity through a specifically located Sumo-interaction motif on Sp100.

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Wednesday, the 30th of August – 11:00 am

Exploring the Keratinocyte SUMOeome

Dr WILSON Van / wilson@medicine.tamhsc.edu

Authors : Van G. Wilson, German Rosas-Acosta, and Adeline Deyrieux
Topic : Ubiquitine/SUMO

Content : Over the past few years, post translational modification with the Small Ubiquitin-Like Modifier (SUMO) has been shown to play an important role in viral infection. A number of viral proteins are known to be SUMO modified, and this modification influences viral reproduction. For papillomaviruses (PV), the E1 protein is SUMO modified, and sumoylation allows E1 to localize to the nucleus where viral replication takes place. Beyond this effect on E1, further roles for sumoylation in papillomaviral infection have not been explored. To investigate the contribution of the sumoylation system to the PV life cycle, we first examined sumoylation during normal differentiation of keratinocytes, the natural host for this virus. Our results indicate that the sumoylation system genes are upregulated at both the RNA and protein level in keratinocyte cells (HaCaT) undergoing calcium-induced differentiation. This upregulation was transient and was synchronous with the expression of the differentiation marker K1. In addition, the increase in the sumoylation components resulted in an overall increase in sumoylation of cellular proteins. 2-D gel analysis revealed that there were extensive and dynamic changes in the SUMOeome during keratinocyte differentiation. Identification and characterization of the major targets observed in the 2-D gels is in progress. In addition, to identify a broader range of SUMO substrates in keratinocytes by mass spectroscopy approaches, a HaCaT cell line inducibly expressing a TAP-tagged SUMO is being constructed. As proof-of-principle, 293 cell lines expressing either SUO1 or SUMO3 have already been developed and characterized by LC-MALDI-MS/MS analysis. This analysis identified 122 putative SUMO substrates, most of them previously not known to be sumoylated. Out of the 122, only 27 appeared to be modified by both SUMO-1 and SUMO-3, suggesting biologically distinct roles for modification by each SUMO type. To further evaluate the functional role of sumoylation in keratinocyte, sumoylation was down regulated using the Gam1 protein from avian adenovirus. Gam1 was previously shown to inhibit sumoylation through direct binding to the SUMO activating enzyme, SAE1/2, which enhances SAE1/2 degradation. Gam1 expression in HaCaT cells perturbed the normal cellular morphology and reduced cell-cell contacts. During calcium-induced differentiation, the presence of Gam1 also led to delayed expression of the K1 differentiation marker. These results suggest Gam1 is adversely affecting the normal process of keratinocyte differentiation through its action on the sumoylation system. This study supports the conclusion that SUMO orchestrates proper keratinocytes differentiation and therefore will be generally important for the HPV life cycle. Identification and functional characterization of specific SUMO substrates will be critical for understanding the regulation and control of keratinocyte differentiation.

Exploring the Molecular Function of PIN1 prolyl *cis*/*trans* isomerase by Nuclear Magnetic Resonance

Dr LANDRIEU Isabelle / Isabelle.Landrieu@univ-lille1.fr

Authors : Isabelle Landrieu, C. Smet, J.-M. Wieruszeski, and G. Lippens
Topic : Phosphorylation

Content : PIN1 participates in the regulation of a number of signalling pathways in the cell involving protein phosphorylation/dephosphorylation. Its role seems to be an essential control level in addition to the protein phosphorylation by proline-directed kinases. We investigate the molecular function of PIN1 at the atomic level based on data obtained by NMR. PIN1 specifically interacts with pThr/pSer-Pro motifs and is constituted by two domains: a WW N-terminal domain that binds pThr/pSer-Pro epitopes and a prolyl *cis*/*trans* isomerase C-terminal catalytic domain. An exception to this organisation is found in the plant PIN1 homologous enzymes, like PIN1At from *Arabidopsis thaliana*, constituted of the sole catalytic domain. The molecular function of PIN1, binding to and isomerization of pThr/pSer-Pro bonds, are thought to lead to several functional consequences. In a first mode of action, exemplified by its competition with the CKS protein, the interaction with PIN1 prevents interaction with other regulatory proteins. In a second mode of action, the largely accepted but never directly demonstrated idea is that the local isomerization modifies the global conformation of the protein substrate and hence it's intrinsic activity. Finally, isomerization catalysis is thought to regulate the (de)phosphorylation of specific pThr/pSer-Pro motifs.
Many PIN1 substrates are described as unstructured proteins or domains. Moreover, most of them can be phosphorylated on multiple sites and therefore present multiple anchorage sites for the PIN1 enzyme. Because of a lack of stable secondary or tertiary structure, these PIN1 substrates are not easily characterized by biological techniques. We have used NMR to study in detail the interaction between PIN1 and the Tau protein as model substrate. The Tau proteins bind the microtubules and help their polymerization and stabilization. This dynamic process is regulated by numerous kinases and phosphatases, the phosphorylation of Tau decreasing its capacity to polymerize the microtubules. PIN1 is thought to be involved in the development of the AD by regulating the function of phosphorylated Tau (Lu et al. Nature 1999; 2003).

Landrieu, I., Wieruszeski, J.-M., Wintjens, R., Inzé, D., Lippens, G. (**2002**) Solution structure of the single domain prolyl cis/trans isomerase PIN1At from *Arabidopsis thaliana J. Mol. Biol.* **320**, 321-332.
Smet, C., Sambo, A.V., Wieruszeski, J.M., Leroy, A., Landrieu, I., Buée, L., Lippens, G. (**2004**) The peptidyl prolyl cis/trans-isomerase Pin1 recognizes the phospho-Thr212-Pro213 site on Tau. *Biochemistry* **43**: 2032-2040.
Smet, C., Duckert, J.-F., Wieruszeski, J.-M., Landrieu, I., Buée, L., Lippens, G., et Déprez. B. (**2005**) Control of protein-protein interactions: structure based discovery of low molecular weight inhibitors of the interactions between Pin1 and phosphopeptides. *J. Med. Chem.* **48**: 4815-4823.
Smet, C., Wieruszeski, J.-M., Buée, L., Landrieu, I. and Lippens, G. (**2005**) Regulation of Pin1 peptidyl-prolyl *cis*/*trans* isomerase activity by its WW binding module on a multi-phosphorylated peptide of Tau protein *FEBS Lett.* **579**: 4159-4164.
Sillen, A., Wieruszeski, J.-M., Leroy, A., Ben Younes, A., Landrieu, I. and Lippens, G. (**2005**) High Resolution Magic Angle Spinning NMR of the neuronal Tau protein integrated in Alzheimer's like Paired Helical Fragments *J.Am.Chem.Soc.* **127**: 10138-10139.
Landrieu, I., Lacosse, L., Leroy, A., Wieruszeski, J.M., Trivelli, X., Sillen, A., Sibille, N., Schwalbe, H., Saxena, K., Langer, T., Lippens, G. (**2006**) NMR analysis of a Tau phosphorylation pattern. *J. Am. Chem. Soc.* **128**: 3575-3583.
Landrieu, I., Smet, C., Wieruszeski, J.-M., Sambo, A.-V., Wintjens, R., Buée L. and Lippens G. (**2006**) Exploring the molecular function of PIN1 by Nuclear Magnetic Resonance *Current Protein Peptide Science* **7**:179-194.

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Solid-Phase Strategy for Improved Phosphopeptide Detection and Phosphorylation Site Determination.

Dr NIKA Heinz / nheinz@aecom.yu.edu

Authors : H. Nika, R.H. Angeletti
Topic : Phosphorylation

Content : Reversible phosphorylation of serine and theronine residues in proteins is recogized as a critical event in modulation of cellular processes including cell signaling, cell cycle progression and differentiation. Existing approaches to map this important post-translational modification rely predominantly on the use of mass spectometry methods to identify and sequence the peptide of interest. However, these methods are challenged by the ionization inefficiency of the phosphopeptides and the limited information content of the MS/MS spectra due to the intrinsic lability of the phosphate group upon collisionally induced dissociation (CID). Immobilized metal ion affinity chromatography (IMAC) is a frequently reported technique to address these problems by phosphopeptide enrichment. Titanium oxide microcolumns have been recently employed as an alternative to IMAC to minimize variable peptide recovery but issues persist with regard to phosphate lability during tandem mass spectrometric analysis. Beta-elimination of phosphate coupled with Michael addition using barium hydroxide as elimination reagent and 2-aminoethanethiol as nucleophile has emerged as a viable chemical strategy to facilitate phosphorylation site determination. In this presentation, we adapted this chemistry to derivatization on C18 ZipTip pipette tips to benefit from the advantages of the combined approach, including ease of operation, effective analyte concentration, efficient in-situ solvent exchange and amenabilty to automation. Phosphoseryl and phosphothreonyl containing model peptides were used to optimize the reaction conditions to afford efficient conversion of both phosphoamino acids. The utility of the final protocol for improved phosphopeptide detection by signal enhancement was demonstrated with low level amonunts of protein in-gel digests. The reaction products proved highly suitable for CID and the resultant increased spectral information content greatly faciltitated mapping of the site(s) of phosphorylation.

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Wednesday, the 30th of August – 02:00 pm

Viewing the complexity and variation of phosphorylation in postsynaptic signaling network

Dr BURLINGAME Alma / alb@cgl.ucsf.edu

Authors : J. Trinidad(1), A. Thalhammer(2), C. Specht(2) and R. Schoepfer(2) and A. L. Burlingame(1)
Topic : Phosphorylation

Content : Recently mass spectrometry has made remarkable and pervasive inroads into macromolecular bioscience and these advances have provided the biomedical research community with a significantly more comprehensive view of the actual molecular players and landscape than was known previously. Indeed, mass spectrometry has demonstrated that it can provide the inherent analytical power and experimental versatility required to define the true composition and complexity of cells. However, there is still a long way to go to discover and decipher the complex machinery of human cells and master an understanding of the molecular workings essential for their homeostasis or the defects underlying states of dysfunction. In addition to protein expression per se, this complexity involves nature's use of a daunting array of dynamic epigenetic covalent processes that modulate protein functions and regulate their associations and activities in a host of protein machines and networks [1]. These cannot be deciphered from genomics per se. Protein phosphorylation is intimately involved in many cellular processes and is one posttranslational modification that we are investigating in the context of the pseudoorganelle, the postsynaptic density. This presentation will focus on what we have learned thus far in these investigations. We have reported some of our initial observations recently [2].

We acknowledge support of this work by NIH NCRR 01614.
1. C.T. Walsh, Posttranslational Modifications of Proteins:Expanding Nature's Inventory, 2006, Roberts and Company, Greenwood Village, CO, 80111.
2. J.C. Trinidad etal., 2006, Mol Cell Proteomics, 5, 914-922.

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Wednesday, the 30th of August – 02:40 pm

Modification specific proteomics: With emphasis on selective isolation of acidic peptides on titanium dioxide nanocolumns.

Pr ROEPSTORFF Peter / roe@bmb.sdu.dk

Authors : Peter Roepstorff, David Selby and Martin R.Larsen
Topic : Mass spectrometry

Content : The complexity of the proteome in terms of dynamic range and number of variants of given gene products due to alternative splicing and co- and post translational modification makes it impossible to cover the complete proteome of a cell or tissue type in a single experiment. To reduce the complexity, many proteomics studies are now performed on isolated organelles. However, the post translational modifications on a given protein may be extremely heterogeneous in terms of site as well as in terms the modifying group. Since the localization of a protein often depend on its modification state, methods are needed to determine the relationship between the modification state of a protein and its localization. We have in our laboratory developed a concept, which we term modification specific proteomics that allows specific assignment of a given type of modification in proteomics studies. By combining this with organelle specific proteomics, it should be possible to determine which modification state of a protein determines its specific localization in the cell. The methods developed in our laboratory for modification specific proteomics and applications to organelle specific proteomics, e.g. membranes, will be described. Specific emphasis will be given to the selective isolation of acidic peptides, e.g. phosphorylated and sialic acid containing glycosylated peptides, using titanium dioxide nanocolumns.

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Wednesday, the 30th of August – 03:20 pm

Peptide-centric tools for studying protein processing and less drastic modifications

Pr GEVAERT Kris / kris.gevaert@ugent.be

Authors: Kris Gevaert, Petra Van Damme, Francis Impens, Bart Ghesquière, Xavier Hanoulle, Lennart Martens, Jozef Van Damme, An Staes, Evy Timmerman and Joël Vandekerckhove
Department of Biochemistry and Medical Protein Research, Faculty of Medicine and Health Sciences, Ghent University and Flanders Interuniversity Institute for Biotechnology, A. Baertsoenkaai 3, B9000 Ghent, Belgium
Topic: Mass spectrometry

Content: Gel-free or peptide-centric proteome analytical techniques aim at compensating inherent drawbacks of 2D-PAGE-centered proteome analyses by characterizing the generally more soluble peptides obtained by digesting an isolated proteome. One obstacle of peptide-centric proteomics is the huge number of peptides requiring MS/MS analysis. As the capacity of contemporary mass spectrometers is inadequate, too many peptides are not identified in a direct analysis. However, two different approaches are applied to compensate for this: orthogonal (IEC and RP) chromatographic separations split the peptide mixture in as many analyzable components as possible prior to MS/MS or specific peptides are affinity-isolated and serve as signatures for their corresponding proteins. As the latter technique reduces the number of analytes, it is generally conceived that a higher part of a given proteome is finally identified.
Based upon diagonal electrophoresis/chromatography techniques described in the 1960's, our lab has recently developed a central technology termed combined fractional diagonal chromatography (COFRADIC) that isolates predefined sets of peptides for further characterization by automated LC-MS/MS. Major differences with alike techniques is that COFRADIC does not rely on affinity anchors and by simply changing the sorting chemistry different sets of peptides are isolated. Routine procedures for isolating methionyl, cysteinyl and amino terminal peptides are available and combined with differential stable isotope tagging allow in-depth analysis of proteomes and their fluxes. Other, more recently developed procedures focus on posttranslational modifications including phosphorylation, N-glycosylation and nitration of tyrosines.
One example of a COFRADIC analysis that will be discussed is a detailed, proteome-wide degradomic view on apoptotic Jurkat T-lymphocytes. This will illustrate the unique characteristic of N-terminal COFRADIC as it is the sole protein technology that identifies protease substrates and delineates the exact processing sites simultaneously in a large, unchanged in vivo proteomic background. Another example deals with the in vitro delineation of granzyme A and granzyme B substrates in freeze-thaw cell lysates. We will furthermore illustrate the versatility of COFRADIC by discussing and exemplifying ways to isolate peptides containing N-glycosylated asparagines, phosphorylated peptides, peptides containing 3-nitrotyrosine residues and peptides encompassing ATP-binding sites in proteins.

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Wednesday, the 30th of August – 04:20 pm

Mechanism and Consequences of Protein Palmitoylation

Dr LINDER Maurine / mlinder@wustl.edu

Authors: Maurine E. Linder
Topic: Other

Content: Protein S-palmitoylation is the thioester linkage of long chain fatty acids to cysteine residues. Addition of palmitate to proteins facilitates their membrane interactions and regulates protein trafficking and stability. The reversibility of palmitoylation makes it an attractive mechanism for regulating protein localization and activity. Our work has focused on the mechanism of palmitate addition to proteins, which is mediated by protein acyltransferases or PATs. Yeast genetics revealed the first candidates for PATs and biochemical confirmation of their activity followed. The PATs discovered in yeast shared a common protein domain, the DHHC-CRD (Asp-His-His-Cys-cysteine rich domain), which is required for palmitoyltransferase activity. A family of seven DHHC proteins is present in yeast; a much larger family with at least 23 members is present in mammals. We hypothesize that multiple PATs exist in order to palmitoylate different classes of substrate proteins in different subcellular compartments. In an effort to define the rules that govern the biological activity of DHHC proteins, we are studying the localization and substrate specificity of DHHC proteins. In yeast, we found that certain DHHC proteins localize to the endoplasmic reticulum or Golgi, whereas others localize to the vacuole or plasma membrane. For example, we found that a DHHC protein we named Pfa3 (protein fatty acyltransferase 3) is a PAT for Vac8, an N-myristoylated, palmitoylated protein required for vacuole fusion. Pfa3 is localized specifically on the vacuole membrane and targets Vac8 to this compartment through its PAT activity. Likewise, in mammalian cells we identified a human PAT comprised of DHHC9 and a Golgi complex protein GCP16 that utilizes a different type of substrate--Ras, a C-terminally farnesylated, palmitoylated protein. We found that DHHC9 and GCP16 codistribute in the Golgi apparatus, a location consistent with the site of mammalian Ras palmitoylation in vivo. We are currently investigating the determinants that allow a PAT to recognize a specific substrate and whether a single PAT recognizes multiple classes of substrates.

Wednesday, the 30th of August – 05:00 pm

Tyrosine phosphorylation in bacteria

Dr GRANGEASSE Christophe / c.grangeasse@ibcp.fr

Authors : Christophe Grangeasse and Alain Cozzone
Topic : Phosphorylation

Content : Phosphorylation-dephosphorylation at tyrosine has long been considered a protein modification restricted to eukaryotes but it is now well established that it participates also in the regulation of bacterial physiology. Of special interest is the recent finding that a number of protein tyrosine kinases from various bacterial species share no sequence homology with their eukaryotic counterparts even though they catalyze the same overall reaction. In addition, the reversibility of the reaction is effected by two main particular classes of protein-tyrosine phosphatases : one includes acidic phosphatases of low molecular weight and the other comprises enzymes of the polymerase-histidinol phosphatase type. These phosphorylating/dephosphorylating enzymes are involved in a diversity of biochemical pathways including antibiotic resistance, carbohydrate production, stress resistance as well as pathogenicity. Recently, a new concept has emerged suggesting the existence of a biological link between protein-tyrosine phosphorylation and bacterial pathogenicity. Therefore, the structural and functional specificity of bacterial tyrosine kinases and phosphatases, compared to eukaryotic kinases, allows to envisage the existence of specific inhibitors that would control the physiology of bacteria.

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Thursday, the 31st of August – 09:00 am

Chromatin and Beyond: Detecting Post-translational Switches in the Human Nucleus by Next-Generation Mass Spectrometry

Dr KELLEHER Neil / kelleher@scs.uiuc.edu

Author: Neil L. Kelleher
Topic: Mass spectrometry

Content: Human cells have evolved a complex language to facilitate cell-cell communications, perform signal transduction, and regulate biophysical access to the genetic code. The prototypical systems that embody this line of thinking are human histones. Chromatin researchers such as Allis, Jenuwein, Grunstein and others have provided many recent articulations of the “Histone Code” hypothesis. Through certain combinations of modifications such as methylation, phosphorylation, and acetylation, central processes such as genome packaging/duplication, gene transcription, and epigenetic information transfer are somehow regulated. Our group’s recent technological advancements are perhaps best understood in the context of combining histone biology, Top Down Mass Spectrometry (TDMS), and computer science. To the extent that cellular logic is written in the language of post-translational modifications, TDMS is well-positioned to read out combinations of PTMs – a key ability to better understand chromatin biology and human diseases at the molecular level. We have established a “basis set” of expressed histone forms for each of the five histones in human cells, with data from TDMS of yeast histones also to be presented.

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Thursday, the 31st of August – 09:40 am

Identification of Intact Proteins, Large Peptides, and Their Post-translational Modifications on a Chromatographic Time-Scale by using a combination of Ion/Ion Chemistry and Tandem Mass Spectrometry.

Dr HUNT Donald F. / dfh@virginia.edu

Author : Donald F. Hunt, Departments of Chemistry and Pathology, University of Virginia, Charlottesville, Virginia 22901
Topic : Mass spectrometry

Content : For the direct analysis of proteins on a chromatographic time scale (1 protein/2-5 sec), we use C4 and C8, nanoflow-HPLC columns, a modified linear ion trap mass spectrometer, and sequential ion/ion reactions to both fragment the intact protein and reduce the charge on the resulting multiply charged fragment ions.1,2 Proteins are converted to gas-phase, positive ions by electrospray ionization and then allowed to react with fluoranthene radical anions. Electron transfer to the multiply charged protein promotes random fragmentation of amide bonds along the protein backbone. Multiply charged fragment ions are then de-protonated in a second ion/ion reaction with the carboxylate anion of benzoic acid. The m/z values for the resulting singly, doubly, and triply charged ions are used to read a sequence of 15-60 amino acids at both the N and C termini of the protein. This information, along with the measured mass of the intact protein, is used to identify unknown proteins, to confirm the amino acid sequence of a known protein, to detect posttranslational modifications, and to determine the presence of possible splice variants. For the comparative analysis of chemically or post-translationally modified proteins, two samples are digested proteolytically and the resulting peptides from each are then converted to d0- and d3-methyl esters, respectively. The two samples are then mixed together and analyzed by nanoflow-HPLC interfaced to electrospray ionization on tandem linear ion trap-Fourier transform (LTQ-FTMS) or tandem linear ion trap-orbitrap instruments. These instruments operate at resolutions in excess of 40,000, measure masses to three decimal places, and record the molecular masses of peptides in each sample at the high attomole level.3 For the analysis of phosphorylated proteins, immobilized metal affinity chromatography (IMAC) is employed to enrich the sample for phosphopeptides prior to analysis by nanoflow HPLC.4 The above approaches will be illustrated with examples from research involving the comparative analysis of: (a) phosphorylation on proteins involved in cell migration, (b) post-translational modifications on histones that control gene expression, gene silencing, DNA damage repair, recombination, etc, and (c) phosphopeptides presented to the immune system on cancer cells that might be used as potential cancer vaccines or therapeutics.

1. Peptide and Protein Sequence Analysis by Electron Transfer Dissociation Mass Spectrometry, Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF, Proc Natl Acad Sci USA 2004;101:9528-9533.
2. Protein Identification Using Sequential Ion/Ion Reactions and Tandem Mass Spectrometry, J.J. Coon, B. Ueberheide, Syka JEP, Dryhurst DD, Ausio J, Shabanowitz J, Hunt DF. Proc Natl Acad Sci USA 2005;102:9463-9468.
3. Novel Linear Quadrupole Ion Trap/FT Mass Spectrometer: Performance Characterization and Use in the Comparative Analysis of Histone H3 Post-Translational Modifications, Syka JEP, Marto JA, Bai DL, Hornung S, Senko MW, Schwartz JC, Ueberheide BM, Garcia BA, Busby SA, Muratore T, Shabanowitz J, and Hunt DF, J Proteome Res 2004;3:621-626.
4.Methods for the Detection of Paxillin Post-Translational Modifications and Interacting Proteins by Mass Spectrometry, Schroeder MJ, Webb DJ, Shabanowitz J, Horwitz AF, Hunt DF. J Proteome Res 2005;4:1832-1841.
5. Dynamic Regulation of HP1/Chromatin Interaction by Methylation and Phosphorylation of Histone H3. Fischle W, Tseng BS, Dormann H, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD. Nature 2005;438, 1116-1122.

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Thursday, the 31st of August – 11:00 am

How phosphorylation controls the activity of nuclear retinoic acid receptors.

Dr ROCHETTE-EGLY Cécile / cegly@igbmc.u-strasbg.fr

Authors : Gaillard E, Bour G, Bruck N and Rochette-Egly C Department of Cell Biology and Signal Transduction. Institut de Génétique et de Biologie Moléculaire et Cellulaire, BP 10142, 67404 Illkirch Cedex, France
Topic : Phosphorylation

Content: Nuclear retinoic acid receptors (RAR) work as ligand-dependent heterodimeric RAR/RXR transcription activators which are targets for phosphorylations. The N-terminal AF-1 domain of RAR? Is phosphorylated by the cdk7/cyclin H complex of the general transcription factor TFIIH and the C-terminal AF-2 domain by the cyclic AMP-dependent protein kinase PKA. Here we report the identification of a molecular pathway by which phosphorylation by PKA propagates cyclic AMP signaling from the AF-2 to the AF-1 domain. The first step is the phosphorylation of S369 located in Loop 9-10 of the AF-2 domain. This signal is transferred to the cyclin H binding domain (N-terminal tip of Helix 9 and Loop 8-9) resulting into enhanced cyclin H interaction and thereby into greater amounts of RAR? phosphorylated at S77 located in the AF-1 domain, by the cdk7/cyclin H complex. This molecular mechanism relies upon the integrity of the LBD and of the cyclin H binding surface. Finally it results in higher DNA binding efficiency and enhanced transcriptional response. We propose a model in which phosphorylation coordinates and fine tunes in cooperation with the ligand the RA response of RAR-target genes

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Thursday, the 31st of August – 11:40 am

Post-translational modifications on lupus autoantigens during apoptosis

Dr DIEKER Jürgen / j.dieker@ibmc.u-strasbg.fr

Authors : Jürgen Dieker, Fanny Monneaux, Johan van der Vlag, Sylviane Muller
Topic : Functional analysis

Content : Autoimmune diseases affect millions of people around the world; however, the underlying mechanisms leading to each type of these diseases are unclear. The break of B- and T-cell tolerance, which leads to the production of autoantibodies directed to self-structures, is a common feature of these diseases. Systemic lupus erythematosus (SLE) is the prototype of a systemic autoimmune disease, which is associated with the production of many different autoantibodies, some of which appear to have pathogenic consequences. However, its etiology is largely unknown. Autoantibodies in SLE patients target almost 100 different, mostly nuclear proteins. The main targets are nucleosomal (i.e. nucleosomes, histones, dsDNA) and spliceosomal proteins (i.e. U1-70K, U1A, and U1C snRNPs, Sm RNPs, SSA/Ro and SSB/La proteins). The reason why particularly these proteins become autoantigens during SLE has remained for most part an unanswered question. An important observation in the early 90s was that many of these autoantigens are clustered in apoptotic blebs which appear on the surface of late apoptotic cells. Additional evidence has shown that nucleosomal and spliceosomal autoantigens are exposed on the outside of apoptotic blebs and are available for the binding of autoantibodies. Apoptosis, or programmed cell death, is an essential mechanism to remove cells without exposing their contents to the immune system. Caspases play an essential role in the execution of the apoptotic process by cleaving many proteins involved in cellular processes, such as transcription, splicing and translation. In addition to cleavage by caspases many proteins undergo post/translational modifications during apoptosis. All these apoptosis-induced protein changes ensure a fast and regulated breakdown of the most important cellular processes. Normally, apoptotic cells are removed in a very early stage by phagocytosing cells, especially macrophages. The fast removal of apoptotic cells by macrophages prevents activation of the immune system. Indeed, studies involving mice deficient for molecules involved in the removal of apoptotic cells have shown that a disturbed removal results in the development of autoimmunity with lupus-like symptoms. In both lupus mice and patients it has been found that the removal of apoptotic cells is impaired. In addition, abnormal apoptosis, such as in the Fas-deficient MRL/lpr mice, has been shown to cause lupus. Apoptotic material, such as nucleosomes, has been detected in plasmas from SLE patients and lupus mice. In addition, immunization of normal mice with apoptotic cells leads to the development of autoimmunity against structures also recognized by lupus autoantibodies. Therefore, it has been proposed that proteins released from apoptotic cells might play an important role in the induction of lupus-like autoimmunity. Cleavage or post-translational modifications of these proteins might enhance their immunogenicity and explain why these proteins are recognized by the immune system. Remarkably, many proteins targeted by lupus autoantibodies are cleaved by caspase-3 and/or Granzyme B during apoptosis. The cleaved protein is also a preferred target for lupus autoantibodies in most cases. In addition, many post-translational modifications have been described on these proteins. These modifications normally play a regulatory role in function of the protein in the cell. Additionally, post-translational modifications on these proteins have also been described during apoptosis. So far (de)phosphorylation, (de)acetylation, methylation, (de)ubiquitination, citrullination, ADP-ribosylation and transglutamination of potential autoantigens have been described during apoptosis. Although, the possibility that these modified proteins might become autoantigens during an impaired removal of apoptotic cells has been very often put forward, experimental data supporting this idea is scarce. The exact nature of post-translational modifications occurring during apoptosis has not been clarified for most lupus autoantigens. Moreover, the nature of the post-translational

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modifications recognized by lupus autoantibodies remains unclear. However, a preferential recognition of phosphorylated proteins by lupus autoantibodies has been shown. Moreover, contradictory results have been found for the spliceosomal SR proteins for which both dephosphorylation and phosphorylation during apoptosis has been shown, which in both cases was recognized by lupus autoantibodies. Recently, several new reports by us and others have shown that nucleosomal and spliceosomal proteins undergo specific post-translational modifications during apoptosis, which can become a target of autoreactive T and B cells during lupus. Therefore, it is expected that future research, using advanced techniques such as mass spectrometry, will provide a better insight into the exact nature of the autoantigens involved in the development of SLE.

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Thursday, the 31st of August – 11:55 am

Prediction of PTM sites and the utility of PTM predictors in mass spectrometry experiments

Dr **RADIVOJAC** Predrag **Email :** predrag@indiana.edu

Authors : Predrag Radivojac, Aaron Buechlein, Wyatt Clark, Ross Cocklin, Kenneth Daily, Keith Dunker, Mark Goebl, Lilia Iakoucheva, Amrita Mohan
Topic : Other

Content : Protein post-translational modifications (PTMs) play an important regulatory role in numerous protein pathways and interactions offering a great functional diversity to the primary structure of a protein and effectively increasing the organism's complexity given its genome. It has been hypothesized that modifications to proteins occur in regions that are easily accessible, and many have been determined to be located within intrinsically disordered regions. However, identifying precise locations of protein modifications still involves expensive and time consuming laboratory work. Thus, automated identification of these sites is important. Here we present our methods for mining protein PTM sites from the literature, building automated models prediction of PTM sites, and finally applying these predictors to achieve fast and improved identification of new modification sites from the mass spectrometry experiments. First, we use semi-automated literature search of the full scientific papers to extract new PTM sites. We develop simple algorithms for ranking the papers according to their probability to contain experimentally verified modification sites. Second, we use newly found experimental sites together with the ones available in various databases to construct predictors of PTM sites from amino acid sequence only. Currently, we constructed models for protein phosphorylation, methylation, and ubiquitination and show that all have useful prediction accuracy, between 63% (lysine methylation) and 81% (serine phosphorylation). The utility of predictors has been demonstrated in an example of ubiquitination, where the model has been used to screen the yeast proteome for most likely ubiquitination sites. Using several whole cell lysate samples from yeast and only about 1% of top predictions for ubiquitination sites, we identified more than 200 unique ubiquitination sites using SEQUEST, of which 25 were with confidence >99%. We propose that a combination of bioinformatics approaches and proteomics searches can result in a discovery of a large number of post-translational modification sites from the existing data that may not be identified using standard approaches (due to the exponentially increased number of background peptides) and in a significantly reduced amount of time typically needed for differential searches. In addition, our methods can be helpful in increasing the numbers of assigned peptide spectra in standard shotgun proteomics experiments which are typically below 30%.

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Thursday, the 31st of August – 14:00 pm

Direct Analysis of Ubiquitin Chain Linkages Regulating Proteasomal Degradation and Proteasome Independent Signaling

Dr KIRKPATRICK Donald / donald_kirkpatrick@hms.harvard.edu

Authors : Donald S. Kirkpatrick, Nathaniel A. Hathaway, Fangtian Huang, John Hanna, Daniel Finley, Alexander Sorkin, Randall W. King & Steven P. Gygi
Topic : Mass spectrometry

Content : Post-translational modification of protein substrates by ubiquitin regulates both proteasomal degradation and proteasome-independent signaling processes. To discriminate between these downstream effects, cells have the capacity to generate a diverse array of unique ubiquitin signals. Mass spectrometry based methods have made it possible to characterize the key structural features of these ubiquitin signals in a substrate specific manner. Typically, samples enriched in ubiquitinated proteins are digested with trypsin and separated by reversed phase HPLC prior to analysis in a mass spectrometer. Digestion of a ubiquitinated protein yields a series of peptides from both ubiquitin and the substrate, including specific –GG signature peptides. These –GG signature peptides can be used to identify ubiquitin modified lysine residues on the substrate. Similarly, -GG signature peptides derived from ubiquitin itself denote the presence of specific ubiquitin-ubiquitin linkages. Using standard shotgun sequencing methods, we have recently identified the major ubiquitination sites on both cyclin B1 and the EGF receptor, two canonical ubiquitin substrates. Previous results have shown that whereas ubiquitination of cyclin B1 targets it for rapid destruction by the proteasome, EGF receptor ubiquitination traffics the protein to the lysosome for proteasome-independent degradation. In each case, ubiquitinated lysines were found to be clustered within a single region of the protein; the N-terminal domain of cyclin B1, or the kinase domain of the EGF receptor. Using the recently developed Ubiquitin-AQUA method, we then examined the forms of ubiquitin conjugated to each substrate. Ubiquitin-AQUA utilizes isotope labeled internal standard peptides and selected reaction monitoring to quantify tryptic peptides of ubiquitin and the substrate. Ubiquitin-AQUA results demonstrated that although it is a classical proteasome substrate, cyclin B1 is modified by poly-ubiquitin chains linked through multiple lysine residues (Lys-11, Lys-48, and Lys-63), rather than simply through Lys-48. Alternatively, ubiquitination of the EGF receptor was found to occur primarily through Lys-63 and mono-ubiquitin. For cyclin B1, we performed a comprehensive kinetic analysis of the ubiquitination process. Assembly of the degradation signal was found to occur in multiple stages, wherein the first stage was characterized by predominantly mono-ubiquitination of distinct lysines on the substrate, and the second stage by the assembly of short poly-ubiquitin chains. Despite the short chains and complex linkage topology, ubiquitinated cyclin B1 was recognized by various ubiquitin receptors and degraded by purified proteasomes. Furthermore, Lys 48-linked poly-ubiquitin chains were found to be non-essential for either ubiquitin receptor binding or proteasomal degradation. Together, these results expand our understanding of both proteasomal and lysosomal degradation signals, while providing unique insights into the mechanisms of substrate ubiquitination.

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Thursday, the 31st of August – 14:40 pm

Analysis of post-translational modifications of Murine Myelin Basic Protein on the LTQ-FT and LTQ-Orbitrap mass spectrometers

Dr WAANDERS Leonie F. / waanders@biochem.mpg.de

Authors : Leonie Waanders, Boris Macek, Jesper V. Olsen, Reinout Raijmakers, Ger Pruijn, Matthias Mann
Topic : Mass spectrometry

Content : Multiple sclerosis (MS) is one of the most important neurological diseases of young adults in the western hemisphere. Its etiology is still very elusive, mainly due to the complexity of the disease and the lack of brain and spinal cord tissue for research purposes. To gain knowledge about the cause and pathology of MS, different animal models have been developed. One of the most widely used is experimental autoimmune encephalomyelitis (EAE). In this study we characterized the post-translational modifications (PTMs) of myelin basic protein (MBP) of normal mice and mice affected by EAE by top-down and bottom-up mass spectrometric analysis. MBP's is a major component making up the insulating myelin sheat surrounding axons and it is the most-studied protein involved in the pathology of MS: in MBP of MS-patients hypercitrullination, hypermethylation and hypophosphorylation have been described. Especially the arginine-deiminating modification 'citrullination' may be important, since the level of citrullination has been correlated to disease progression. MBP was extracted from brain and spinal cord tissue and the various splice and differentially modified isoforms were separated by strong cation exchange chromatography and gel electrophoresis. Tandem mass spectrometry using the LTQ-FT and LTQ-Orbitrap were used to characterize the PTM types and sites. Western blots provided biochemical support of the mass spectrometric findings. No in-depth characterization has been performed on murine MBP before. We detected many different types of post-translational modifications (PTMs), including phosphorylation, deamidation, acylation, methylation and citrullination, but focused on the types that are thought to be most important in multiple sclerosis: phosphorylation, methylation and citrullination. We confirmed many expected sites (previously only reported as 'by similarity' in databases like SWISSPROT) and identified a series of new modification sites with very high confidence. When comparing MBP from healthy and EAE mice, we observed similar tendencies as described for human MBP. These results provide further support for EAE as a disease model to study the pathogenesis of MS on a molecular level, as subject of considerable ongoing controversy. They also indicate that the pattern of modification may be more complex than previously thought. This analysis also offered the possibility to look closely at the characteristics of two modern and high performance mass spectrometers, the hybrid linear quadrupole ion trap Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FT, Thermo Electron Corp., Bremen, Germany) and the linear quadrupole ion trap orbitrap mass spectrometer (LTQ-Orbitrap, Thermo Electron Corp.) in the analysis of PTMs. The LTQ-FT has been commercially available for a few years and has proven to be very useful for PTM studies, mainly thanks to the excellent mass accuracy and high resolving power in the ICR cell. For validation of most of the PTMs it is sufficient to measure very accurate precursor mass value in the FT mass analyzer and to detect the fragment ions in the ion trap with relatively low resolution and accuracy but very high sensitivity. However, the LTQ-FT is less suitable for high performance mass spectrometry in the MS/MS stage, mainly due to low sensitivity and time-of flight effects during transfer of fragment ions into the ICR cell. As we show here, the latter is readily possible in the novel LTQ-Orbitrap, which features more than ten fold higher sensitivity for MS/MS measurements. In the orbitrap ion packages circle between two concentric electrodes, where their axial motion is detected as a frequency and - as in the FT-ICR instrument – converted into mass spectra by Fourier transformation. Features like mass accuracy and

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resolution are therefore comparable, but thanks to better transmission, the LTQ-orbitrap is more sensitive than the LTQ-FT, and allows for acquisition of MSn spectra in the orbitrap. For modifications like citrullination (with a mass difference of 0.98402 Da) the detection of full and fragmentation spectra in the orbitrap simplified the identification and increased the confidence of the modification site assignment. Since the LTQ-orbitrap requires no magnetic field or special maintenance it may also be more attractive to use for less specialized laboratories. Also for top-down analysis the orbitrap outperforms the LTQ-FT. Top-down mass spectrometry, the analysis of intact proteins (without digesting them first into peptides), makes it possible to evaluate the heterogeneity of proteins and study combinatorial effects of PTMs. As we have shown recently for proteins of 10-25 kDa, the orbitrap mass spectrometer has enough resolving power (set to 60,000 at m/z 400) to reveal the isotopes of all protein charge states given total amounts of 50 to 250 fmole, and it was capable of detecting all proteins with mass accuracies of 0.9 to 2.8 ppm. Thanks to the fast filling and scanning time it is also possible to measure the proteins in an on-line setup with a HPLC prior to the mass spectrometer. The LTQ-Orbitrap allows for routine, high accuracy and high confidence protein characterization and opens the door for 'top-down proteomics' of small proteins.

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Thursday, the 31st of August – 14:55 pm

Extending Top Down Mass Spectrometry from <50 kDa to >200kDaProteins

Pr McLafferty Fred W / fwm5@cornell.edu

Authors : Fred W. McLafferty,[1*] Xuemei Han,[1] Mi Jin,[1] and Kathrin Breuker[2]

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Topic : Mass spectrometry

For characterization of sequence and posttranslational modifications, molecular and fragment ion mass data from ionizing and dissociating a protein in the mass spectrometer are far more specific than such data of peptides from the protein's digestion. We now extend the ~50 kDa dissociation limitation of the top down methodology by using electrospray additives, heated vaporization, and separate noncovalent and covalent bond dissociation. This cleaves 287 interresidue bonds in the termini of a 144 kDa protein, assigns presence/absence of disulfide bonding at 21 cysteine residues in a 200 kDa protein, and corrects sequence predictions in 144 and 229 kDa proteins.

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Thursday, the 31st of August – 15:35 pm

Exploring and Exploiting Chemical Protein Glycosylation

Pr DAVIS Benjamin / Ben.Davis@chem.ox.ac.uk

Authors : Benjamin G. Davis
Topic : Glycosylation

Content : Sugars are critical biological markers that modulate the properties of proteins and may be an important molecular origin of biological complexity. Our work studies the interplay of proteins and sugars. This lecture will discuss recent developments our laboratory in the area of Glycoprotein & Glycoconjugate synthesis: Precisely glycosylated enzymes & proteins can be constructed through highly selective synthetic chemistry and used in • preparative bio-catalysis • drug delivery • selective protein degradation • in vivo sensing. New classes of glycoconjugate, glycodendriprotein and glycoviruses act as powerful nanomolar inhibitors of bacterial interactions or gene delivery vehicles, respectively. Antioxidant glycopolymers enhance cellular lifetimes and enhance function.

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MPSA 2006 chairman

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Thursday, the 31st of August – 16:35 pm

Title not communicated

Dr Pappin Derryl / Applied Biosystems

Authors : Derryl Pappin
Topic : Mass spectrometry

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Friday, the 1st of September – 09:00 am

Mass spectrometric strategies for glycomics and glyco-proteomics

Pr DELL Anne / a.dell@imperial.ac.uk

Authors : Anne Dell, Mark Sutton-Smith, Maria Panico, Sara Chalabi, Nyet-Kui Wong, Paul Hitchen, Jihye Jang-Lee, Simon North, Victoria Ledger, Simon Parry, Stuart Haslam and Howard Morris
Topic : Glycosylation

Content : Imperial College of Science, Technology and Medicine, Division of Molecular Biosciences, Faculty of Natural Sciences, London SW7 2AZ

Ultra-high sensitivity mass spectrometric (MS) strategies for defining the primary structures of highly complex mixtures of glycoproteins are revolutionising structural glycobiology in the post-genomic era. MS strategies incorporating MALDI-MS and nano-electrospray(ES)-MS/MS enable very complex mixtures from biological extracts and/or glycopolymer digests to be screened thereby revealing the types of glycans present and, importantly, providing clues to structures that are likely to be functionally important. We have devised MS strategies which enable the glycome of cells, tissues and organs to be examined and the glycoforms of individual glycoproteins to be identified. Thus, we are employing MALDI mapping and MALDI or ES MS/MS sequencing of permethylated N-and O-glycans in order to define the glycomes of individual glycoproteins, cells, tissues and organs. Our strategies will be illustrated by data from collaborative research aimed at defining the glycosylation repertoire of model organisms and establishing the roles of glycans in cell-cell communication. The methodologies which we have developed have been adopted by the NIH Consortium for Functional Glycomics whose Analytical Core is carrying out high throughput analyses of mice and human tissues in order to provide a glycomics data resource for the glycobiology community. In our glyco-proteomics studies we largely exploit nanospray and on-line nanoLC-ES-MS/MS technology. These methodologies have yielded important new information on the O-glycosylation of zona pellucida glycoproteins from normal and transgenic mice, and the N-glycosylation of a variety of novel bacterial glycoproteins. Data from some of these studies will be described. In collaboration with David Goldberg at the Palo Alto Research Centre in Stanford, algorithms are being developed for the automatic interpretation and annotation of glycomics and glycoproteomics data. Progress in this area will be described. This work is supported by the BBSRC, the Wellcome Trust and the NIH.

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Friday, the 1st of September – 09:40 am

Exploring complexity of *O*-glycosylation

Dr J. Peter-Katalinić / jkp@uni-muenster.de

Authors : J. Peter-Katalinić
Topic : Glycosylation

Content : Cell surface and extracellular proteins are *O*-glycosylated, where the most abundant type of *O*-glycosylation in proteins is the GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an α glycosidic linkage. Most eukaryotic nuclear and cytoplasmic proteins modified by a β-linked *O*-GlcNAc to Ser or Thr exhibit reciprocal *O*-GlcNAc glycosylation and phosphorylation during the cell cycle, cell stimulation and/or cell growth. *O*-fucosylation, *O*-mannosylation and *O*-glucosylation belong to less investigated types of *O*-glycosylation, but functionally of high relevance for early stages of development and for vital physiological functions of proteins [1,2]. Glycosaminoglycans are β-linked to proteoglycans via a xylose-containing tetrasaccharide, represented by linear chains of repetitive disaccharides modified by carboxylates and *O*- or/and *N*-linked sulfates.
In the context of novel technologies and strategies related to the high-throughput glycomics, automatization and miniaturization of analytical devices to gain sensitivity, speed of data acquisition and enhance data interpretation are required. Identification of glycoconjugate structure calls for efficient analytical methods similar to those for identification of proteins, like separation by 1- and 2D-electrophoresis or HPLC, accompaniied by determination of the size of the macromolecule, building block analysis and their sequence by mass spectrometry (MS). However, additional structural parameters of oligomeric glycoconjugates relevant for their biological interaction specificity in receptor-ligand recognition, cell transport and cell-cell interactions include determination of patterns of branching, site of glycosidic bond attachments, stereochemistry at the anomeric centre and the aglycone.
For mapping and sequencing of complex glycomixtures in glycomics on-line capillary electrophoresis and automated chip-based sample admission to MS analyzers were shown to be highly efficient for different type of *O*-glycan identification by mapping and sequencing in a data dependent acquisition [3-6]. High mass resolution and accuracy can be achieved by Fourier Transform Ion Cyclotron Resonance (FT-ICR) MS at 9.4 T along with the possibility to apply distinct mechanisms of fragmentation [7-9]. A computational method was developed for interpretation of the composition, based on assignment of monosaccharide unit building blocks in oligomers and their sequences obtained by tandem MS experiments. This concept was verified on highly complex mixtures of glycopeptides and oligosaccharides obtained from urine of patients suffering from congenital disorders of glycosylation (CDG) and Schindler's disease, both hereditary diseases showing severe clinical symptoms [8,9].
References
1. J. Peter-Katalinić, Methods Enzymology ***405*** (2005) 139-171. Gonzalez de Peredo, A., D. Klein, B. Maček, D. Hess, J. Peter-Katalinić, J. Hofsteenge, Mol. Cell. Proteomics ***1*** (2002) 11-18. 2. Zamfir, A., N. Lion, Ž. Vukelić, L. Bindila, J. S. Rossier, H. H. Girault, J. Peter-Katalinić, Lab on the Chip ***5*** (2005) 298-307. 3. Zamfir, A., L. Bindila, N. Lion, M. Allen, H. H. Girault, J. Peter-Katalinić, Electrophoresis ***26*** (2005) 3650-3673. 4. Zamfir, A., S. Vakhrushev, A. Sterling, H.-J. Niebel, M. Allen, J. Peter-Katalinić, Anal. Chem. ***76*** (2004), 2046-2054. 5. A. Zamfir, D. G. Seidler, H. Kresse, J. Peter-Katalinić, Glycobiology ***13*** (2003), 733-742. 6. M. Mormann, B. Maček, A. Gonzalez de Peredo, J. Hofsteenge, J. Peter-Katalinić, Int. J. Mass Spectrom. ***234*** (2004) 11-21. 7. M. Froesch, L. Bindila, A. Zamfir, J. Peter-Katalinić, Rapid Comm. Mass Spectrom. ***17*** (2003) 2822-2832. 8. S. Y. Vakhrushev, M. Mormann, J. Peter-Katalinić, Proteomics ***6*** (2006) 983-992.

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Friday, the 1st of September – 11:00 am

High-throughput glycan analytics using DNA-sequencers

Dr CALLEWAERT Nico / nico.callewaert@dmbr.ugent.be

Authors : Wouter Laroy, Nico Callewaert
Topic : Glycosylation

Content : We have developed protocols for the analysis of protein-linked glycans on DNA sequencing equipment. This technique satisfies the glyco-analytical needs of many projects, and can form the basis of 'glycomics' studies, in which robustness, high throughput, high sensitivity and reliable quantification are of paramount importance. The technique routinely resolves isobaric glycan stereo-isomers, which is much more difficult using mass spectrometry. Earlier protocols made use of polyacrylamide gel-based sequencers, but we have now adapted it for the multi-capillary DNA-sequencers that are the state-of-the-art today. Moreover, we have integrated an option for HPLC-based fractionation of 8-amino-1,3,6-pyrenetrisulfonic acid-labeled glycans before rapid capillary electrophoretic profiling of the collected fractions. This enables both two dimensional profiling of complex glycan mixtures and exoglycosidase sequencing, or mass spectrometric analysis of particular compounds of interest rather than of the total pool of glycans in a sample. We will illustrate the utility of this technology for the development of liver disease diagnostics.

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Friday, the 1st of September – 11:40 am

Disease Glycomics and N-Glycan Branching Structures and Functions

Pr TANIGUCHI Naoyuki / proftani@biochem.med.osaka-u.ac.jp

Authors : Naoyuki Taniguchi
Topic : Glycosylation

Content : Among various posttranslational modification reactions of proteins, glycosylation is most abundant and actually almost over 50% of those proteins are glycosylated. Glycosylation reactions are catalyzed by the catalytic reaction of glycosyltransferases and sugar chains are added to various complex carbohydrates such as glycoproteins, glycolipids and proteoglycans. Functional glycomics using sugar remodeling technique using glycosyltransferase genes provides a new insight into the post genomic era to characterize the molecular mechanism of various diseases. N-Acetylglucosaminyltransferase V (GnT-V), N-acetylglucosaminyltransferase III (GnT-III) and alpha 1-6 fuosyltransferase (Fut 8) catalyze the formation of biologically important branching N-linked sugar chains of glycoproteins. These three enzymes catalyze the formation of beta 1-6 GlcNAc branching, bisecting GlcNAc and core fucose (alpha 1-6 fucose), respectively and play pivotal roles under various physiological and pathological conditions. Functional glycomics by which identifies the target proteins for glycosyltansferases is an important approach to characterize the function of sugar chains. We could identify matriptase for GnT-V which carries beta 1-6 GlcNAc branching. Addition of beta 1-6 GlcNAc branching to matripatase became resistant to auto-digestion and proteolysis and became a constitutively active form which may be implicated in cancer invasion and metastasis. GnT-V has bifunctional proteins and in addition to its catalytic function the enzyme acts as an angiogenesis releasing factor. Bisecting GlcNAc addition to various signaling molecules or adhesion molecules such as E-cadherin suppresses cancer metastasis. KO mice of Fut 8 bring about disorders of growth and development and emphysema of the lung. We could identify the target molecules as TGF –beta and EGF receptors and found the deletion of the core fucose from TGF-beta receptor upregulates the matrix metalloproteinase to degrade the lung alveoli to induce the lung emphysema. These data clearly show that the branching structures of N-glycans are biologically important sugar chains.

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Friday, the 1st of September – 02:00 pm

Post-translational modification of peptide toxins from cone snail venoms

Dr OLIVERA Baldomero / olivera@biology.utah.edu

Authors : BM Olivera, P Bandyopadhyay and O Buczek,
Topic : Functional analysis

Content : BM Olivera, P Bandyopadhyay and O Buczek, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA The 700 different species of carnivorous cone snails (genus Conus) use their complex venoms to capture their prey, defend against predators and deter competitors. The venom of each Conus species has a distinctive complement of 100 – 200 different peptide toxins, mostly 10-30 amino acids in length, with multiple disulfide cross-links. These are synthesized through the normal ribosomal translation pathway, and secreted by specialized cells lining the venom duct. Conus peptides can exhibit an unprecedented diversity and frequency of post-translational modification (PTM). These include the hydroxylation, epimerization and glycosylation of various amino acids, as well as the sulfation of tyrosine, bromination of tryptophan, g-carboxylation of glutamate and more conventional post-translational modifications found in many neuropeptides (proteolysis, C-terminal amidation, N-cyclization to pyroglutamate, disulfide bond formation). Unequivocal evidence for the functional importance of several PTMs has been obtained; there is also indirect evidence that PTMs play a role in directing the proper folding of a subset of Conus peptides. A general paradigm has emerged for post-translational modification of Conus venom peptides based on studies with g-glutamylcarboxylase, the enzyme that carries out the g-carboxylation of glutamate. An ancient enzyme that arose early in metazoan evolution has been recruited to act on a subset of venom peptide precursors (~10% of all Conus peptides, in the case of g-glutamyl carboxylase). A recognition signal in the propeptide region of the targeted precursors recruits the enzyme, which then catalyzes the g-carboxylation of specific glutamate residues in the mature peptide region. The highly sophisticated, neuropharmacologically active peptides in the venoms of different Conus species were clearly evolved under considerable selective pressure to be effective and potent pharmacological agents. This led to the recruitment of post-translational modification enzymes to act on the venom peptides as they are processed. In some ways, the evolution of Conus peptide genes has parallels to the drug development strategy of a large pharmaceutical company; a combination of combinatorial library methodology and medicinal chemistry is used in most modern drug development. Post-translational modification in Conus peptides is, in effect, equivalent to the medicinal chemistry carried out after a lead compound has been identified; it refines the potency, efficacy and selectivity an unmodified venom peptide.

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Friday, the 1st of September – 02:40 pm [TP1]***TP1***

Protein-Protein Interactions during Oxidative Protein Folding and Quality Control in the Endoplasmic Reticulum

Pr RUDDOCK Lloyd / Lloyd.ruddock@oulu.fi

Topic : Functional analysis

Content : Disulphide bonds are covalent linkages formed between two cysteine residues in proteins, whose primary function is to stabilise the folded structure of the protein. Since any two cysteine residues in a protein have the potential to form a disulphide bond, the correct formation of native disulphide bonds is not trivial. Hence, it is unsurprising that native disulphide bond formation is often the rate-limiting step in the folding of proteins in vitro and in vivo. The process of native disulphide bond formation in the endoplasmic reticulum (ER) is known to be catalysed by several families of enzymes. However, while some of the participants in the cellular process are known, their individual roles are still largely confused. This situation not only inhibits our understanding of the biogenesis of a range of important proteins and hence associated disease states, but also prevents the effective manipulation of the cellular environment by the biotechnology industry for the efficient production of therapeutic proteins. Native disulphide bond formation can occur via multiple parallel pathways and this significantly complicates the interpretation of in vivo data. What is clear is that the rate-limiting step for native disulphide bond formation in proteins that contain multiple disulphides is late-stage isomerisation reactions, where disulphide bond formation is linked to conformational changes in protein substrates with substantial regular secondary structure. These steps are thought to only be catalysed by proteins belonging to the protein disulphide isomerase (PDI) family and hence an understanding of the mechanisms of action of the PDI-family is critical for our understanding of native disulphide bond formation. PDI-family members differ considerably between different organisms. There are at least 17 human PDI-family members, most of which are poorly characterized with little or nothing being known about their physiological function(s). PDI was the first protein folding catalyst reported nearly 40 years ago, but as yet significant details of its mechanism of action are unknown. PDI is an excellent catalyst of disulphide bond isomerisation reactions (especially late-stage isomerisation reactions). However, the mode of action of PDI on folding protein substrates is complicated by the fact that PDI also catalyses oxidation and reduction reactions and has molecular chaperone like properties, being able to catalyse the refolding of proteins which contain no disulphide bonds. Only by dissecting out these different functions of PDI can their relative contribution to protein folding be understood The mechanism of action of PDI as a catalyst of native disulphide bond formation in folding polypeptides depends on the ability to catalyse disulphide-dithiol exchange, to bind non-native proteins and to trigger conformational changes in the bound substrate allowing access to buried cysteine residues. While the ability of PDI to catalyse disulphide-dithiol exchange has been long studied the ability to bind non-native proteins probably presents the most significant question in the area of PDI catalysis, how does PDI recognise and bind every single protein folding intermediate tested and yet not bind native proteins? Recent progress in this area with the identification and characterisation of the substrate binding sites of PDI, PDIp, PDIp and ERp27 will be discussed. The ability to trigger conformational changes in bound non-native protein substrates is again an area that has been poorly pursued due to the extreme difficulty in isolating/identifying intermediates and in studying the process. We have recently reported an intra-domain conformational change in the catalytic domains of PDI which is required for catalysis disulphide-dithiol exchange. Further, we have identified intra-domain and inter-domain conformational changes in PDI which are linked to the ability to trigger changes in the conformation of non-native protein substrates. We believe this conformational flexibility is responsible for the inability to obtain high resolution structures of the human PDI family by crystallographic methods. In addition to a role in protein folding, PDI-family members also play other roles in the ER, including prolyl-hydroxylation, quality control and MHC-class I loading. The potential role of a previously uncharacterised human PDI-family member, ERp27 in the later two processes will be discussed, in particular that ERp27 is bound by ERp57 both in vitro and in vivo by a similar mechanism by which ERp57 binds the ER lectins calnexin and calreticulin and that this interaction may be modulated by ERp27-substrate interactions.

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Friday, the 1st of September – 03:20 pm **TP2**

FACIT Collagens XII and XIV and Matrilins is Mediated by the Interaction of the SVMP Cysteine-Rich Domain and the Matrix Protein Von Willebrand Factor A Domains

Dr SERRANO Solange M. T. / solangeserrano@butantan.gov.br

Authors : Solange M. T. Serrano[1], Junho Kim[2], Deyu Wang[3], Bojan Dragulev[3], John D. Shannon[3], Henning H. Mann[4], Raimund Wagener[4], Manuel Koch[4,5], and Jay W. Fox[3]*
[1]Laboratório Especial de Toxinologia Aplicada/CAT-CEPID, Instituto Butantan, Brazil;[2]Department of Chemistry, Sangji University, Wonju-si, Kangwon-do, South Korea; [3]University of Virginia, Charlottesville, VA; [4]Center for Biochemistry, University of Cologne; [5]Department of Dermatology, University of Cologne, Germany.

Content: Snake venom metalloproteinases (SVMPs) are the predominant toxins in Viperid venom which give rise to local and systemic hemorrhage. They are members of the Reprolysin M12 family of metalloproteinases to which the ADAMs proteins also belong. The class PIII SVMPs containing disintegrin-like/cysteine-rich domains carboxy to the metalloproteinase domain are typically more potent hemorrhagins than SVMPs with only a metalloproteinase domain suggesting a functional role for one or both of the carboxy domains. The disintegrin-like/cysteine-rich domains of the ADAMs proteins have also been implicated in contributing to their function. In the case of the SVMPs, we hypothesized that these domains could function to target the metalloproteinases to key extracellular matrix proteins or cell surface proteins whose proteolysis could lead to capillary basement membrane disruption. We initiated this investigation by assaying for cell surface proteins on fibroblasts that could bind to *Crotalus atrox* venom proteins, particularly PIII SVMPs. Collagen XIV, was identified as binding to the *Crotalus atrox* PIII SVMP catrocollastatin. Collagen XIV is a FACIT collagen containing von Willebrand factor A domains. Based on our previous studies we decided to investigate whether other VWA domain containing matrix proteins could support the binding of PIII SVMPs and which domain in the SVMPs was binding to the VWA domain. Using surface plasmon resonance, full length SVMPs and a recombinant cysteine-rich domain from a PIII SVMP were demonstrated to bind to collagen XIV, collagen XII and matrilins 1, 3 and 4. Furthermore, when the PIII SVMP jararhagin was incubated with these proteins the predominant sites of cleavage were localized at or near the VWA domains in those proteins suggesting that it is in fact the VWA domains in the matrix proteins to which the PIII SVMPs are binding via their cysteine-rich domain. In light of the fact that these extracellular matrix proteins function to stabilize matrix, targeting the SVMPs to these proteins, followed by their specific cleavage could promote the destabilization of the extracellular matrix and cell-matrix interactions and in the case of capillaries could contribute to their disruption and hemorrhage. Given the structural homology shared by the cysteine-rich domains of the PIII SVMPs and the ADAMS our results may suggest an analogous function for the cysteine-rich domains in the ADAMs family of proteins to target them to VWA domain-containing proteins. Further exploration of ADAM protein function in terms of these potential interactions could provide an enhanced understanding of the ADAMs family members' role in the many biological processes in which they appear to be involved.

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Friday, the 1st of September – 04:20 pm

Complex methylation of HBHA, an important virulence factor and antigen of *M. tuberculosis*.

Dr Camille Locht/ Camille.Locht@pasteur-lille.fr

Authors : Camille Locht[1], Carine Rouanet[1], Hélène Host[1], Franco D. Menozzi[1], Anne-Sophie Debrie[1], Hervé Drobecq[2], Jean-Michel Hougardy[3], Samy Place[3] and Françoise Mascart[3]

[1]INSERM U629, [2]CNRS, UMR 8161, IBL, Institut Pasteur de Lille, IFR142, Lille, France; [3]Laboratory of Vaccinology and Mucosal Immunity, Hôpital Erasme, ULB, Brussels, Belgium

Topic : Methylation

Although common in Eucaryotes, natural post-translational modifications of proteins in Procaryotes are more unusual, and relatively little is know about their role. We have discovered that the heparin-binding haemagglutinin (HBHA) of *Mycobacterium tuberculosis* undergoes post-translational methylation at its C-terminal end. Tuberculosis is one of the major causes of death world-wide, and new tools for controlling the disease are desperately needed. HBHA is an important adhesin, involved in attachment of *M. tuberculosis* to epithelial cells and in extrapulmonary dissemination. In addition, it is a powerful diagnostic and vaccine antigen and is currently considered to be a promising vaccine candidate against tuberculosis. The adherence activities of HBHA rely on a C-terminal lysine-rich region of the protein. This region is prone to proteolytic degradation, and its post-translational methylation protects the protein against degradation. The methylation pattern of this domain is very complex and composed of several mono- and di-methyl-lysines. This unique pattern is the result of specific HBHA:methyltransferase(s) produced by the mycobacteria. In addition to its role in stabilising the protein, the methylation also plays a crucial role in the immunological properties of the protein. Only the methylated protein is protective in mouse protection studies against tuberculosis, and the methylated protein is recognised much better than the non-methylated form by T cells from healthy *M. tuberculosis*-infected human subjects. Thus, this novel, so far unique complex methylation pattern of HBHA plays an important role in the function and immunological properties of this important *M. tuberculosis* virulence factor.

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Friday, the 1st of September – 05:00 pm

Closing lecture : Cellular organization through modular protein-protein interactions.

Dr PAWSON Tony / pawson@mshri.on.ca

Authors : Tony Pawson. Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, ON M5G 1X5, Canada.
Topic : Phosphorylation

Content : Many intracellular signaling pathways, and related regulatory systems, are controlled by protein-protein interactions, mediated by a dedicated family of modular interaction domains. Interaction domains also play an important role in dynamic cellular organization, through their ability to selectively recognize post-translational modifications such as phosphorylation. In addition to protein ligands, interaction domains can bind other types of biomolecules, including phospholipids, nucleic acids, metabolites and small molecules. We have used a variety of approaches, including proteomic analysis, to explore protein interaction networks mediated by interaction domains, including SH2/SH3 domains in tyrosine kinase signaling, 14-3-3 proteins in phosphoserine/threonine signaling, and PDZ domain-based proteins involved in cell polarity. Three specific systems will be discussed:
1) We have explored the binding properties of 14-3-3 proteins, using both experimental and computational approaches, with a focus on regulation of the actin cytoskeleton.
2) In polarized epithelial cells, we have identified a series of PDZ-, BAR- and SH3 domain-based interactions that link apical polarity proteins and the Cdc42 GTPase to the maintenance of tight junctions and protein trafficking. This defines a novel network of proteins that regulate apical cell polarity.
3) We have investigated SH2/SH3-containing Nck adaptor proteins, which couple specific phosphotyrosine signals to the actin cytoskeleton, and thus to complex architecture of specialized cells such as kidney podocytes and spinal cord neurons. We have also shown that pathogenic bacteria can exploit Nck proteins of infected cells to respecify cellular morphology.

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16th meeting of Methods in Protein Structural Analysis

P o s t e r s

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Poster ***Abstract 1***

Cysteine oxidation of an anti-lipopolysaccharide factor (rALFPm3) during its over expression in Pichia pastoris. Solution structure of rALFPm3

Dr AUMELAS André / aumelas@cbs.cnrs.fr

Authors : Hélène Boze1, Patrick Chemardin1, Yinshan Yang2, André Padilla2, Guy Moulin1, Yannick Gueguen3, Evelyne Bachère3, André Aumelas2
Dr AUMELAS André Email **:** aumelas@cbs.cnrs.fr
Topic : Structural Methods

Content : Anti-lipopolysaccharide factors (ALFs), originally characterized from horseshoe crabs (LALF), bind to the lipid A moiety of lipopolysaccharides (LPS) to prevent sceptic shock. New ALFs have been recently identified from haemocytes of the black tiger shrimp, Penaeus monodon, by a genomic approach. One of them, ALFPm3, is a 98-residue protein that shares 34% of sequence identity with LALF. To determine its biophysical properties, its antimicrobial activities and its 3D structure, rALFPm3 was overexpressed as a 102-residue fusion protein in Pichia pastoris, using the methanol-inducible alcohol oxidase (AOX1) promoter. Surprisingly, the mixture of the folded and unfolded proteins was obtained. The folded protein (262 mg/l) was purified to homogeneity by a single chromatography step on expanded-bed Streamline SP6XL and characterized by mass spectrometry and NMR (1). ESI-MS analysis of the unfolded protein revealed a posttranslational modification corresponding to the oxidation of the two cysteines into cysteic acid (-SO3H). Changes in concentrations of divalent cations, pH, culture duration and stirring, to control the oxygenation of the culture medium, did not decrease the oxidative process. We concluded that oxidation was probably due to the hydrogen peroxide produced in situ by the yeast metabolism. Consequently, due to the efficient cysteine oxidation, by using this promoter for a low scale production of the 15N labelled protein (5-10 mg scale), only the unfolded rALFPm3 was obtained. Therefore, another Pichia pastoris transformant, using the constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter, growing in glycerol and that does not produce hydrogen peroxide, was used instead. In this case, despite a lower yield, the correctly folded and 15N labelled protein was mainly obtained. Its solution structure was determined by NMR and consists of three alpha-helices packed against a four-stranded beta-sheet in which the two central strands are tightly linked by the 34-55 disulphide bond. Moreover, we demonstrated that this unique disulphide bond was essential to maintain the rALFPm3 fold. Indeed, upon its reduction the rALFPm3 3D structure was lost. Since numerous proteins containing disulphide bonds were previously and successfully produced in Pichia pastoris by using the AOX1 promoter, we concluded that the environment of the two cysteines of rALFPm3 particularly favours the irreversible cysteic acid formation by hydrogen peroxide. The fact that the essential disulphide bond cannot be formed would explain why, in this case, the unfolded protein was obtained. The identification of the cysteic acid, performed by selective enzymatic cleavages analyzed by ESI-MS, as well as the determination of the 3D structure of the rALFPm3 protein will be described.

Reference:
1. Somboonwiwat K, Marcos M, Tassanakajon A, Klinbunga S, Aumelas A, Romestand B, Gueguen Y, Boze H, Moulin G, Bachère E. (2005) Dev. Comp. Immunol. 29 841-51.

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Poster ***Abstract 2***

Implication of *O*-linked N-acetylglucosaminylation in the contractile activity in skeletal muscle.

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Authors : J. HEDOU, C. CIENIEWSKI-BERNARD, Y. LEROY*, L. STEVENS, M. FALEMPIN, JC. MICHALSKI*, Y. MOUNIER, B. BASTIDE.
Topic : Glycosylation

Content : *O*-linked N-acetylglucosaminylation (*O*-GlcNAc) is a dynamic cytosolic and nuclear glycosylation. We have previously reported that, in skeletal muscle, proteins of the glycolytic pathway and energetic metabolism, and contractile proteins were *O*-GlcNAc modified and that *O*-GlcNAc variations could control the muscle protein homeostasis. We studied a potential rôle of *O*-GlcNAc post-translational modification in skeletal muscle contraction. Proteomic analysis demonstrate that key contractile proteins, myosin heavy and light chains, actin and tropomyosin are modified by *O*-GlcNAc. Many of these proteins also display lectinic properties toward *O*-GlcNAc. Moreover, our results clearly showed that incubation of skeletal muscle fibers in a solution of N-acetyl-D-glucosamine, which could inhibit *O*-GlcNAc dependant interactions, induces a decrease in calcium sensivity and affinity of muscular fibers. Thus, our results strongly suggest that *O*-GlcNAc is involved in many contractile protein interactions and could therefore modulate muscle contraction.

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Poster ***Abstract 3***

Phosphorylation of human syncytial virus P protein

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Authors : Ana Asenjo, Enrique Calvo, Emilio Camafeita and Ni
Topic : Phosphorylation

Content : Human respiratory syncytial virus (HRSV) P protein has been described to be phosphorylated at various serine and threonine residues with different turnover rates. In this work we investigate the role of phosphothreonines in viral RNA synthesis using P protein substitution variants and the HRSV-based minigenome pM/SH. Liquid chromatography coupled to ion-trap mass spectrometric analysis showed Protein to be phosphorylated at T108 with a high turnover rate. Phosphorylation of T108 takes place both in P protein expressed transiently and upon HRSV infection. Results indicate that M2-1transcriptional activity is influenced by phosphorylation at T108, as this post-translational modification hampers the interaction between P and M2-1 proteins, therefore playing a significant role in viral transcription and replication processes.

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Poster ***Abstract 4***

A chemical proteomics approach reveals the taxoid binding site on tubulin

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Topic : Other

Content : The mitotic spindle is an important target in cancer chemotherapy. Spindle function is dependent on microtubule dynamics, which involves the loss and gain of α/β–tubulin heterodimers from microtubule ends. By interfering with microtubule dynamics, compounds that bind to tubulin arrest cells in mitosis and cause apoptotic cell death. Microtubule stabilizing agents (MSAs), such as paclitaxel, bind preferentially to assembled tubulin, minimizing dissociation of tubulin-GDP from microtubule ends. Other structurally diverse taxoid site MSAshave been discovered, as cyclostreptin (FR182877), a natural product we found to have unusual biochemical properties. Cyclostreptin weakly stimulated tubulin assembly, but it avidly bound to microtubules. Kinetic evaluation of cyclostreptin binding indicated an irreversible, covalent binding mechanism, as revealed by biochemical and competition experiments with radiolabeled taxoid site ligands. We also found that cyclostreptin bound irreversibly to cellular microtubules in vivo, being fully active in MDR cells overexpressing P-gp. Using the different scan modes of a hybrid triple quadrupole mass spectrometer, we have characterized the covalent binding of cyclostreptin to microtubules and oligomeric and dimeric tubulin. We found 1:1 modification of β-tubulin at two residues, either Thr-220 or Asn-228. While Asn-228 is part of the taxoid binding site facing the microtubule lumen, Thr-220 is a component of pore type I on the outer microtubule wall. Our data support the hypothesis that taxoids reach the relatively inaccessible luminal site through transient binding to the pore site I, followed by translocation of the drug to its luminal site.

Poster ***Abstract 5***

The Protein glycoconjugated extracts from mastic gum var

Pr CHOLI-PAPADOPOULOU Theodora / Tcholi@chem.auth.gr

Authors : Ph. Kottakis1, N. Kouzi-Koliakou2 and T. Choli-Pap
Topic : Glycosylation

Content : Mastic gum var. Chia is a resinous, aromatic substance that comes from the trunk and the thickest branches of the gum mastic tree Pistacia lentiscus species. Its bactericidal activity against Helicobacter pylori has been reported by Huwez et al., (1998) and Marone et al., (2001). Its high content on 69 essential oils and a-pinene (Magiatis et al., 1999) in conjunction with the existence of Zn and high molecular weight glycoconjugates (Kottakis et al., unpublished results) indicates a multifunctional natural product against Helicobacter pylori. The Helicobacter Pylori Neutrophil Activating Protein (HPNAP) was shown to induce neutrophil adhesion to endothelial cells in vitro (Yosida et al., 1993) and in vivo (Kurose et al., 1994). HPNAP stimulates the production of reactive oxygen intermediates (ROI) of human neutrophils and monocytes and therefore can be related to mucosal damage (Evans et al., 1995, Tonello et al., 1999, Satin et al., 2000). In an attempt to elucidate the mechanism of action of mastic gum glycoconjugates against H. pylori experiments concerning neutrophil activation as well as their inhibition were performed. Neutrophils isolated from healthy donors who had either received mastic gum orally before or not were treated with HPNAP and mastic gum protein glycoconjugates separately. In addition, preincubated HPNAP with glycoconjugates was further incubated with human neutrophiles Their activation in all cases was measured by performing a neutrophil activation assay (activation of NADPH oxidase –super oxide anions production). Our results concerning the neutrophil activation show an inhibition of neutrophil activation indicating a possible mechanism of action of the glycoconjugates. Both HPNAP and glycoconjugates separately induce the super oxide anions formation in both donors' categories. On the contrary, only the neutrophils -isolated from the second donor group (that had been consuming with mastic gum orally previously) – show a remarkable inhibition after their treatment with pre incubated HPNAP-glycoconjugates. Their binding to neutrophil membranes that has been observed by optical microscopy experiments showed binding of both HPNAP and mastic glycoconjugates separately to the membranes but HPNAP and mastic extracts together abolish binding. At the moment it is evidenced only for the second donor group. This observation could point out a possible way of protection of the human neutrophils from the oxidative stress induced by HPNAP but other experiments are needed for a further elucidation of the exact mechanism of action of mastic gum extracts against H.pylori. The authors would like to thank the "CHIOS GUM MASTIC GROWERS ASSOCIATION" for supporting this work.

REFERENCES

Yosida et al., (1993), Gastroenterology, 105, 1431-1440
Huwez et al. Path M.R.C., (1998)
Marone et al., J Chemoth, 2001, 13(6), 611-614
Kurose et al., Gastroenterology. 1994 Jul, 107(1), 70-9
Evans et al, (1995), Gene, 153,123-127
Tonello et al. , (1999), Mol. Microbiol., 34,238-246
Satin et al. , (2000), J.Exp.Med. 191, 1467-1476

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Poster ***Abstract 6***

Functional and structural Studies of the Helicobacter Pylori Neutrophile Activating Protein (HPNAP).

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Authors : F. Kottakis1, G. Papadopoulos2, K. Kuzi-Koliakou3
Topic : Functional analysis

Content : The neutrophil activated protein of H. pylori (HP-NAP) was originally purified from water extracts of H. pylori and was shown to induce neutrophil adhesion to endothelial cells in vitro (1) and in vivo (2). It induces migration and activation of human neutrophils and monocytes (3) and is a potent stimulant of mast cells. HP-NAP is a dodecameric protein consisting of 17kDa monomers with a central cavity where iron can bind (4). According to three dimensional structure determination (5), its quartenary structure is similar to that of the dodecameric bacterial ferritins (Dps-like family) but it has a different surface potential charge distribution. HPNAP protein lacks the lysine-containing N-terminal extension and therefore it is expected to abolish binding to DNA. On the other hand experiments concerning gel retardation assays and DNA protection assays protects DNA from oxidative cleavage due to its capacity to bind iron and to inhibit hydroxyl radical formation. After mutation of the amino acids that are localized within the ferroxidase activity center, namely His25, His37 to Ala and Asp52, Lys134 also to Ala, the protein was overexpressed heterologous in E. coli BL21 cells and purified by using the affinity Ni sepharose beads. Experiments concerning the ferroxidase activity shown that it abolishes its activity as well as its DNA protection capacity. In addition, after the mutations it is evidenced, that the protein does not form dodecameric structure but only its monomeric one and therefore lacks its capacity to bind iron and to inhibit radical formation. Surprisingly the neutrophils are activated by both forms of HPNAP the wild type and the mutated. From this unexpected result it is concluded that for its implication on the oxidative stress does not require its ferroxidase activity.

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REFERENCES

1.Yoshida,N., et al., Gatsroenterology, 1993, 105: 1431-1440
2.Kurose,I., et al., Gastroenterology 1994, 107:70-79
3.Satin,B., et al., J. Exp.Med. 191, 1467-1476
4.Evans,. Infect. Immun. 1995. 63: 2213-2220
5.Zanoti, et al., JMB,2002, 323: 125-130

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Poster ***Abstract 7***

Protein Phosphorylation Analysis Using "Phosphorylation Motif" Specific Monoclonal Antibody

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Topic : Phosphorylation

Content : Post-translational modification is a major mechanism, by which protein function is regulated in a wide variety of signal transduction pathways, such as cell cycle progression, development and differentiation [1, 2]. Especially, it is well known that phosphorylation serve as a common mechanism for regulating protein function in eukaryotes [3]. The phosphorylation level of proteins is stringently controlled by kinases and phosphatases. Antibodies specific for modified sites are essential tools for studies of protein phosphorylation. In general, protein kinases recognize and phosphorylate specific sequences of substrate proteins to produce the corresponding "phosphorylation motif". For instance, DNA-PK, ATM, and ATR kinases, members of the PI-3 kinase family, recognize sequences of Ser-Gln in substrate proteins including p53, Chk1 and Brca1 and consequently phosphorylate the Ser residues to give Ser(P)-Gln motifs. In this study, we generated the monoclonal antibody 3G9-H11 that specifically recognizes the Ser(P)-Gln phosphorylation motif and applied it for detection of phosphorylated proteins in mammalian cells.
ELISA analysis for various p53 phosphopeptides demonstrated that the 3G9-H11 antibody recognized only phosphopeptides containing Ser(P)-Gln motif and did no react with other p53 phosphopeptides. In order to evaluate whether 3G9-H11 can recognize universal Ser(P)-Gln sequence, we synthesized and analyzed the Ser(P)-Gln-containing phosphopeptides derived from Brca1 and Chk1, which were known as target proteins of ATR/ATM kinase. 3G9-H11 also recognized both phosphopeptides specifically, although the affinity for each phosphopeptide was influenced by flanking residues of the Ser(P)-Gln sequence. Furthermore, we applied the 3G9-H11 antibody for phosphatase assay. Analysis of phosphatase activity of PPM1D using phosphorylated p53 peptide demonstrated that the 3G9-H11 could detect the phosphatase activity with higher sensitivity than the common method using malachite green for phosphatase activity analysis.
In order to explore whether 3G9-H11 antibody can detect the protein phosphorylation in mammalian cells after ATR activation, 3G9-H11 was applied for Western blotting analysis and immunohistochemistry against UV-treated cells. Western blotting analysis against lung carcinoma H1299 cells over-expressed p53 exhibited that 3G9-H11 could detect the phosphorylation of p53 and 47 kDa unknown protein after UV stimulation following ATR activation, suggesting that 3G9-H11 should be useful not only to detect the phosphorylation level of ATR/ATM target proteins such as p53 but also to find the novel target proteins of ATR/ATM kinases. Furthermore, immunohistochemistry of breast adenocarcinoma MCF7 cells using 3G9-H11 showed that proteins containing Ser(P)-Gln sequence accumulated in nucleus after UV stimulation. These data suggested that 3G9-H11 should be powerful tool to evaluate the function of ATR/ATM kinases both of *in vitro* and *in vivo*.

References

1. Mann, M., et al. *Nat. Biotechnol.,* **21**, 255-261, (2003)
2. Nalivaeva, NN., et al. *Proteomics*, **1**, 735-747, (2001)
3. Holmberg, CI., et al. *Trends Biochem. Sci.,* **27**, 619-627, (2002)

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Poster ***Abstract 8***

Are O-N-acetylglucosaminylation and phosphorylation implicated in heart diseases, like post-infarction left ventricular remodeling ?

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Authors : Caroline CIENIEWSKI-BERNARD, Paul MULDER, Christian THUILLEZ, Philippe AMOUYEL, Vincent RICHARD and Florence PINET.
Topic : Glycosylation

Content : There are many ways for a cell to increase the complexity of its proteome from DNA to functional proteins. Among them, post-translational modifications such as phosphorylation, glycosylation or acetylation, provide additional levels of functional complexity to the cell's proteome. One of them, O-linked N-acetylglucosaminylation (termed O-GlcNAc), constitutes an abundant and reversible form of glycosylation on numerous cytoplasmic and nuclear proteins ; it consists on the linkage of a unique monosaccharide, N-acetyl-D-glucosamine, on serine and threonine hydroxyl group of a protein. The transfer of this monosaccharide is realized by the UDP-GlcNAc-peptide-β-GlcNAc transferase while its removing is performed by the N-acetyl-β-D-glucosaminidase. This enzymatic system could be compared with the kinase/phosphatase system in the phosphorylation process. Indeed phosphorylation and O-GlcNAc modifications are often reciprocal at the same or at neighbouring sites, O-GlcNAc appears as a regulatory modification that has a complex dynamic interplay with phosphorylation. Many proteins have been identified to be O-GlcNAc modified, which belong to various classes of proteins, suggesting that O-GlcNAc could be implicated in several key cellular processes. Numerous evidences suggest the importance of O-GlcNAc in many pathologies including diabetes, cancer, neurodegeneratives diseases, muscular atrophy, and also in adaptation processes, underlying its crucial role in cell life. Recent studies clearly showed the emerging of O-GlcNAc in heart, but its implication in cardiovascular diseases remains to be defined.
One of the major determinants of the severity of heart failure is the extent of left ventricular (LV) remodeling after myocardial infarction. Indeed, in patients, the severity of LV remodeling cannot be fully predicted based on its known determinants, such as myocardial infarction size. Recent developments in the field of proteomic technology allow to analyse the entire cellular proteome, leading to the identification of proteins involved in a pathological condition and so to determine markers of the pathology. In order to perform proteomic studies in the context of experimental post-infarction remodeling, we set up techniques for differential proteomic approach using an experimental model of heart failure. After measurement of hemodynamic parameters, proteomic analysis was performed in order to identify O-GlcNAc bearing proteins and phosphoproteins differentially expressed between normal and pathological tissues. Glycosylated proteins were purified from ventricular extract prior to their analysis using bidimensional gel (2-D) electrophoresis, with separation of proteins from a biological sample according to their isoelectric point and their molecular mass, followed by silver staining. The Platinum® bioinformatic software was used to analyse 2D-gel in order to determine O-GlcNAc proteins and phosphoproteins differentially expressed between normal and pathological conditions. Phosphoproteins were analysed using two approaches: the first one is 2D-gel electrophoresis followed by specific revelation of phosphoproteins using ProQ Diamond coloration; the second approach was the SELDI-TOF analysis or ProteinChip® system, in which phosphoproteins were specifically captured on IMAC surface. These two techniques are associated with mass spectrometry in order to identify O-GlcNAc proteins and phosphoproteins differentially expressed between normal and pathological tissues. This work will allow the identification of proteins involved in post-infarction remodeling, and this work will determined if a balance between phosphorylation and O-GlcNAc occurs on proteins implicated in left ventricular remodeling.

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Poster ***Abstract 10***

Posttranslational Modification in Ordered and Disordered Structures

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Topic : Functional analysis

Content : A novel data mining tool that identifies functions in the SwissProt database that correlate with intrinsic disorder and order was elaborated. This bioinformatics analysis is based on testing the null hypothesis that for a particular function, the fraction of proteins with long disordered regions or without long disordered regions is the same as the fractions expected by chance. Contrarily, a small p-value for the correlation should indicate a strong association between protein intrinsic disorder or intrinsic order and functions. A statistical measurement was used to rank the significance of the associations. Application of this tool identifies 275 and 298 non-overlapping Swiss-Prot functional keywords to be strongly correlated with intrinsic disorder or intrinsic order, respectively. These functions cover a large variety of biological activities, including posttranslational modifications. Overall, these analyses suggest two distinctive groups of posttranslational modifications: those associated with structured (ordered) proteins and those associated with disordered proteins. The first group involves modifications that are associated primarily with structured proteins and regions. These include the following modifications: formylation, protein splicing, oxidation and covalent attachment of quinones and organic radicals. These modifications are important for providing moieties for catalytic functions, for modifying enzyme activities or for stabilizing protein structure. For example, activity of several crucial enzymes (e.g., ribonucleotide reductase containing a stable organic free radical located on a tyrosine residue in the small subunit of the enzyme) depends on organic radicals covalently bound to the protein moiety. Several quinoproteins are involved in the long-range interprotein electron transfer. Quinoproteins that possess pyrroloquinoline quinone (PQQ), tryptophan tryptophylquinone (TTQ), and cysteine tryptophylquinone (CTQ) are dehydrogenases. PQQ is tightly but non-covalently bound to the enzyme, whereas TTQ and CTQ are derived from amino acid residues of the polypeptide chain. Finally, proteins are known to undergo different types of oxidation. These include carbonylation (an irreversible process that targets different amino acids including lysine, arginine, proline and threonine), nitration of tyrosine and oxidation of methionine to methionine sulphoxide. Furthermore, protein cysteines can undergo various forms of oxidation, some of them reversible (disulphide formation, glutathionylation, cysteinylation, S-nitrosylation and formation of sulphenic and sulphinic acids). Intraprotein disulphide bonds are viewed, in classical textbooks, as part of the tertiary structure of the protein and their formation is an important step in protein folding. Similarly, interprotein disulphide bonds are important in the quaternary structure of proteins, in the formation of homo- or hetero-multimers. The second group involves modifications that are associated primarily with intrinsically unstructured or disordered proteins and regions. These include the following modifications: phosphorylation,acetylation, acylation, adenylylation, ADP ribosylation, amidation, carboxylation, formylation, glycosylation, hydroxylation, methylation, sulfation, palmitoylation, prenylation, ubiquitination, Ubl-conjugation (i.e.,covalent attachment of ubiquitin-like proteins, including SUMO, ISG15, Nedd8, and Atg8). These modifications involve low affinity, high specificity binding interactions between a specific enzyme and a substrate (a protein that has to be modified). Combination of high specificity with low affinity, being ideal for signaling, can be achieved via the coupled binding and folding. The low net affinity arises because the positive free energy associated with the disorder-to-order transition reduces the magnitude of the negative free energy arising from the interactions within the contact surface. Importantly, posttranslational modifications associated with intrinsically disordered proteins and regions are especially important for signaling and regulation. For example, protein phosphorylation is known to represent a

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crucial regulatory mechanism in eukaryotic cells. In fact, at least one-third of all eukaryotic proteins are estimated to undergo reversible phosphorylation. Phosphorylation modulates the activity of numerous proteins involved in signal transduction, and regulates the binding affinity of transcription factors to their coactivators and DNA thereby altering gene expression, cell growth and differentiation. Another illustrative example is Ubl-conjugation, which is critical for many cellular processes, including transcription, DNA repair, signal transduction, autophagy, and cell-cycle control. Ubl-conjugation cascades are initiated by activating enzymes, which also coordinate the ubls with their downstream pathways. In fact, conjugation of ubiquitin-like proteins (the Ubl conjugation pathway) to components of the transcriptional machinery is an important regulatory mechanism allowing switching between different activity states. While ubiquitination of transcription factors is associated with transcriptional activation, their SUMOilation is most often connected with transcriptional repression. Overall, comparison of the posttranslational modifications in structured and intrinsically disordered regions revealed that later are essentially more diverse and more often tend to be reversible. Thus, the irreversible posttranslational modifications are mostly used to increase stability and allow catalytic functions of the ordered proteins, whereas the reversible modifications are more frequently used for the signaling activity of intrinsically disordered proteins.

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Poster ***Abstract 11***

Specificity of Phosphopeptide-Metal Ion Complex Formation Analyzed by MALDI-MS

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Topic : Phosphorylation

Content : During time-of-flight analysis of peptides ionized by matrix assisted laser desorption/ionization cationized species are often detected in addition to the predominant protonated ones. The ubiquitous alkali metals, sodium and potassium, are most commonly observed. In solution, numerous peptides exhibit affinities for transition metals such as copper or zinc. Phosphopeptides exhibit high binding affinity for a number of metal ions including iron(III) and gallium(III). We have investigated whether matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) allows the detection of cationized phosphopeptides. A number of metal ions, including Fe(III), Ga(III) and Ca(II), were tested in a variety of matrices ranging from acidic to basic. It was found that Fe(III) binds preferentially to phosphopeptides in the presence of non-phosphorylated peptides under acidic conditions. Methods: Peptides (beta-casein [48-63], synthetic tyrosine phosphorylated peptides and the BSA digest) were dissolved in aqueous 0.05% TFA/30% acetonitrile. Samples were spotted on Bruker Anchorchip MALDI targets and analyzed on a Bruker Ultraflex TOF/TOF mass spectrometer. All spectra were measured in positive reflectron TOF mode. The beta-casein fragment was spotted with different matrices and concentrations of metals (1mM to 20mM). Alpha-casein was digested with trypsin and analyzed for phosphopeptides. Titanium dioxide microcolumns were utilized to bind phosphopeptides from the alpha-casein digest and eluted with aqueous piperidine. Samples were dried, redissolved and desalted on ZIP Tips before analysis. The matrices are listed in the Results Section. Preliminary results: When incubating phosphopeptides with millimolar concentrations of metal salts (FeCl3, Ga(NO3) 3, CaCl2), cationized species were observed in MALDI-MS spectra with all matrices tested. The major cationized species carried a single positive charge, e.g [M -2H +Fe]+. The cationized species typically exhibited a signal intensity 10-20% that of the protonated one ([M+H] +). To test the specificity of the metal ion binding, model serine and tyrosine phosphorylated peptides (beta-casein 48-63, actin 48-61, src 521-533) were mixed with a BSA tryptic digest and incubated with the metal salts on the MALDI target in the presence of matrix. The matrices used included alpha-cyano 4-hydroxycinnamic acid (ACHC), sinapinic acid (SA), dihydroxybenzoic acid (DHB), 6-aza-2-thiothymine (ATT) and para-nitroaniline (PNA). Cationization was observed to a varying extent in all cases but only one metal ion/matrix combination exhibited specificity for the phosphopeptide. Iron(III) bound to the phosphopeptide exclusively when employing ACHC as the matrix. A variety of phosphopeptides containing phosphorylated serine, threonine and tyrosine residues were analyzed in the same manner with similar results. To expand this investigation, an alpha-casein tryptic digest was incubated with FeCl3 in ACHC and analyzed by MALDI-MS. The phosphopeptides in the crude digestion mixture showed only a weak signal. Therefore, titanium dioxide affinity was employed to enrich the phosphopeptides. When analyzing the enriched peptide mix, two phosphopeptides gave rise to a cationized species. In addition, an acidic peptide that bound strongly to the titanium dioxide column also formed an Fe(III) complex, albeit at a weaker relative intensity. Discussion and Summary: Phosphopeptides exhibit a high affinity for metal ions such as iron(III) and gallium(III), a fact that is exploited in immobilized metal affinity chromatography enrichment procedures. Here we have investigated the formation and the stability of peptide-metal ion complexes under MALDI-MS conditions. Cationized species were observed in the mass spectra when using a wide variety of matrices and conditions. We investigated conditions that would allow discrimination of phosphopeptides from non-phosphopeptides based on their metal binding properties. The highest degree of specific cationization was observed when adding low millimolar concentrations of iron (III) chloride to peptides in the matrix ACHC. This procedure can potentially lead to the identification of phosphopeptides in complex mixtures by detection of a cationized signal.

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Poster ***Abstract 12***

An H3 Modification Pattern that Marks and Configures Mitotic Chromatin

Dr GEORGATOS Spyros / sgeorgat@cc.uoi.gr

Authors : A. S. Politou, S.D. Georgatos Y. Markaki, A. Christogianni, G. Papamokos
Topic : Methylation / Acetylation

Content : Investigating the site-specific phosphorylation of histone H3 at threonine-3, we have identified a combinatorial modification pattern (the PMM “signature”) established in the beginning of mitosis and completely erased at the end of cell division. Geometry optimization calculations, biophysical analysis and cross-linking experiments show that histone tails possessing the PMM combination can accommodate a certain type of secondary structure and has a tendency to self-associate in vitro. Furthermore, comparative mapping of PMM-sites in extended chromatin fibers and condensed chromosomes reveals close lateral packing in pericentromeric or peritelomeric regions and suggests formation of “stem-and-loop” microdomains. Consistent with a role of these microdomains in transient interactions with the mitotic spindle, disruption of the PMM modification/de-modification cycle leads to chromosome segregation defects and ectopic distribution of G1 chromatin.

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Poster ***Abstract 13***

Protein Kinase C pathway promotes the farnesoid X receptor transcriptional activity

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Authors : Romain GINESTE and Audrey SIRVENT, Réjane Paumelle
Topic : Phosphorylation

Content : The farnesoid X receptor (FXR, NR1H4) belongs to nuclear receptor superfamily and is activated by bile acids such as chenodeoxycholic acid (CDCA) or synthetic ligand as GW4064. FXR is implicated in the modulation of bile acids, lipid and carbohydrate metabolism. Although its mechanism of action is well characterized, post-translational modifications regulating its activity have not yet been investigated. Here, we demonstrate for the first time that calcium dependant PKC inhibitor prevents the ligand-mediated regulation of FXR target gene. Additionally, using transactivation assay, we show that the FXR transcriptional activity is modulated by calcium dependant protein kinase C (PKC) and particularly the PKCa isoform. Furthermore, in HepG2, the phorbol 12-myristate 13-acetate (PMA), a PKC activator, induces the phosphorylation of endogenous FXR and, in vitro, PKCa phosphorylates FXR in its ligand binding domain (LBD). Finally, by using two mammalian hybrid assay and GST pull-down, we show that phosphorylation of the LBD promotes the recruitment of the steroid receptor coactivator 1 (SRC1). These results demonstrate, for the first time, a link between FXR activity and the PKC phosphorylation pathway.

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Poster ***Abstract 14***

FSBA-COFRADIC: a new functional, chemical proteomics technology to identify purine nucleotide binding sites in complex proteomes.

Dr HANOULLE Xavier / xavier.hanoulle@UGent.be

Authors : Xavier Hanoulle, Jozef Van Damme, An Staes, Lennart Martens, Marc Goethals, Joël Vandekerckhove & Kris Gevaert
Topic : Functional analysis

Content : Purine nucleotides are small, very abundant molecules binding various proteins involved in many key cellular processes. These nucleotides are co-factors or substrates for enzymes, regulators of protein function or form structural binding motifs. The identification of nucleotide-binding sites on a proteome-wide level is challenging, not only in view of the high number of nucleotide-binding proteins and their large in vivo concentration differences but also due to the various functions they exert. We developed a new functional gel-free technology permitting the characterization of protein purine nucleotide binding sites starting from un-fractionated cell lysates. Our technology combines a synthetic ATP analogue, 5'-p-fluorosulfonylbenzoyladenosine (FSBA), as an affinity/activity-based probe for purine-binding sites and diagonal reverse-phase peptide chromatography. We first validated this technique with an in vitro experiment involving a known ATP-binder, the c-AMP-dependent protein kinase, and then analyzed a whole human Jurkat T-lymphocytes proteome identifying 185 different SBA-modified sites. We furthermore show here for the first time that FSBA also labels known in vivo tyrosine phosphorylation sites.

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Poster ***Abstract 16***

High-throughput on-chip identification and interaction mapping

Dr HIRANO Hisashi / hirano@yokohama-cu.ac.jp

Authors : Yuko Iwafune1, Jian-Zhong Tan1, Yoko Ino1, Akiko O
Topic : Mass spectrometry

Content : In proteome analysis, protein chip is one of the most promising tools to profile the expressed proteins such as disease-associated proteins and to analyze protein-protein, protein-DNA and protein-drug interactions. In conventional technique, many proteins expressed by DNA (RNA) or natural proteins were purified and immobilized in a 2D addressable grid on non-conductive plates such as glass, silicon and plastic plates. However, purification of a number of proteins was laborious and time-consuming, and was often impossible, limiting to perform the high-throughput on-chip analysis of the expressed proteins and protein-protein interactions.Two-dimensional gel electrophoresis (2-DE) using isoelectric focusing in the first dimension and SDS polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension is one of the most important techniques to separate a number of proteins. This allows simple, sensitive and reproducible separation of thousands of proteins for one or two days. Previously, to identify proteins separated by 2-DE, we digested protein spots in the gel with proteases such as trypsin or lysylendopeptidase, and the resultant digests were measured by mass spectrometry. Based on the mass spectra, the gel-resolved proteins were identified. This technique has been widely used, but more efficient techniques have been required for proteome analysis. If proteins separated by 2-DE could be transferred on the protein chip plates by electroblotting just as Western blotting, high-density of protein chips with thousands of immobilized proteins can be produced rapidly. However, such chip plates, to which we can directly transfer the proteins from the gels, have not been available. We developed a novel protein chip plate, which is made of stainless steel coated with diamond-like carbon film by ion assisted deposition method. The surface of DLC plate was modified with N-hydroxysuccinimide ester or amino groups. Proteins separated by SDS-PAGE or 2-DE were successfully electroblotted and immobilized covalently or non-covalently on the chemically modified DLC plate to produce easily and rapidly high-density of protein chip. High blotting efficiency of proteins from the gels onto the DLC plate (30-70 %) was obtained. Proteins electroblotted onto the DLC plate were digested on the plate with protease prior to mass spectrometric analysis. The DLC plate was applied to MALDI-TOF MS to identify the proteins on the DLC plate. On the other hand, analysis of the protein-protein interaction is important to determine the function of proteins. Protein-protein interaction is usually determined by several methods such as yeast two-hybrid system, immunoaffinity purification, tandem affinity purification and protein chip in proteome analysis. Among them, the protein chip technique has a potential to analyze protein-protein interactions at protein level in a large scale. In this study, the proteins electroblotted from the gel onto the DLC plate were interacted with peptides or proteins in the extract. The interacted peptides were identified directly by MALDI-TOF MS, and the interacted proteins by MALDI-TOF MS after digestion of the proteins with protease, showing that the DLC plate can be used for analysis of protein (or peptide)-protein interaction. The established technique could be applied to analysis of protein-DNA and protein-drug interactions as well as protein-protein interaction. The developed techniques have a great potential to make possible the high-throughput proteome analysis.

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Poster ***Abstract 17***

Enrichment of Phosphorylated Proteins

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Authors : Lilya Schumann, Daniel Weis and Roza Maria Kamp
Topic : Phosphorylation

Content : Enrichment of Phosphorylated Proteins Liliya Schumann, Daniel Weis and Roza Maria Kamp*

Introduction

The analysis of phosphorylated proteins plays an important role in the diagnostic of many diseases, such as cancer (1), Alzheimer (2) or cardiovascular diseases (3). The identification of phosphoproteins is helpful for the understanding of cellular processes, in which phosphorylation is involved and for the development of new diagnostic markers in clinical research. The phosphate groups play a role as switches for enzymatic activity. The steric and electrostatic effects of the negatively charged phosphate groups cause changes of the protein conformation, the catalytic center and enzymatic activity. The phosphorylation is a reversible process with interactions of many enzyme groups, such as kinases and phosphatases. About 500 kinases and 200 different phosphatases were identified (4). The phosporylation and dephosphorylation is a very important part for all communication during the cell growth, cell differentiation, cell interaction, apoptosis, gene expression, protein secretion, signal transfer or metabolismus of carbohydrates or lipids (5). Phosphorylation reactions participate also in the regulation of the neurotransmitter dopamin. The disorder in metabolism of dopamin causes following diseases: Morbus Parkinson, Morbus Huntigton or schizophrenia.

Methods

Because the cell contains phosphorylated proteins only in very low amounts, usually less than 10% ,specific enrichment methods for selective separation of phosphorylated proteins are necessary. The phosphorylation and dephosphorylation reaction is reversible. Phosphoproteins are not very stable and there is always risk of alteration during purification processes. For this reason it is important to protect the phoshoproteins by use of phosphatate inhibitors. Purification without inhibitors the phosphate groups might be partially or completely removed and a quantitative analysis is no possible (6). For the enrichment of phosphorylated proteins different methods can be aplied: Affinity chromatography using antibodies Different monoclonal or polyclonal antibodies for serine, threonine and tyrosine are commercially available. This method is very expensive and the achieved results showed non specific separation of phosphorylated proteins. Only very limited number of proteins can be separated using this method. Chemical enrichment using beta-elimination The beta-elimination can be used for the enrichment of phosphorylated proteins.This method has limitations, because phosphorylated serine reacts faster than phosphorylated threonine and phosphorylated tyrosine does not react with chemicals, such as hydroxide (7). This method is cheaper than affinity chromatography using antibodies, but there is observed very often degradation of proteins and the differentiation between carbohydrates and phosphate groups is non possible.

- IMAC immobilized metall affinity chromatography

Phosphorytaled proteins contain negatively charged phosphate groups, which are the reason for their high affinity to positively charged metal ions. Neville et al. used NTA Sepharose loaded with Fe^{3+} for the enrichment of cystic fybrosis transmembrane conductance regulator. The analysis of separated proteins was performed using mass spectrometry. But the problem of this method is simultaneously enrichment of acidic proteins and free phosphate groups in biological samples. These cross reactions cause contamitation of the purified protein. Fe^{3+} is not the only metal ion, which is used for IMAC, other bi- and trivalent ions, such as Ga^{3+}, Zr^{3+}, Al^{3+}, Zn^{2+}, ruthenium, antimony, cerium, indium, germanium, yttrium, tantalium, lutenium, can also be applied for purification of phosphoproteins. The bigest problem in the enrichment of phosphorylated proteins is the

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selectivity of this method. We developed a new adsorber for IMAC chromatography, which is useful for the separation of phosphorylated proteins. The method is very easy to use and the cost are low. The separation conditions, such as buffer, pH, capacity, specifity were optimized. The selectivity was proven by standard phosphoproteins and phoshate free proteins. We also use Saccharomycces cerevisiae as a test organism for phosphoproteome analysis. The identification of phosphorylated standard proteins was performed using Pro-Q Diamond phosphoprotein gel stain and mass spectrometry. The results show high recovery and specific enrichment of phosphorylated standard and Saccharomyces cerevisiae proteins. It is important to use only optimized conditions, specially binding buffer should contain aspartic and glutamic acid, to hinder unspecific affinity of acidic proteins (8).

Literature

1. Bachman KE., and Park BH. Curr Opin Oncol. Jan. 17(1): 49-54 (2005)
2. Rank KB., Pauley AM., Bhattacharya K., Wang Z., Evans DB., Fleck TJ., Johnson JA., Sharma SK. FEBS Lett. Mar 13; 514 (2-3): 263-8 (2002)
3. Müllner C., Vorobiov D., Bera AK., Uezono Y., Yakubocich D., Frohnwieser-Steinecker B., Dascal N., Schreibmayer WJ. Gen Physiol. May; 115(5):547-58 (2000)
4. Cohen P. Trends Biochem Sci 17, 408-413 (1992), Blom N., Sicheritz-Ponten, T., Gammeltoft S., Brunak S. Proteomics 4, 1633-1649 (2004)
5. Thaler F., Valsasina B., Baldi R., Xie J., Stewart A. Anal Bioanal Chem 276, 366-373 (2003)
19/38
6. Mann M., Ong S., Gronborg M., Stehen H., Jensen O.N., Panday A. Trends in Biotechnology 20, 261-268 (2002)
7. Neville DCA., Rozanas CR., Price EM., Gruis DB., Verkamn AS., Towsend RR. Prot Sci. 1997 Jun: 6:2436-2445 (1997)
8. Wolschin F., Wienkoop S., Weckwerth W. Proteomics 5, 1-9 (2005)

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Poster ***Abstract 18***

Phosphoproteome Analysis of the Human Mitotic Spindle

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Authors : Xiuling Li, Marjaana Nousiainen, Herman Silljé, Guido Sauer, Erich Nigg, Roman Körner
Topic : Phosphorylation

Content : During cell division, the mitotic spindle segregates the sister chromatids into two nascent cells, such that each daughter cell inherits one complete set of chromosomes. Errors in spindle formation can result in both chromosome missegregation and cytokinesis defects and hence lead to genomic instability. To ensure the correct function of the spindle, the activity and localization of spindle associated proteins has to be tightly regulated in time and space. Reversible phosphorylation has been shown to be one of the key regulatory mechanisms for the organization of the mitotic spindle. The relatively low number of identified in vivo phosphorylation sites of spindle components, however, has hampered functional analysis of regulatory spindle networks. A more complete inventory of the phosphorylation sites of spindle-associated proteins would therefore constitute an important advance. Here, we describe the mass spectrometry-based identification of in vivo phosphorylation sites from purified human mitotic spindles. In total, 1696 phosphorylation sites were identified, using IMAC, SCX, and TiO2. A comparison of the sensitivity and specificity of these phosphopeptide enrichment methods will be presented. Furthermore, we show preliminary results on the quantitative comparison of spindle protein phosphorylation events in metaphase and anaphase/telophase.

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Poster ***Abstract 19***

CDC25B phosphorylation by p38 and MK-2: a study combining MASS SPECTROMETRY and the use of SPECIFIC ANTIBODIES

Mr LEMAIRE Matthieu / lemaire@cict.fr

Authors : Matthieu LEMAIRE, Carine FROMENT, Rose BOUTROS, Odile MONDESERT, Odile BURLET-SCHILTZ, Angel R. NEBREDA, Bernard MONSARRAT and Bernard DUCOMMUN
Topic : Phosphorylation

Content : The CDC25B phosphatase is a key regulator that controls entry into mitosis through the dephosphorylation and subsequent activation of the cyclin-dependent kinases (CDKs). CDC25B appears to play a key role in the checkpoint response to DNA injury. Here we present our work dedicated to the identification of phosphorylation of CDC25B by p38SAPK and MAPKAP Kinase-2. To this aim we combined the use of mass spectrometry and the use of specific antibodies against phosphorylated CDC25B residues. We report that MAPKAP Kinase-2 phosphorylates CDC25B on multiple sites including S169, S323, S353 and S375, while p38 phosphorylates CDC25B on S249. We show that the S323-phosphorylated form of CDC25B is detected at the centrosome during a normal cell cycle.Since most of these sites are also phosphorylated by several other kinases, our observations highlight the difficulty in characterising and understanding in vivo phosphorylation patterns.

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Poster ***Abstract 20***

Post-Translational Protein Mapping by Automated Nanoelectrospray Combined with an Orbitrap

Dr LU Aiping / lu@biochem.mpg.de

Authors : Ai-Ping Lu [1]; Leonie F.Waanders [1]; Reinaldo Almeida [2]; Jesper V. Olsen [1]; Guoqing Li [1]; Matthias Mann [1]
1 Department of Proteomics and Signal Transduction, Max-Planck Institute for Biochemistry, Munich, Germany
2 Advion Biosciences Limited, Norfolk, UK
Topic : Mass spectrometry

Content : Research on post-translational modification is increasingly important, but their diversity and substochiometric level makes the characterization of all PTM sites of a single protein challenging. With the aim of full characterization, we here introduce a sensitive method with high dynamic range, involving automated nanoelectrospray (TriVersa NanoMate, Advion BioSciences, Ltd) coupled to a linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap, Thermo Electron Corporation). In this method, a normalized full survey spectrum is acquired by combining multiple survey scans of partial m/z ranges. The intermediate C-trap is filled to capacity by multiple injections with only the ions of each m/z range. Each m/z range is acquired several times, to improve the mass accuracy and sensitivity of the composite full spectra. In this way, the resulting composite spectrum is 'normalized' and features a very high dynamic range. Low ppm mass accuracy was achieved, and the dynamic range was more than 1000 in the composite full spectra. Peaks identified in the composite spectrum are fragmented differently depending on their intensity. For intense peaks (at least 50,000 ions per second) MS/MS is performed by high energy injection into the C-trap and the fragment mass spectrum is acquired in the orbitrap with a high mass accuracy and without low mass cut-off. For the less intense peaks, MS/MS and MS3 can still be performed in the ion trap. MS and MS/MS data are extracted by in-house written software and can be searched in 'real time' during the nanoelectrospray experiment. We have successfully applied the nanoelectrospray – orbitrap combination for the identification of modifications such as phosphorylation. Advantages of this method are that no LC separation is needed, and the whole procedure can be finished in a few minutes. Compared to direct MALDI TOF or MALDI TOF/TOF characterization, much higher data quality is obtainable. The sensitivity and signal to noise ratio of the low intense peaks are improved in the composite full spectra (composed of multiple SIM scans) compared to normal full survey spectra.

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Poster ***Abstract 21***

Detection and Identification of Unexpected/Novel Covalent Modifications in Mass Spectrometry Datasets using Protein Prospector

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Mass Spectrometry Facility, Deparment of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94143-0446, USA.
Topic : Mass spectrometry

Content : Enzymatic digestions followed by exhaustive mass spectrometric analysis are frequently utilized for the qualitative and quantitative characterization of the proteins in complex samples of biological origin (different body fluids, cell lines, cell organelles or protein complexes). Using the resulting large datasets, databases are interrogated by search engines such as ProteinProspector, Mascot, or Sequest to characterize the protein mixture composition. During such interrogations only a relatively small portion of the good quality CID spectra are matched with suitable scores and thus assigned. Some spectra are not matched due to methodological errors in the determination of a peptide's mass and/or charge state. However, more interestingly, some unmatched spectra correspond to: a) proteins that are not present in the database; b) peptides that contain covalent modifications (post-translational, xenobiotic or artifactual by sample-handling); c) products of non-specific enzyme cleavages or d) amino acid substitutions. In this poster we present a search strategy which can consider a wide range of integer mass shifts on any selected amino acids for the list of proteins identified from an initial strict database search. Furthermore an expectation value is associated with each hit to facilitate assessment of its potential validity. Combining searches of different levels of complexity can lead to a much more comprehensive sample analysis and to the identification of unexpected/novel covalent modifications. We present examples for the identification of sample-preparation related side-products, post-translational modifications, and identification of cross-linked peptides. We acknowledge support from the Vincent J. Coates Foundation and NCRR RR01614.

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Poster ***Abstract 22***

Characterization of the TSPN domain of the Collagen V N-terminal region

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Authors : I. Zanella-Cleon, E. Vaganay, M. Beraud, C. Bonod- Bidaud, C. Geourjeon, M. Becchi and F. Ruggiero
Topic : Mass spectrometry

Content : Fibrillar collagens represent the principal structural proteins of connectives tissues. Assembly, growth and organization of collagen fibrils in tissues are mainly controlled by the quantitatively minor fibrillar collagens, type V and XI. These two collagens are closely related and present a common N-terminal noncollagenous domain that is thought to play a crucial role in the regulation of fibrillogenesis. This region contains a domain called TSPN originally identified in thrombospondin. With the aim to further elucidate the function and mechanism of action, we have decided to undertake a biochemical characterization of the TSPN domain. This domain was recombinantly expressed in 293 HEK cells and purified by two steps of ion exchange chromatography. TSPN construct starts with the signal peptide, includes the 217 amino acids of TSPN and stops at the BMP-1 cleavage site. Electrophoretic analysis of the purified domain [37-254] under reduced conditions showed a band at 34 KDa whereas the expected molecular weight was 24.3 KDa indicating that post-translational modifications may occur. Two potential N-glycosylation site were predicted using the NetNGlyc 1.0 Server for Asn159 and Asn176. This was confirmed by the mass shift observed on SDS-PAGE after N-Glycosidase F treatment and by mass spectrometry of TSPN. Determination of N-glycosylation sites was performed by LC/MS/MS analysis (Q-STAR XL, Applied Biosystems) of trypsin peptides after PNGase F digestion. Only one site of N-Glycosylation was effective as only the Asn176 was changed in Asp176 in the trypsin peptide [176-184]. The characterization of the N-Glycated part of TSPN is under investigation. TSPN contains 4 conserved cystein residues that are thought to form disulfide bonds. Disulfide mapping was thus performed on the non-reduced N-deglycosylated TSPN (PGNase F treatment in non-reduced conditions) after trypsin digestion. The MS/MS spectra of the tetra sulfide bonds tripeptide at m/z 884.41 (+5) and m/z 1105.26 (+4) allowed the Cys62_Cys244 and the Cys183_Cys237 disulfide bonds characterization. Molecular modeling reveals a compact structure of this N-terminal domain that was confirmed using rotary shadowing likely reinforced by the formation of the disulfide bonds. Collagens form a super family of proteins of 28 members. Of these, sixteen share a TSPN domain. This domain may be a key factor in the establishment of molecular interactions that play a role in cell interactions with its surrounding extracellular matrix, as well as interactions between the molecular components of the extracellular matrix.

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Poster ***Abstract 26***

MALDI TOF/TOF de novo sequence analysis of 2D-PAGE separated proteins from *Halorhodospira halophila*, a bacterium with unsequenced genome

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Authors : Samyn, B.; Sergeant,K.; Memmi, S.; Debyser,G.; Devreese B.; Van Beeumen J.
Topic : Mass spectrometry

Content : Because protein identifications rely on matches with sequence databases, high-throughput proteomics is currently largely restricted to those species for which comprehensive sequence databases are available. The identification of proteins derived from organisms with unsequenced genomes mainly depends on homology searching. Recently, we reported the use of a simplified, gel-based, chemical derivatization strategy for de novo sequence analysis using a MALDI TOF/TOF mass spectrometer. This approach allows to determine de novo peptide sequences of up to 20 amino acid residues in length (1,2). Here, as a proof of principle, we applied our improved MS identification approach to identify a number of 2D-PAGE separated proteins from *Halorhodospira halophila*, an extremophilic eubacterium with yet unsequenced genome. (Partial) sequences of tryptic peptides were submitted to homology-search for identification of the corresponding protein. For this purpose, we applied three different homology-based search algorithms, FASTS, MS-Blast and MS-Homology. Using these search algorithms, we were able to identify more than 30 proteins from this organism using sub-picomole quantities of protein (3).

(1) Samyn, B.; Debyser, G.; Sergeant, K.; Devreese, B.; Van Beeumen, J. J. Am. Soc. Mass Spectrom. 2004,15, 1838-1852.
(2) Sergeant, K.; Samyn, B.; Debyser, G.; Van Beeumen, J. Proteomics 2005, 5, 2369-2380.
(3) Samyn, B.; Sergeant, K.; Memmi, S.; Debyser, G.; Devreese, B.; Van Beeumen J. Electrophoresis 2006, 27, 2702-2711.

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Poster ***Abstract 27***

Analysis of Glycopeptides by LC-MALDI-MS/MS and combined ETD/CID on the Ion Trap

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Authors: Markus Lubeck, Anja Resemann, Arndt Asperger, Ralf Hartmer, Andreas Brekenfeld, C. Baessmann, Lars Vorwerg and Detlev Suckau
Topic : Glycosylation

Content :

Introduction

The detailed characterization of glycopeptides remains being one of the greatest challenges for mass spectrometry based protein structure characterization. The structural complexity and variability of glycopeptide structures and the heterolog susceptibility to mass spectrometric fragmentation techniques of the carbohydrate part and the peptide part of glycopeptides are amongst the greatest challenges. In addition, the great complexity of glycoproteomes or glycoprotein digests requires its reduction by pre-fractionation and chromatographic efforts. We describe here a simple and rapid method for the analysis of glycoproteins, combining reduction of complexity of the digest mixtures by means of glyco-specific magnetic nanoparticles with subsequent LC-MALDI-TOF MS/MS analysis with DHB on spatially refined hydrophilic sample patches and with ESI-ETD/CID in a novel scan scheme. Although MALDI is well suited for such analysis, glycopeptides are difficult to analyse by MALDI using the standard peptide matrix -cyano-4-hydroxycinnaminc acid (CCA), albeit this is the typical matrix for high throughput peptide analysis. Dihydroxybenzoic acid (DHB) is the matrix of choice for carbohydrate and glycopeptide analysis. We used hydrophobic target surfaces with prestructured hydrophilic patches of small dimensions (AnchorChips) to achieve homogeneous surface morphologies of MALDI samples prepared with DHB suitable for automated measurements. While CID conditions for electrosprayed ions can often be optimized to prefer sugar fragmentation to a certain degree, a selective analysis of the peptide chain itself usually requires enzymatic removal of the carbohydrate residue. The recently introduced electron transfer dissociation (ETD) option for ion trap mass spectrometers shows selective fragmentation of the peptide backbone while leaving post translational modifications widely intact. Thus, a combination of both fragmentation techniques is a highly promising tool for the analysis of glycopeptides.

Methods

Glycopeptides were extracted from protein digests using various types of glyco-specifically functionalized magnetic nanoparticles: Concanavalin A, wheat germ agglutinin, boronic acid. Captured glycopeptides were analyzed by MALDI using a Bruker Ultraflex II TOF/TOF with DHB as matrix. At higher sample complexity, LC-MALDI analyses were used for protein identification and detection of glycopeptides. All samples were prepared on hydrophobic sample plates with hydrophilic anchors 400 or 600 µm in diameter confining the sample dimensions. Part of the glycopeptide eluate was treated with PNGase F to separate N-glycans from the peptide. The PNGase treated eluate was then analyzed by MALDI with or without LC separation. A quadrupole ion trap MS with ETD option was used to control in a non-redundant fashion the fragmentation techniques ETD and CID. For fragmentation via electron transfer dissociation (ETD), radical anions of fluoranthene were generated in a negative chemical ionization source and added to previously isolated multiply charged peptide cations inside the ion trap. Tryptic digests of horseradish peroxidase were separated by preparative reversed phase HPLC. Fractions containing glycopeptides were analyzed using offline nano spray in positive ion mode.

Results

The developed LC-MALDI method with the DHB matrix was applied to model protein mixtures and to digested human serum after treatment with glyco-specific beads on the intact protein level. After digestion, a second-round glyco extraction was followed by LC-

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MALDI with and without PNGase F pre-treatment. Information regarding the glycan structure of detected glycopeptides and of the protein/peptide ID could be derived from the respective MALDI-MS/MS spectra from the untreated and from the PNGase treated aliquot of the sample, providing glycoprotein and peptide IDs together with respective glycosylation sites. MALDI-LID-MS/MS spectra allowed to unequivocally assigning the peptide-only MH+ information in the MS/MS spectra by specific fragment ion patterns. This information permitted the definition of protein sequence database searches using glycopeptide spectra for the direct identification of the respective peptide. After considering the peptide part of the structure the carbohydrate part could be characterized from the same spectrum Glycopeptides from a pre-fractionated tryptic digest of horseradish peroxidase as well as complete digests of other glycoproteins were investigated using various ETD and CID techniques. Complementary data on these high mannose glycopeptides was obtained with the combination of both fragmentation methods: ETD allowed for highly selective fragmentation of the peptide backbone even of large, highly charged peptides resulting in a broad distribution of c and z fragments due to random fragmentation of N-C (alpha) bonds without internal heating of the peptide. CID in positive mode could be adjusted to fragment preferably sugar structures showing subsequent losses of individual sugars. This combined approach enabled substantial characterization of glycopeptides with regard to determination of peptide sequence and modification sites, as well as comprehensive information about composition and structure of the carbohydrate moiety.

Conclusion

Both described approaches yield selective information about the protein sequence and the carbohydrate structure from single glycopeptides and will allow mapping identified detailed carbohydrate structures to the characterized glycopeptides.

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Poster ***Abstract 28***

Neutral Loss triggered ETD (electron transfer dissociation) for the Characterization of Post translational Modifications (PTMs)

Authors : Ralf Hartmer; Markus Lubeck; Carsten Baessmann; Andreas Brekenfeld; Arnd Ingendoh
Topic : Phosphorylation

Content : MS analysis of protein phosphorylation is still one of the most challenging tasks in proteomic research. One inherent reason for the limit of phosphorylation identification is the relative low abundance of phosphorylated peptides. Second, the peptide characterization by CID (collision induced dissociation) MS/MS is difficult, as CID results preferably in the neutral loss of phosphate, i.e. peptide chain fragments are generally low abundant. Electron transfer dissociation (ETD), a new MS/MS fragmentation technique, leads to a prompt peptide fragmentation, leaving the PTMs intactly bound. However, the duty cycle for ETD (80-120 msec, including trapping of anion reagents) is longer than for CID MS/MS (40 msec), which limits the application for ETD for analyzing a huge number of precursor ions on a chromatographic time scale. A non-linear Paul trap, which is capable to perform either CID or ETD, has been modified to enable a data dependent neutral loss triggered ETD acquisition mode. CID MS/MS is used as the preceding fragmentation technique for PTM detection. If a phosphate loss from a doubly or triply charged precursor ion is detected, then the previous selected parent ion is automatically chosen for an additional and more selective ETD experiment. The neutral loss triggered algorithm combines the advantages of two different MS/MS ion activation techniques: a) Fast and sensitive CID within a high capacity ion trap b) Peptide backbone selective ETD fragmentation, which is particularly suitable for PTM characterization So, we present here for the first time a neutral loss triggered autoMS/MS acquisition mode by using an orthogonal and more suitable ETD fragmentation for PTM characterization. The general concept of the present approach has been evaluated analyzing standard samples as well as digests of natural phosphorylated proteins. By applying the neutral loss triggered ETD we were able to identify and characterize several previous unknown phosphorylation sites. Particularly the ETD has proved its capability for PTM identification, as uncertain CID results of phosphorylated peptides were unambiguously characterize by the more selective ETD. The advantage of the current approach in comparison to common neutral loss triggered CID MS/MS and to ETD MS/MS without a preceding neutral loss triggering will be presented.

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Poster ***Abstract 29***

Evaluation of Different Stable Isotopic Labelling Approaches to Protein Quantification in LC-MS/MS

Dr SUCKAU Detlev / dsu@bdal.de

Authors : Wolfgang Jabs; Stephanie Hahner; Sven Brand; Lars Vorwerg; Ulrike Schweiger-Hufnagel; Detlev Suckau
Topic : Mass spectrometry

Content : Experiments based on stable isotope labeling and LC-MS/MS attract more and more attention in quantitative proteomics. A wealth of different labeling chemistries like ICAT, ICPL, iTRAQ, 18O/16O, and SILAC have gained popularity and are increasingly applied in studies of post translational modifications (PTMs), and biomarker discovery. The labelling strategies provide a broad proteome coverage and increased statistical relevance of quantitative results and can be combined with many different types of mass spectrometers like MALDI-TOF/TOF, Ion Trap, Q-OTOF and FTMS. Furthermore, different statistical models and presentations can be applied to gain quantitative information from such experiments. Evaluation of some of these options was undertaken to choose viable approaches for quickly obtaining the most reliable quantitative results. In this study we compare protein regulations of standard protein mixtures derived from different labeling strategies (iTRAQ and ICPL), different types of mass spectrometers (MALDI-TOF/TOF, high capacity ion trap, QTOF), area or intensity of isotopic peak clusters, and different statistical parameters. All quantitative determinations of protein abundance ratios were calculated by applying equations following form lognormal theory. Peptide abundance determinations derived from MALDI measurements were based on the SNAP algorithm which determines the exact monoisotopic mass, resolution, fitted intensity of the monoisotopic peaks, and the area under the isotopic peak clusters. Mixtures containing 15 standard proteins at varied quantitative composition, covering a broad dynamic range were modified for quantification either with ICPL or iTRAQ labels on the protein and the peptide level, respectively. They were analyzed with the different mass spectrometers in capLC-MS/MS experiments. Peak intensities or peak areas were used to derive the desired peptide and protein regulation ratios. Average values as well as median values were considered as parameters for the report of protein regulations. For a fast assessment of the different experimental workflows and parameters a novel integrated software platform was deployed which presents raw LC-MS/MS data, protein identification, and protein quantification results in several combined graphical views, charts, and tables. Under this single platform, all the required parameters described in this study, were evaluated.

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Poster ***Abstract 30***

Screening for glyco-proteins in human serum using glyco-specific magnetic beads for enrichment and LC-MALDI

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Authors : Katrin Sparbier, Arndt, Asperger, Irina Kessler, Thomas Wenzel, Markus Kostrzewa, Yann Hebert
Topic : Glycosylation

Content : In the course of comprehensive proteomic analyses, high sample complexity and the enormous dimensions of the concentration range to be covered are the main difficulties. Thus, direct analysis of post-translationally modified peptides and proteins is often impossible. Selective enrichment and purification of peptides and proteins can be attained by chromatographic methods as supported by specifically functionalized magnetic particles. Glyco-proteins and-peptides can be isolated by lectin affinity chromatography or covalent binding to boronic acids. According to the type of glycosylation and the presented carbohydrate motif, binding to different functionalities is favoured. Human serum contains a high number of glyco-protein species comprising several orders of magnitude in concentration. Thereby, isolation and subsequent identification of low abundant glyco-proteins from complex serum samples is a challenging task. Human serum (20 µl) was incubated with different magnetic micro particles functionalized with Concanavalin A (ConA), wheat germ agglutinin (WGA) and boronic acids, respectively. Isolated proteins were released from the beads under acidic conditions, dried and subsequently re-dissolved and digested with trypsin over night at 37°C. The resulting complex mixture of peptides was separated by LC on a PepMap C18 column applying a gradient from zero to 45 % acetonitrile within 110 min. 384 fractions were directly spotted on a disposable AnchorChip target pre-spotted with HCCA matrix and analyzed by an Ultraflex II TOF/TOF. Applying this fast and simple approach, 101 proteins were identified from human serum comprising 84 known glycosylated proteins. According to the specific binding preferences of the different types of beads, complementary results were obtained from experiments using magnetic ConA-, WGA- and boronic acids beads, respectively. An overlap of 14 glyco-proteins was detected by all three bead functionalities together. The two lectins in use, ConA and WGA, as expected, showed a certain similarity in their binding behavior, revealing 10 shared glyco-proteins. In contrast, the overlap of the identified glyco-proteins from WGA and ConA with boronic acids was only 3 and 5 proteins, respectively. The fraction of glyco-proteins uniquely captured by boronic acid beads comprised four proteins with C-linked glycosylation. The approach described here could be extended to the more detailed analyses of glycosylation in complex biological samples in the sense of mapping N-glycosylation sites or full characterisation of glycan structures.

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Poster ***Abstract 31***

Protein Extractor - from peptide ID to protein ID

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Authors : Martin Blueggel; Gerhard Koerting; Katrin Marcus; Daniel Chamrad; Ulrike Schweiger-Hufnagel; Jörg Glandorf; Meyer Helmut E.; Herbert Thiele
Topic : Mass spectrometry

Content : Today's proteomics workflows can be divided into two strategies: One strategy consists of a first step of protein fractionation followed by proteolytic digestion to peptides. The other strategy is based on peptide separation. Here the proteins are first digested and peptides are then separated and subjected to identification by mass spectrometry. In the latter case the peptide assignment to a protein is lost and has to be rebuilt by bioinformatics methods. Current approaches show very little transparency, disregard isoform distribution, utilize rough estimates or sophisticated training is needed. We present Protein Extractor which is a module of the ProteinScape Bioinformatics Platform and uses an empiric method to derive protein identification lists from peptide search results. The algorithm was implemented as a SQL stored procedure operating on the relational database system which serves as a backbone for ProteinScape. Protein Extractor uses an iterative approach to generate a minimal protein list. It could be shown that this approach obeys the rules setup by mass spec experts. ProteinExtractor was tested with Mascot, Sequest, Protein Solver and the IPI or NCBInr database elongated by a decoy database. For algorithm training, two MS/MS data training sets with known proteins were analysed. The algorithm was then applied to a bigger data set from an E. Coli lysate (24482 spectra). Generating a MS/MS data training set is not trivial. Isolated or recombinant proteins have non-product related impurities (typical 0,1% to 5%.) Product related impurities like different isoforms (e.g. alternative splice products) are still often unknown for well characterized protein samples. We generated two protein standards with 5 and 19 proteins and identified their correct mapping to the most suitable protein sequence entries of the sequence database with several MS/MS datasets acquired on the same sample and intensive manual data interpretation by several mass spectrometry experts. With standard search algorithms (MASCOT, SEQUEST) the correct protein sequence database entries of the training set 1 (5 proteins, no isoforms) are distributed over the top 230 protein ranks of the search results (sequence database NCBInr). This is because of high similarities of protein sequence entries and false positive protein identification. Therefore the expert has to manually validate a factor of 46 more protein candidates that can be approved by the underlying ms/ms data (non provable identification factor NPIF=46). Together with application specialists a set of rules was determined to define a minimal protein list, which contains only those proteins, which can be distinguished by the MS/MS data. Applying these rules the correct 5 proteins of training set 1 are ranked on the top 7 protein candidates list (NPIF= 1.4). For training set 2 the NPIF value could be reduced from 13.5 (without Protein Extractor) to NPIF= 1.4. The algorithm was then applied to real life ms/ms datasets from unknown protein mixtures. To measure efficiency and compare results to existing approaches a decoy database was utilized. In the HUPO BPP the ProteinExtractor was used on app 4000 ms/ms datasets with overall 750 000 MS/MS spectra to generate maximal protein list content with a defined false-positive rate.

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Poster ***Abstract 32***

MALDI Imaging: Solutions for Sample Preparation an

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Authors : Detlev Suckau, Soeren-Oliver Deininger, Martin Sch
Topic : Mass spectrometry

Content : MALDI imaging is a technique with increasing importance for marker discovery and in clinical research. The sample preparation, especially the application of matrix onto the sample is of utmost importance on the quality of the results. The main parameters in judging the results are the achievable spatial resolution and the spectra quality, unfortunately these parameters seem to be negatively correlated. Here, various preparation methods, such as pneumatic spray, robotic and manual spotting were evaluated. The images are usually analysed visualizing the spatial resolution of selected peaks on an image. The data however contain more complex information, such as overall changes in the proteomic pattern. Here we discuss how such information can be tapped. Tissue sections (human gastric cancer) were prepared on a microtome, transferred onto electrically conductive glass slides were used for the experiments. Matrix was applied either by pneumatic spray, by manual deposition of matrix droplets or by robotic spotting of matrix arrays. Images were acquired with a MALDI-TOF/TOF instrument equipped with a 200 Hz smartbeam all-solid state laser with changeable focus size (~120 µm to 10 µm). Data were evaluated using dedicated software packages: FlexImaging for the direct image analysis of the MALDI data and ClinProTools for generating multivariate statistical models which were employed to distinguish tissue areas of different health status. The best spectra quality was achieved by straight forward pipetting matrix droplets onto the tissue. Local differences on one matrix spot were equalized by accumulating spectra from different raster points, providing very reproducible spectra. Robotically spotted micro droplets provided reproducible spectra as well and more peptide signals compared to spray preparation and less compared to manual preparation. We assume that this is directly correlated with the time of solvent exposure and the amount of matrix. To achieve good quality of spectra at maximal spatial resolution it proved necessary to optimize preparation parameters such as the number of repeated matrix applications, solvent composition and matrix concentration. It was easily possible to distinguish tumor from non-tumor areas, because regulated peaks differ significantly in intensity. When tissues were compared that contained only homogenous tumor or non-tumor regions the simple presence or absence of a peak was not sufficient to classify the tissue because of the lack of an “internal control”. The peak profile in the spectrum as such was used in this case for unambiguous classification of the tissue using multivariate statistical models.

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Poster ***Abstract 34***

Disulfide Structure and Post Translational Modifications

Dr WEN Dingyi / dingyi.wen@biogenidec.com

Authors : Dingyi Wen, Craig P. Wildes, and R. Blake Pepinsky
Topic : Mass spectrometry

Content : Title: Disulfide Structure and Post Translational Modifications of the Leucine-Rich Repeat C-Terminal Cap and C-Terminal
Authors: Dingyi Wen,* Craig P. Wildes, Laura Silvian, Lee Walus, Sha Mi, Daniel H. S. Lee, Werner Meier, and R. Blake Pepinsk
Nogo-66 receptor (NgR1) is a leucine-rich repeat (LRR) protein that forms part of a signaling complex modulating axon regeneration. Previous studies have shown that the entire LRR region of NgR1, including the C-terminal cap, LRRCT, is needed for ligand binding, and that the adjacent C-terminal region (CT stalk) of NgR1 contributes to interaction with its co-receptors. To provide structure-based information for these interactions, we analyzed the primary structure and disulfide linkages of full-length NgR1. Mass spectrometric analysis of a typtic digest of full length NgR1 confirmed 97% of the predicted NgR1 sequence and revealed eight unexpected sites of posttranslational modification: hydroxylation at Pro-352 and O-linked glycosylation at seven sites in residues 378-414. The hydroxylation site was identified by an MS/MS sequencing experiment and the glycosylation sites were determined by mass spectrometric analysis of the endo-Glu-C digest of sialidase-treated tryptic glycopeptide. Most interestingly, our disulfide structure analysis revealed a novel disulfide structure in the C-terminal region of the NgR1, wherein the two Cys residues, Cys-335 and Cys-336, in the CT stalk are disulfide-linked to Cys-266 and Cys-309 in the LRRCT region: Cys-266 is linked to Cys-335, Cys-309 to Cys-336. The other two Cys residues, Cys-264 and Cys-287, in the LRRCT region are disulfide-linked to each other. The analysis also showed that Cys-419 and Cys-429, in the CT stalk region, are linked to each other by a disulfide bond. Although published crystal structures of a recombinant fragment of NgR1 had revealed a disulfide linkage between Cys-266 and Cys-309 in the LRRCT region and we verified its presence in the corresponding fragment, it turns out that this is an artifact caused by the truncation of the protein, since this linkage was not detected in intact NgR1 or a slightly larger fragment containing Cys-335 and Cys-336. A structural model of the LRRCT with the extended residues 311-344 from the CT stalk region is proposed and its function in co-receptor binding is discussed.

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Poster ***Abstract 36***

The transmembrane domain of hTPST1 is the key element for enzyme dimerisation and Golgi targeting

Dr HARTMANN-FATU / cristina.hartmann-fatu@uni-due.de

Authors : Cristina Hartmann-Fatu, Christoph Wotzlaw, Sandra Goettsch and Peter Bayer
Topic: Other **Presentation type:** Poster

Content: Tyrosyl protein sulfotransferases (TPSTs) are a class of membrane-bound enzymes able to catalyse the transfer of a sulphate group from the universal sulphate donor, 3'phosphoadenosine-5'phosphosulphate (PAPS), to a Tyr rest of a target protein. Humans possess two isoforms of the enzyme, termed hTPST1 and 2. Both TPST isoforms are located in the membrane of the Golgi network being anchored by a single span type II helix, the transmembrane domain (TMD). The catalytic domain is facing the lumen of the Golgi compartment. Despite the fact that TPSTs were studied for decades, many questions concerning their biochemical and structural characterisation remain open. Here we bring evidence that TPST1 TMD forms dimers *in vivo*, inside Golgi membranes of live mammalian cells and that it represents an inbuilt targeting signal for enzyme localisation to this compartment.

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Poster ***Abstract 37***

Loss of Pin1 expression increases phosphorylation of Amyloid Precursor Protein at Threonin 668 in human cell cultures

Dr ANDO KUNIE / ando@lille.inserm.fr

Authors : Kunie Ando, Valérie Vingtdeux, Pierre Dourlen, Olivier Kerdraon, Séverine Bégard, André Delacourte, Malika Hamdane, Nicolas Sergeant, Claude-Alain Maurage, Marie-Christine Galas, and Luc Buée
Topic : Phosphorylation

Content : AD is characterized by a global cognitive decline and memory impairment. The two neuropathological hallmarks of the etiology of AD are neurofibrillary tangles (NFT) composed of Tau protein aggregates and amyloid beta peptides (Abeta) that compose amyloid plaques. Abeta derives from successive cleavages of a transmembrane precursor named APP for Amyloid Protein Precursor. APP is first cleaved by alpha-secretase or beta-secretase to generate membrane bound C-terminal fragments (CTFs), namely alpha- and beta- CTFs, respectively. Soluble N-terminal ectodomain are also realised from those cleavages. APP-CTFs are subsequently processed by gamma-secretase, releasing a 3-kDa peptide called p3 and a 4-kDa Abeta, from alpha- and beta-CTFs, respectively. A growing body of evidence suggests that phosphorylation of APP regulates its cleavage by secretases. Alzheimer brains show an increased Threonine 668 (Thr668) phosphorylation of APP-CTFs. Moreover, phosphorylation of APP-CTFs at Thr668 facilitates their processing by alpha and beta-secretases, thus resulting in increased Abeta production. Peptidyl prolyl isomerase Pin1 isomerizes the peptide bond between specific phosphorylated serine or threonine residues followed by a proline residue (pSer/Thr-Pro). APP Thr668 corresponds to such a motif. Moreover, Phosphorylation of APP at Thr668 changes the conformation of APP and affects the binding of molecular partners. Therefore, supposedly phosphorylation of APP at Thr668 might act as a conformational switch and is a potential target of Pin1. Recently, an interaction between APP and Pin1 has been evidenced, but discrepancies exist between the effects observed. Overexpression of Pin1 leads to either an increased or reduced Abeta production. To investigate the function of Pin1, we used SY5Y human neuroblastoma cells overexpressing APP wild type (SY5Y APPWt cells) and Human Embryonic Kidney cells overexpressing APP wild type (HEK APPWt cells). We provide evidence that Pin1 co-immunoprecipitates alpha-CTF and conversely, APP-CTFs pull-down Pin1. Loss of Pin expression by siRNA showed an increased phosphorylation of APP at the Thr668 site. However, in our study, using 4 different RNA duplex against Pin1, large discrepancies were observed. A decrease or increase of Abeta secretion was observed depending on the siRNA used, although a reduced expression of Pin1 was evidenced with all duplexes. From these results, it is assumed that Pin1 play an important role in the balance between phosphorylation-dephosphorylation of APP at Thr668 but discrepancies remain to fully explain the consequences toward Abeta secretion.

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Poster ***Abstract 38***

Profiling proteins co-aggregating with PrPres in the brain of experimentally infected hamster

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*Dipartimento di Scienze Biochimiche "A. Rossi Fanelli"
Topic : Other

Content : Prion diseases or transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders affecting humans (e.g., Creutzfeldt–Jakob disease) and animals (e.g., scrapie of sheep). Humans are affected by several forms of TSEs with different aetiology: sporadic Creutzfeldt–Jakob disease (sCJD) apparently arises as a spontaneous disease; genetic forms (e.g., familial CJD) are invariably associated with mutations of the PrP gene; infectious forms derive from a documented episode of accidental transmission. The presence of amyloid plaques accumulating in the brain is one of the pathological hallmarks of TSEs. Many lines of evidence have converged to argue persuasively that prion amyloids are composed largely, if not exclusively, of a host-encoded protein (PrPc), conformationally modified into a detergent-insoluble and protease-resistant isoform (PrPsc). Detailed molecular mechanism that translates this cellular protein into plaques and into neuronal damage and functional brain impairments is still largely unknown. Moreover, there is very little information on the participation of other cellular proteins in PrPsc aggregation. Specific host protein expression impairments have been reported both in scrapie infected neuronal cells and in diseased brain tissues. Immunohistochemical detection of specific proteins within prion-associated lesions in squirrel monkey brains was also reported. The aim of this study is to identify non-PrP components in the prion amyloid and shed light on the cellular microenvironment of the pathological form of PrP. Therefore, we employed a proteomic approach to identify proteins that interact with the protease-resistant prion protein accumulating in the brain amyloid of Syrian Hamster experimentally infected with scrapie. To give insight into the sub-proteome that associates with PrP and to setup a methodological approach to highlight possible difference in co-aggregating proteins among prion strains, the protein profiling was attempted by two complementary proteomic approaches: a 2DE mapping and an in toto MALDI-ToF fingerprinting. Apart the unambiguous identification of the hamster PrP, fingerprints of ferritin, CAM kinase, apolipoprotein E were unambiguously resolved. Overall, our results revealed for the first time the sub-proteome of prion amyloid plaques, a critical step in the study of the molecular mechanisms of TSE pathogenesis. For these non-PrP components, validation by immunodetection and immunocoprecipitation is in progress. Discussion on their possible role in PrP amyloidogenesis or in the PrP infective transmission will also be provided.

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Poster ***Abstract 39***

Phosphorylation of the microtubule-associated Tau protein in situ: regulation by the peptidyl prolyl cis/trans isomerase Pin1

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Authors : Dourlen P, Hamdane M, Bégard S, Buée L, Galas MC
Topic : Phosphorylation

Content : Posttranslational modifications include some molecular additions such as phosphorylation and conformational changes such as peptide bond isomerisation. In neurodegenerative disorders, major alterations were reported in the regulation of these posttranslational modifications. For instance, in Alzheimer's disease, hyperphosphorylated microtubule-associated Tau proteins aggregate into filaments in neuronal cells. Consequently, any regulator of Tau phosphorylation is of interest in this pathology. Recently, a peptidyl prolyl cis/trans isomerase named Pin1 has been shown to be involved in the regulation of some phosphorylation sites in Tau proteins. Its two domains, the WW domain and the catalytic domain specifically recognize phosphorylated Ser-Pro or Thr-Pro motives (p(Ser/Thr)-Pro). This interaction leads to isomerisation of the peptide bond between the cis and trans conformations within target proteins. Tau proteins contain 17 p(Ser/thr)-Pro sites. Some of them are hyperphosphorylated and present abnormal conformation-dependent phosphorylated epitopes in Tau aggregates. Pin1 has been previously shown, in vitro, to restore phosphorylated Tau function on microtubules and to facilitate Tau dephosphorylation. In addition, brains of Pin1 knock-out mice were shown to display an accumulation of hyperphosphorylated and abnormally phosphorylated Tau. Such observations would be related to the conformational changes produced by Pin1 on Tau proteins that would allow or prevent the activity of conformation-specific kinase or phosphatase. Indeed, Pin1 has been shown to facilitate the dephosphorylation of Tau by the trans-specific phosphatase PP2A. In vitro, Pin1 was reported to present a high affinity for both Thr231, a crucial site for microtubule binding, and Thr212. However, whether Pin1 binding to Tau leads to isomerisation of a single site or of multiple pSer/Thr-Pro sites in situ is still unknown. We have investigated the activity exerted by Pin1 on the dephosphorylation of endogenous full length Tau in two cellular models, the neuroblastoma SY5Y cell line and primary cortical neuronal cells. First, during the differentiation of the SY5Y cells, we observed a concomitant increase of Pin1 expression and the dephosphorylation of the particular Tau Thr231. In a Pin1 inducible SY5Y cell line, we confirmed that Tau dephosphorylation at Thr231 was a consequence of Pin1 overexpression. Then, in primary cortical neuronal cells, Pin1 involvement was investigated in a model of Tau dephosphorylation induced by oxidative or heat stresses, known to activate PP2A. In this system, Pin1 pharmacological inhibition or Pin1 deletion specifically reduced Tau dephosphorylation at Thr231. This was rescued by transfecting a cDNA coding for wild type Pin1 but not for a mutant of the catalytic domain, which assessed the isomerase activity of Pin1. Altogether, our results demonstrate that Pin1 is involved in a differential dephosphorylation of the full length multiphosphorylated Tau protein in situ.

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Poster ***Abstract 40***

Glycosylation of recombinant human C1 inhibitor: characterization and batch-to-batch consistency

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Authors : M. Germano, R.A.A. Zoetemelk, Y. Van Geertruy and M.L.M. Mannesse
Topic : Glycosylation

Content : Pharming Technologies B.V. is developing recombinant human C1 inhibitor (rhC1INH) produced in the milk of transgenic rabbits, as a treatment for Hereditary Angioedema. Human C1INH is a glycoprotein with six predicted sites for N-glycosylation and at least seven sites for O-glycosylation[1]. The occupancy of all the N-glycosylation sites in rhC1INH has been demonstrated by peptide mapping combined with mass spectrometry. The structures of the N- and O-glycans on rhC1INH have been determined by NMR[2] and mass spectrometry. RhC1INH contains a broad array of N-glycans, made-up of oligomannose-, hybrid- and complex-type structures. The complex-type structures are mono- or diantennary, mono- or disialylated, and some are core- or antenna- fucosylated (Lewis X). The O-glycans on rhC1INH are of the core 1-type, mono- or disialylated. For the characterization of rhC1INH batches, the glycans are analyzed routinely by four different chromatographic methods, all based on high-pH anion-exchange chromatography with pulsed-amperometric detection. Two of these methods are for glycan mapping of the N- and O-linked glycans. The other two methods are for quantification of neutral monosaccharides and sialic acid. The glycosylation analysis of rhC1INH batches as described above shows a high level of batch-to-batch consistency, demonstrating that the manufacturing of this complex recombinant therapeutic glycoprotein in the milk of transgenic animals is under control.

[1] http://www.expasy.org/uniprot/P05155
[2] Koles, K., van Berkel, P.H.C, Pieper, F.R., Nuijens, J.H., Mannesse, M.L.M., Vliegenthart, J.F.G. and Kamerling, J.P. (2004) Glycobiology 14(1), 51-64.

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Poster ***Abstract 43***

Role of the PKC phosphorylation in the regulation of the activity nuclear receptor PPAR alpha

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Authors : Benoit Pourcet, Christophe Blanquart, Jean-Charles Fruchart, Bart Staels and Corine Glineur
Topic : Phosphorylation

Content : PPARα (Peroxisome Proliferator-Activated Receptor alpha) belongs to the nuclear receptor superfamily. PPARα is activated by natural ligands such as polyunsaturated fatty acids (PUFAs) and synthetic ligands such as fibrates. PPARα regulates the transcription of genes involved in the metabolism of fatty acids, lipids, lipoproteins, and therefore plays an important role in anti-atherosclerotic processes. As numerous nuclear receptors, the activity and the stability of PPARα are tighly regulated by binding of ligands, recruitment of cofactors and also by post-translational modifications. PPARα stability is regulated by the ubiquitin-proteasome system (Blanquart et al., J Biol Chem 277: 37254-9). We have also shown that the interaction with cofactors modulates the stability of PPARα (Blanquart et al., Biochem Biophys Res Commun 319: 663-70). Finally, PPARα is phosphorylated by kinases such as cAMP-dependent protein kinase (PKA) and MAP Kinase and these phosphorylations regulate its transcriptional activity. The function of PPARα is also regulated by AMP Kinase. Interestingly, we have shown that PPARα is phosphorylated by protein kinase C, a kinase activated by fatty acid (Blanquart et al., Mol Endocrinol 18: 1906-18). By using directed mutagenesis technology, we have constructed mutant PPARα proteins by replacing the PKC target residues by non phosphorylable alanine residue or by aspartate residue mimicking the negative charge of the phosphate. Our result suggest that the phosphorylation of PPARα on serine 179, 230 could influence the function of PPARα by modulating its stability and its interaction with cofactors. The phosphorylation of PPARα by PKC could favor the nuclear storage of the protein in the absence of ligands.

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Poster ***Abstract 45***

A Proteomic Determination of the Whole Cell Proteins in Two Different Halomonas Strains

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3 TUBİTAK, Research Institute for Genetic Engineering and Biotechnology PK:21, 41470 Gebze Kocaeli, TURKEY
4 WITA GmbH, Warthestr.21 D-14513 Teltow, GERMANY
Topic : Functional analysis

Content : Halophiles are salt-loving organisms that inhabit hypersaline environments. They can balance the osmotic pressure of the environment and resist the denaturing effects of salt. Their novel characteristics and ability of culturing make them valuable for biotechnology. Halophiles can be classified according to their salt requirement. Slight halophiles grow optimally at 2-5% NaCl, moderate halophiles at 5-20% NaCl and extreme halophiles at 20-30% NaCl. Because of phylogenetic and physiological diversity among the halophiles, they may have appeared early during evolution. One of the most important environmental factor controlling the evolution and biodiversity of life is temperature. Temperature also limits the biological activity on earth. Other environmental factors that effect the biological activity are salinity, acidity, desiccation, radiation, pressure, and time. The aim of this work is to investigate the state of two Halomonas type strains and the halomonas isolates from Turkey by comparing whole cell proteins during the growth at different temperatures as 15^{o}C and 39^{o}C. The effect of salt concentration (%20 and %5) on protein expression is also studied. After growing the microorganisms at different temperatures (15^{o}C and 39^{o}C) and different salt concentrations (%5 and %20), high-resolution 2-DE maps of whole cell proteins are prepared according to the tube gel system with NEPHGE technique in the first dimension and SDS-PAGE in the second dimension. Acidic character of the whole cell proteins is detected in both type strains and the isolates. The 2-DE maps of several selected cultures at different environmental conditions are compared and selected spots are identified using MALDI-TOF MS (Waters/Micromass). The research presented in this work is conducted in the Chemical Engineering Department of Marmara University and supported by TUBITAK-MAG Research Project No: 104M236.

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Poster ***Abstract 46***

Posttranslational modification of cysteine or serine to formylglycine (FGly) in the active site of bacterial sulfatases

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Authors : Prem Kumar Sinha, Qinghua Fang and Thomas Dierks
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Topic : Other

Content : Sulfatases are members of a highly conserved gene family sharing extensive sequence homology and a unique posttranslational modification. This novel protein modification generates a unique amino acid, formylglycine (FGly), which directly participates in catalysis. FGly is post-translationally generated by oxidation of cysteine (eukaryotes and prokaryotes) or serine (prokaryotes), located in a conserved (C/S) XPXR motif. In mammals, this oxidation is carried out by the FGly-generating enzyme (FGE) during translocation of sulfatases into the endoplasmic reticulum. We recently identified FGE and determined its 3D structure. FGE defines a new protein family that is conserved in evolution. Structural and functional data suggest that FGE is an unusual oxygenase utilizing molecular oxygen as terminal electron acceptor without requirement of metals or other activating cofactors.

Earlier we have shown that AtsB, a SAM iron-sulfur protein, is the serine-modifying enzyme of Klebsiella pneumoniae sulfatase (AtsA) and SAM is the key cofactor for this modification involving radical redox chemistry. However, FGly-modification of Pseudomonas aeruginosa sulfatase (PAS), a cysteine-type sulfatase does not require co-expression of AtsB. Two kinds of sulfatases can be discriminated with respect to the amino acid residue that is converted to FGly, namely serine-type and cysteine-type sulfatases. Normally atsB and its orthologs are adjacent to serine-type sulfatase gene (atsA). However, interestingly we found that in the archaebacterium Methanosarcina mazei, an atsB ortholog (mm-atsB) is adjacent to a putative cysteine-type sulfatase (mms) ORF. In this work, we have tried to get answers for following questions: 1. Specificity of AtsB: Whether AtsB can only modify serine-type sulfatases (SxPxR) or it can modify cysteine-type (CxPxR) too? 2.What happens with Archaebacterium Methanosarcina mazei, a natural Cysteine-type sulfatase with unique modification motif (CTAGR): Can MM-AtsB and/or AtsB modify Cysteine-type sulfatase MMS? 3. What determines Serine- and/or Cysteine- specificity: Conserved modification motif or other sulfatase sequence elements (background) or both? 4. Role of signal peptide: whether sulfatase modification is temporally/ spatially related to sulfatase export to the periplasm?

Our experiments shows that AtsB-mediated modification is not restricted to serine-type sulfatases, and cysteine-type sulfatases can also be modified by AtsB. Moreover, another cysteine-modifying enzyme must be operative in E. coli modifying PAS. Experiments to define the structural elements of the sulfatases that determine this specificity are presented.

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Poster ***Abstract 47***

Fully automated off-line multidimensional LC methods in proteomics

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Authors : Robert van Ling, Bas Dolman, Evert-Jan Sneekes and Remco Swart
Topic : Other

Content : Multidimensional liquid chromatography (MDLC) coupled to mass spectrometry is a valuable strategy for bottom-up or top-down workflows in proteomics. A large number of different MDLC approaches has been described for the separation of intact proteins and peptides. Off-line MDLC techniques have several advantages over on-line approaches: i) higher flexibility with respect to column dimensions and mobile phase selection, ii) easier method development and iii) the ability to perform re-analysis of the fractionated effluent. Here we present a fully automated method for off-line MDLC of peptides and proteins. The method allows for combination of different column dimensions and chemistries to optimize the MDLC separation. The effluent from the first dimension column is re-directed to an 8-port injection valve and fractionated through the injection needle in a well plate or sample vials. The second dimension separation comprises a column switching configuration for on-line desalting, sample concentration and direct interfacing to MS. Alternatively for protein separations a second fractionation step can be implemented after the RP separation to allow for proteolytic digestion. Methods have been developed for 2D-LC of peptides and proteins using ion-exchange and reversed phase chromatography. Fractionation of the ion-exchange separation and subsequent injection of the fractions is performed automatically onto capillary PS-DVB monolithic columns. The fast mass transfer kinetics of the monolithic columns allow fast gradient separations and a reduction of the total analysis time of the 2D-LC method. Interfacing the 2D-LC separation with tandem MS sequencing of peptides showed that peptides eluted typically in one or two SCX fractions. In addition the system shows good precision performance for injection and fractionation of samples.

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Poster ***Abstract 50***

Differential expression profiling of *Trypanosoma brucei* glycosomal proteins by label-free multidimensional LC-MS.

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Topic : ?

Content : Trypanosomes are parasitic protozoa which cause important tropical diseases in humans such as African sleeping sickness. They possess several unusual biological features such as compartmention of several glycolytic enzymes in specific organelles called glycosomes. Furthermore, the energy metabolism changes drastically during the life cycle of these parasites and is reflected in the enzymatic content of glycosomes. A highly enriched glycosomal fraction was purified by differential centrifugation, followed by gradient centrifugation, from bloodstream (BS) and procyclic (PC) forms of *T. brucei*. Proteins were first separated according to their net charge on a strong anion exchange membrane and eluted by a drop in pH. After digestion with trypsin, peptides were separated on a strong cation exchange column with a linear salt gradient. Fractions were collected and further analysed on a reverse phase C18 column coupled to an ion trap mass spectrometer. Protein identification was performed with the Sequest algorithm and an “in-house” *T. brucei* protein database. Relative quantification of protein abundance was estimated by calculating the ratio of spectral counts determined within the BioWorks software package. This parameter was shown to follow a linear relationship over two orders of magnitude, as determined from spiked internal standard proteins, within a dynamic rang of at least 10^3.
Comparison of the glycosomal proteins from the two life-cycle stages revealed that the glycolytic enzymes were on average one order of magnitude more abundant in BS-form than in PC-form glycosomes. This is entirely in agreement with previous determinations based on activity measurements. Glyceraldehyde-3-phosphate dehydrogenase, aldolase, hexokinase and phosphofructokinase were the most abundant enzymes in the sample. Proteins that were equally abundant in both forms were phosphoenolpyruvate carboxykinase, arginine kinase, the glycosomal membrane protein GIM5B, mevalonate kinase and ribose-5-phosphate isomerase. Proteins that were exclusively associated with PC-form glycosomes were fumarate hydratase, pyruvate phosphate dikinase, malate dehydrogenase and transketolase. It could clearly be demonstrated that *T. brucei* glycosomes, in addition to glycolysis and hexose-monophosphate pathway activity, are also involved in the protection against oxidative stress (superoxide dismutase, trypanothione reductase, trypanothione peroxidase and peroxyredoxin) and purine- and pyrimidine metabolism (hypoxanthine-guaninephosphoribosyl transferase, adenylosuccinate synthase, inosine-monophosphate dehydrogenase and orotidine-monophoshate decarboxylase / orotidine-phosphoribosyltransferase). It is concluded that differential expression profiling using multidimensional label-free LC-MS is a powerful tool for better understanding trypanosome glycosomal metabolism.

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Poster ***Abstract 55***

Mapping of protein N-glycosylation sites by COFRADIC

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Authors :. Bart Ghesquière, Jozef Van Damme, Joël Vandekerckhove, Kris Gevaert
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Topic : Glycosylation

Content : The process of protein N-glycosylation is highly conserved during evolution. This reflects the importance of this co/post-translational modification that, amongst others, regulates protein secretion, antigenicity, folding, solubility and the half-life of glycoproteins. Mapping sites of N-linked glycans should aid in understanding the mechanisms that affect several (patho)physiological processes. Today, several methods are available for the enrichment of N-glycosylated proteins prior to analysis: glycosylated proteins can be affinity-captured by lectins (Geng *et al*, 2001) or, following oxidation, are chemically 'trapped' on a hydrazide resin and subsequently released by PNGaseF (Zhang *et al*., 2003).
We here present a novel method for the analysis of N-glycosylation sites in which the isolation of N-glycan containing peptides is based upon the gel-free, peptide-centric COFRADIC (COmbined FRActional DIagonal Chromatography) proteomics technology (reviewed by Gevaert *et al*., 2005).
Here, peptides carrying a glycosylated asparagine are separated from non-N-glycosylated peptides in two consecutive, identical RP-HPLC runs. A proof-of-concept study was carried out on alpha-1-acid glycoprotein, leading to the identification of several known glycopeptides. We then analysed the glycoprotein repertoire in MARS-depleted mouse serum and postulate that the COFRADIC sorting mechanism is well suited for the analysis of N-glycosylated peptides by identifying 117 known or predicted sites in addition to 10 novel sites of glycosylation in 10 µL of depleted mouse serum. Next to the analysis of several glycosylated sites we also show that the COFRADIC approach holds the potential for pinpointing to different forms of protein N-glycosylation in complex mixtures.

References : Geng M, Zhang X, Bina M, Regnier F, Proteomics of glycoproteins based on affinity selection of glycopeptides from tryptic digests, *J. Chromatogr. B. Biomed. Sci. Appl.* 2001, *752*, 293-306.
Gevaert K, Van Damme P, Martens L, Vandekerckhove J, Diagonal reverse-phase chromatography applications in peptide-centric proteomics: ahead of catalogue-omics? *Anal. Biochem.* 2005, *345*, 18-29.
Zhang H, Li X, Martin DB, Aebersold R, Identification and quantification of N-linked glycoproteins using hydrazide chemistry, stable isotope labeling and mass spectrometry, *Nat. Biotechnol.* 2003, *21*, 660-666.

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Poster ***Abstract 66***

Detection of cAbl kinase-promoted phosphorylation of Rad51 by specific antibodies reveals that the phosphorylation of Tyr54 is stimulated by that of Tyr315

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Authors : Milena Popova, Fabrice Fleury, Hiroko Shimizu, Ken-ichi Yamamoto and Masayuki Takahashi
Topic : Phosphorylation

Content : Rad51 plays a crucial role in homologous recombination and recombinational DNA repair, and is thus related to the resistance of cancer cells to radio- and chemotherapies. Its activity is regulated by phosphorylation by cAbl kinase. However, there is controversy about the position and effect of phosphorylation: some report that Tyr54 is the target of phosphorylation and other report that Tyr315 is the target. To clarify this point, we have prepared two antibodies using the peptides containing phosphotyrosine in the amino acid sequence either around Tyr54 or Tyr315 of Rad51. After two successive affinity chromatographies with the phosphorylated peptide and with the corresponding non-phosphorylated peptide, we obtained the antibodies which detected only the corresponding phosphorylated peptide. Western blotting of Hela cells transfected with the plasmid containing Rad51 gene or co-transfected with the plasmid containing cAbl and that containing Rad51 revealed that the both antibodies detected phosphorylated Rad51 but not non-phosphorylated Rad51. The results indicate that both Tyr54 and Tyr315 are phosphorylated by cAbl. To further examine the specificity of these antibodies, we co-transfected the cells with the plasmid containing cAbl and either with that containing Rad51-Y54F or Rad51-Y315F. Western blotting of these cells showed that the antibody against phosphorylated Tyr315 detects selectively the phosphorylation of Tyr315 in Rad51 and the antibody against the phosphorylated Tyr54 detects selectively that of Tyr54. We also observed that the phosphorylation of Tyr54 is significantly decreased by mutation Y315F, suggesting that the phosphorylation of Tyr315 stimulates that of Tyr54.

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Poster ***Abstract 68***

Sumoylation and Ubiquitination of the Ets-related transcription factor ERM have opposite effects on its transcriptional activity

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Topic : Other

Content : ERM is a transcription factor of the Ets family which belongs to the PEA3 group. This group is composed of three proteins: PEA3/E1AF, ER81/ETV1 and ERM/ETV5. These factors are involved in a number of developmental processes and are implicated in breast cancer. PEA3 group members are often overexpressed in different types of cancers which also overexpress matrix metalloproteases (MMP, enzymes degrading extracellular matrix) and present a disseminating phenotype. It has been shown that post-translational modifications such as phosphorylation or acetylation are important for regulating PEA3 group members transcriptional activity. Since, in addition to acetylation, other lysine residue modifications are known to affect properties of transcription factors. So we have investigated whether ERM was a target of SUMO and ubiquitin pathways and whether these modifications had an impact on its transcriptional activity. Here, we showed that ERM is sumoylated on four lysine residues located at consensus sumoylation motifs conserved among PEA3 group proteins. This modification inhibits ERM transcriptional activity without affecting the protein subcellular localization, stability or DNA-binding. We also determined that ERM degradation depends on the 26S proteasome pathway and we propose that ubiquitin-proteasome pathway enhances the transcriptional activity of the protein.

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Poster ***Abstract 69***

Poster ***Abstract 72***

The transcriptional activity of human delta-lactoferrin is regulated by the *O*-acetylglucosaminylation/phosphorylation balance

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Topic : Functional Analysis

Content : Delta-lactoferrin (ΔLf) mRNA is underexpressed in cancer and is a good prognosis indicator in human breast cancer. Its expression in doxycycline-inducible ΔLf-HEK293 cells led to cell cycle arrest. Using macroarrays, the expression of genes involved in the G1-S transition was examined. Among these, *Skp1* showed 2-3 fold increased expression both at the mRNA and protein levels. Skp1 (S-phase kinase associated protein) belongs to the SCF (Skp1/Cullin-1/F-box ubiquitin ligase) complex responsible for the ubiquitination of cellular regulators leading to their proteolysis.
Analysis of the *Skp1* promoter detected two sequences identical to those previously shown to interact with Lf, the secretory isoform of ΔLf. EMSA demonstrated that ΔLf interacts specifically with these sequences, and reporter gene analysis confirmed that ΔLf recognizes both sequences within the *Skp1* promoter. Deletion of both sequences totally abolished ΔLf transcriptional activity identifying them as ΔLf responsive elements. ΔLf localizes to the nucleus *via* a short bipartite NLS sequence which was demonstrated to be functional using mutants. Our results show that ΔLf binds to the *Skp1* promoter and these interactions lead to its transcriptional activation. By upregulating *Skp1* gene expression, ΔLf may survey cell cycle progression *via* a control of the proteasomal degradation of S phase actors.
The presence of four putative *O*-acetylglucosaminylation sites might imply a control of the nuclear trafficking, the transcriptional activity or the half-life of □Lf *via* the balance between *O*-acetylglucosaminylation and phosphorylation as already described for other factors. These sites are located in or near domains such as DNA binding domains, NLS sequences or a putative ATP binding site. In order to demonstrate the implication of this balance in □Lf activity, inhibitor/activators as well as mutants have been used. We showed that increased concentrations of glucosamine inhibit □Lf transcriptional activity whereas okadaic acid increases it. This impact on □Lf transcriptional activity does not seem to involve a control of its nuclear trafficking or its stability. The use of mutants confirms that □Lf is regulated by this balance and among the four *O*-GlcNAc/P sites, Ser 10 and Thr 559 are particularly important.

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Poster ***Abstract 73***

Structural analysis of O-glycosylation in amphibian mucins: characterization of species-specific carbohydrate chains from oviducal mucins of *Rana clamitans*.

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Topics : Glycosylation

Content: Previous studies undertaken in amphibians demonstrated a wide structural diversity in O-glycans. The jelly coats surrounding amphibian eggs contain highly O-glycosylated proteins. These oviducal mucins have an important role in the fertilization process and their carbohydrate chains appear remarkably species-specific.
Alkaline reductive treatment of the egg-jelly coats of *Rana clamitans* led to the release of neutral and acidic oligosaccharide alditols. In a previous study, neutral oligosaccharide alditols were isolated and the structures of twenty seven of them, ranging from three to sixteen monosaccharides, were established using NMR spectroscopy, MALDI-TOF MS and methylation analyses.
Most of these structures are novel and characteristic of the species, even if they include some known structural patterns, such as Le^x or P_1 determinants. However, it is noteworthy that these determinants, in *R. clamitans*, can be extended with a monosaccharide or a sequence. The occurrence of a peripheral additional sugar or sequence linked to a determinant has been already observed for several amphibian species.
The combination of NMR spectroscopy and mass spectrometry allowed us to complete the characterization of the O-glycosylation in this species, by the structural analysis of about forty five acidic oligosaccharide alditols, whose size can also reach sixteen monosaccharides. In most of these compounds, the anionic character is due to a glucuronic acid residue, which can be substituted with a □-GlcNAc residue.
Moreover, the novel type of chain termination, constituted by the sequence Fuc(α1-2)Gal(α1-3)Gal(α1-4)Galβ, first evidenced in neutral compounds, was also shown in some of the acidic compounds.
These structural analyses confirm the species-specificity of the O-glycans in amphibians, and suggest therefore that the diversity in glycan structures may reflect the diversity of glycosyltransferases involved in their biosynthesis.

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Poster **Abstract 74**

Comparative study of *O*-linked oligosaccharides released from the oviducal mucins of two very closely related amphibian species, *Hyla meridionalis* and *Hyla arborea*

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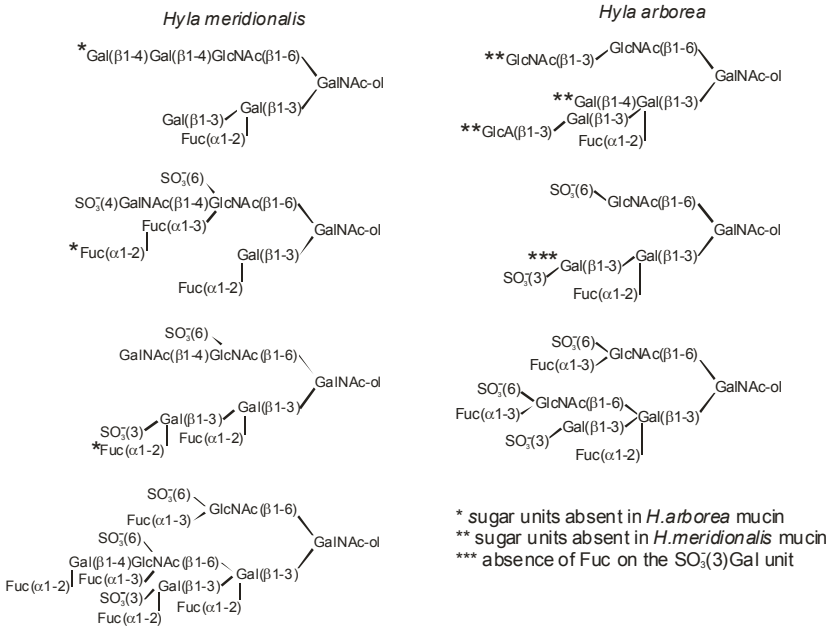
Topics : Glycosylation

Content :



The eggs of amphibians are surrounded by an extracellular matrix, termed jelly coat, which is mainly composed of hydrated mucin-type glycoproteins. The jelly coat has been suggested to function in the induction of sperm capacitation for fertilization and establishment of the structural polyspermy block. Previous studies have shown that the released *O*-glycans are remarkably species-specific. According to the role ascribed to carbohydrates as an evolutionary potential of information, this species-specificity could be involved in a great number of carbohydrate-based specific recognition systems, such as the species-specificity of gamete interaction and species-specific parasitism. The material obtained from *H. meridionalis* and *H. arborea* was submitted to reductive β-elimination and *O*-glycans were further fractionated by HPLC. The structural characterization of 125 different oligosaccharides was performed through a combination of two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR and ESI-MS/MS analyses. As expected, the differences observed between these two species confirm the species-specificity of this carbohydrate material, whereas a sufficient number of common elements clearly shows that these two species belong to a same genus.

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Poster ***Abstract 75***

Species specificity of O-linked carbohydrate chains of the oviducal mucins in amphibians: structural analysis of oligosaccharide alditols released by β-elimination from the jelly coats of the anuran *Pelobates cultripes*

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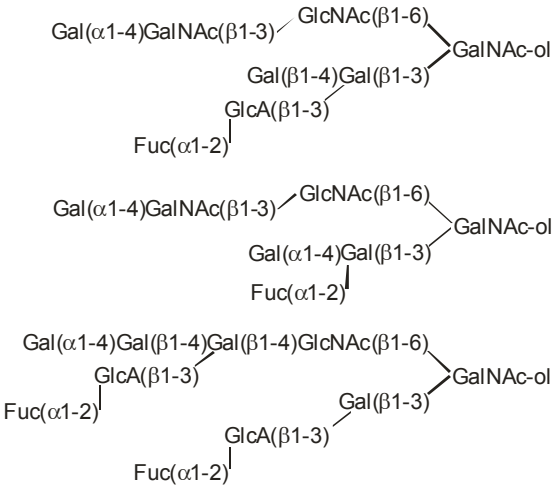
Topics : Glycosylation

Content : The functions attributed to amphibian jelly coats include sperm binding, sperm capacitation, induction of the sperm acrosom reaction, prevention of species cross-fertilization, the blocking of polyspermy and provision of a protective environment for the developing embryo. Previous reports on fish and amphibia oviducal mucins have shown that *O*-glycans are highly species-specific. Considering the role ascribed to carbohydrates as an evolutionary potential of information, our aim consists in establishing a molecular phylogeny based upon the correlation between host parasite co-evolution and *O*-glycan biosynthesis.
The material obtained from *Pelobates cultripes* was submitted to reductive β-elimination and the released oligosaccharide-alditols were isolated by preparative HPLC. Structural characterization was performed through a combination of ^{1}H and ^{13}C NMR and ESI-MS/MS analyses.
As expected, the *O*-glycans of *Pelobates cultripes* are specific to this species, as shown by these three examples selected among the list of novel compounds:



It is worthy of note that the sequence Fuc(α1-2)GlcA(β1-3) has been merely observed until now in *Rana temporaria*.

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Poster ***Abstract 76***

Comparative study of O-glycosylation of oviducal mucins from three related amphibian species: *Rana ridibunda, Rana lessonae* and *Rana esculenta*.

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Topics : glycosylation

Content : *Rana esculenta* (LR) are natural hybrids between *Rana ridibunda* (RR) and *Rana lessonae* (LL) that reproduce by hybridogenesis: LR-females and males exclude the L-genome premeiotically, clonally transmit the R-genome to eggs and sperm, and then regain the L-genome by mating with LL-individuals. Larvae from different mating combinations differ markedly in their performance. Tadpole survival can range from 0% in the case of LR x LR matings to 100% in the other combinations (LL x LL; LL x LR; LR x LL).
In order to verify our hypothesis about the species-specificity of amphibian O-glycans, it appeared interesting to compare the structure of glycans from oviducal mucins of these related species RR, LL and LR.
The egg-jelly coats from genetically controlled matings of these three species were submitted to reductive β-elimination and the released oligosaccharide-alditols were isolated. Structural characterization was performed using a combination of NMR spectroscopy and mass spectrometry.
A feature of these three species is the presence of the blood-group B determinant: Gal(α1-3)[Fuc(α1-2)]Galβ. However, in *R. ridibunda*, the B determinant is extended at the α-Gal residue with a Gal(β1-2) residue, which constitutes the particularity of this species. Considering the hybridogenesis mechanism, the potential presence of this sequence in *R. esculenta* was searched. Structural analyses by MALDI-TOF MS and ESI-MS/MS, confirmed by NMR spectroscopy, allowed to detect the Gal (β1-2) linked to the α-Gal of the B determinant only in the case of *R. ridibunda*.
Nevertheless, the comparative structural study of the O-glycans of these closely related amphibian species confirms the species-specificity and shows therefore the occurrence of particular structures.

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Poster ***Abstract 77***

Structure elucidation of NeuAc, NeuGc and Kdn-containing *O*-glycans released from *Triturus alpestris* oviductal mucins. Characterization of the poly LacdiNAc sequence: HSO_3(4)(GalNAcβ1-4GlcNAcβ1-3)$_{1-3}$GalNAcβ1-4(GlcNAcβ1-3)$_{0-1}$GlcNAcβ1-6GalNAc-ol

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Topics : Glycosylation

Content : Eggs from Amphibia are surrounded by several layers of jelly that are needed for proper fertilization. Jelly coat is composed of highly glycosylated mucin-type glycoproteins containing up to 60% of carbohydrates, which display a remarkable species-specificity. This material obtained from *Triturus alpestris* was submitted to reductive β-elimination and the released oligosaccharide-alditols were further fractionated by HPLC. Structural characterization was performed through a combination of two dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR and ESI-MS/MS analysis. Numerous carbohydrate chains are characterized by the presence of the Cad (Sd^{a}) determinant, including respectively NeuAc, NeuGc or Kdn as a sialic acid. But the most significant *O*-glycan sequences which mark the difference between the jelly of *T. alpestris* and other studies amphibian jellies are polymers of GalNAc(β1-4)GlcNAc (LacdiNAc) which form part of the following sequence:

HSO_3(4)(GalNAcβ1-4GlcNAcβ1-3)$_{1-3}$GalNAcβ1-4(GlcNAcβ1-3)$_{0-1}$GlcNAcβ1-6GalNAc-ol

The LacdiNAc determinant is now considered as a common constituent of *N*- and *O*- linked glycans and largely widespread in invertebrates and particularly in many human pathogens. It has been found in unsubstituted as well as terminally 4-*O*-sulphated, *α*1,3-fucosylated, or *α*2,6-sialylated forms at the distal end of conjugated glycans, but has not been reported previously at truly internal position of polylactosamine chains.
Authentic poly-LDN structures were recently produced *in vitro* by Chinese Hamster Ovary Lec8 cells transfected with the *Caenorhabditis elegans* β1-4-*N*-acetylgalactosaminyltransferase (Kawar *et al.*). To our knowledge, the poly-LDN *O*-glycans isolated from *T. alpestris* egg jelly mucins represent the first reported occurrence of this type of polymer naturally synthesized by animal cells.

Kawar ZS, Haslam SM, Morris HR, Dell A, Cummings RD, Novel poly-GalNAcβ1-4GlcNAc (LacdiNAc) and fucosylated poly-LacdiNAc *N*-glycans from mammalian cells expressing β1,4-*N*-acetylgalactosaminyltransferase and α1,3-fucosyltransferase, *J Biol Chem* **280**, 12810-12819 (2005).

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Poster ***Abstract 78***

Snake venomics of *Bitis gabonica gabonica*. Protein family composition and subunit organization of the dimeric disintegrins gabonin-1 and gabonin-2

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Topics :

Content : Snakes of the family *Viperidae* (vipers and pitvipers) produce a complex mixture of a large number of distinct proteins. However, venom proteins belong to only a few major protein families, including enzymes (serine proteinases, Zn^{2+}-metalloproteases, L-amino acid oxidase, group II PLA_2) and proteins without enzymatic activity (disintegrins, C-type lectins, natriuretic peptides, myotoxins, CRISP toxins, nerve and vascular endothelium growth factors, cystatin and Kunitz-type protease inhibitors). In additon to understanding how venoms evolve, characterization of the protein content of snake venoms also has a number of potential benefits for basic research, clinical diagnosis, development of new research tools and drugs of potential clinical use, and for antivenom production strategies. Despite its potential value, little is known about the venom protein composition of most vipers. Recent reports surveyed gene transcriptional activity (transcriptome) of the snake venom glands of *Bothrops insularis*, *Bothrops jararacussu*, *Bitis gabonica*, and *Agkistrodon acutus* by generation of expressed sequence tags (ESTs) or construction of a cDNA library followed by sequencing of the clones. These works have provided catalogs of full-length venom gland mRNAs. Our proteomic approach complements these studies by showing the relative abundance of the proteins that are actually secreted into the venoms.We have analyzed the protein composition of the crude venom of *Bitis gabonica* by RP-HPLC, N-terminal sequence analysis, MALDI-TOF MS, and in-gel tryptic digestion followed by CID-MS/MS. This approach allowed us to assign unambiguously all of the isolated venom fractions to protein families present in the non-redundant databases. Our proteomic approach complements transcriptomic studies by showing the relative abundance of the proteins that are actually secreted into the venoms. On the other hand, comparison of the proteomic versus the transcriptomic data showed significant differences for a number of protein families, indicating lack of correlation between the transcriptional and the translational activity of the venom gland. The use of protein-chemical techniques allowed us also to determine the subunit composition of expressed venom proteins.

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Poster ***Abstract 79***

Molecular cloning of a non-venom-secreted PII disintegrin-like transcript, BA-5A, from a *Bitis arietans* cDNA library reveals a pathway for the evolution of the long-chain disintegrin bitistatin from a PIII disintegrin-like precursor

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Topics :

Content : Disintegrins represent a family of integrin receptor antagonists that are released in viper venoms by proteolytic processing of PII snake venom metalloproteinase (SVMP) precursors or synthesized from short-coding mRNAs. Functional diversification between disintegrins is mainly due to amino acid substitutions within the active loop, whereas structural diversification was driven through a disulfide bond engineering mechanism involving the selective loss of pairs of cysteine residues engaged in the formation of disulfide bonds. The evolutionary pressure acting to promote high levels of variation in venom proteins may be part of a predator-prey arms race that allows the snake to adapt to a variety of different prey, each of which are most efficiently subdued with a different venom formulation. However, the molecular details of the mechanisms leading to snake venom toxin diversification remains unclear.We report the cloning and sequence analysis of BA-5A from *Bitis arietans*, a novel and unique ECD-disintegrin-like domain.It contains the 16 cysteine residues that are conserved in all PIII disintegrin-like domains but lacks the cysteine-rich domain. These features suggest that BA-5A represents an intermediate in the evolutionary pathway of the long disintegrin bitistatin, and that removal of the cysteine-rich domain and loss of the PIII-specific disulfide bond were separate events along the structural diversification pathway of disintegrins, the former predating the latter. BA-5A could not be detected in the venom proteome of *Bitis arietans* using proteomic techniques. The occurrence of this very low abundance (<0.05%) or non-expressed disintegrin transcript indicates a hitherto unrecognized structural diversity of this protein family. These evidences point to the existence of very low abundance, non-expressed, or non-venom-secreted disintegrin transcripts, which may play a hitherto unrecognized physiological function, or may simply represent orphan molecules which eventually could serve as scaffolds for evolving novel biological activities, perhaps becoming functional proteins of relevance for the adaptation of snakes to changing ecological niches and prey habits.

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Poster ***Abstract 80***

Loss of introns along the evolutionary diversification pathway of snake venom disintegrins evidenced by sequence analysis of genomic DNA from Macrovipera lebetina transmediterranea

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Topics :

Content : Venom toxins likely evolved from endogenous proteins with normal physiological functions that were recruited into the venom proteome. Notably, most venom toxins are extensively cross-linked by disulfide bonds and have flourished into functionally diverse, toxin multigene families that exhibit interfamily, intergenus, interspecies, and intraspecific variability. The existence in the same venom of a functionally diverse isoforms of the same protein family reflects accelerated Darwinian evolution. The evolutionary pressure acting to promote high levels of variation in venom proteins may be part of a predator-prey arms race that allow the snake to adapt to a variety of different prey, each of which are most efficiently subdued with a different venom formulation. Disintegrins, a broad family of small, cysteine-rich polypeptides isolated from venoms of vipers and rattlesnakes, are released in viper venoms and selectively block the function of cell surface adhesive receptors of the integrin family. Analysis of cDNAs from Macrovipera lebetina transmediterranea and Echis ocellatus venom gland libraries encoding disintegrins strongly argued for a common ancestry of the messengers of short disintegrins and those for precursors of dimeric disintegrin chains. We now report the sequence analysis of disintegrin-coding genes from Macrovipera lebetina transmediterranea. Genomic DNAs for dimeric disintegrin subunits Ml_G1 and Ml_G2 contain single 1 kb-intron exhibiting the 5´-GTAAG (donor)/3´-AG (acceptor) consensus signature of group I self-splicing introns. On the other hand, the short RTS-disintegrin Ml_G3 is transcribed from an intronless gene, indicating that the evolutionary pathway leading to the emergence of short disintegrins involved the removal of all intronic sequences. The insertion position of the Ml_G1 and Ml_G2 intron is conserved in the genes for vertebrate ADAM´s disintegrin-like domains and within the gene for the medium-size snake disintegrins halystatin 2 and 3. However, a comparative analysis of currently available disintegrin (-like) genes outline the view that a minimization of both the gene organization and the protein structure underlie the evolution of the snake venom disintegrin family.

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Poster ***Abstract 81***

Venom Proteomes of Sistrurus Rattlesnakes

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Topics :

Content : Venoms represent the critical innovation that allowed advanced snakes to transition from a mechanical (constriction) to a chemical (venom) means of subduing and digesting prey larger than themselves, and as such, venom proteins have multiple functions including immobilizing, paralyzing, killing and digesting prey. Given the central role that diet has played in the adaptive radiation of snakes[14], venom thus represents a key adaptation that has played an important role in the diversification of these animals.The protein composition of the venoms of the three subspecies of Sistrurus catenatus (S. c. catenatus, tergeminus, and edwardsii) and a basal species, Sistrurus miliarius barbouri, were analyzed by RP-HPLC, N-terminal sequencing, MALDI-TOF peptide mass fingerprinting and CID-MS/MS. The venoms of the four Sistrurus taxa contain proteins from 11 families. The protein family profile and the relative abundance of each protein group in the different venoms are not conserved. Myotoxins and 2-chain PLA_2s were present only in S.c. catenatus and S.c. tergeminus, whereas C-type BPP and Kunitz-type inhibitors were exclusively found in S.c. edwardsii and Sistrurus miliarius barbouri. Among major protein families, taxa were most similar in their metalloproteases (protein similarity coefficient value: 34%) while most divergent in PLA_2s (12%), with values for disintegrins and serine proteases lying between these extremes (25% and 20% respectively). The patterns of venom diversity points to either a gain in complexity in S. catenatus taxa or a loss of venom diversity occurring early on in the evolution of the group involving the lineage connecting S. milarius to the other taxa. The high degree of differentiation in the venom proteome among recently-evolved congeneric taxa emphasizes the uniqueness of the venom composition of even closely related species which have different diets. Comparative proteomic analysis of Sistrurus venoms provides a comprehensive catalogue of secreted proteins, which may contribute to a deeper understanding of the biology and ecology of these North American snakes, and may also serve as a starting point for studying structure-function correlations of individual toxins.

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Poster ***Abstract 82***

Proteomics and Bioinformatics Characterization of the Biogenesis and Function of Melanosomes

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Authors : An Chi, Julio C.Valencia, Zhang-Zhi Hu, Jeff Shabanowitz, Vincent J.Hearing, Cathy Wu, Ettore Appella and Donald F.Hunt Univ. of Virginia, Georgetown Univ., NIH
Topics : Mass spectrometry

Melanosomes are membrane-bound organelles that specialize in the production and distribution of melanin pigment. Although methods for isolating melanosomes at various developmental stages have been established, obtaining them in sufficient quantities and removing endogenous melanins has remained a challenge. Global melanosome proteome characterization was made possible by a novel method for conventional in-gel digestion coupled with LC/MS following removal of melanin by immobilized metal affinity chromatography (IMAC). To solubilize melanosomes, we found that a combination of urea and 10% acetonitrile was highly effective. We then took advantage of the heavy metal ion sequestering property of melanin by loading the tryptic peptides onto an IMAC column ctivated with an excess volume of FeCl3, assembled back-to-back with an RP pre-column. The melanin was retained on the IMAC column due to the high affinity of Fe (III) for the o-diOH groups of melanin, while the peptides passed through and were subsequently caught on the C18 reverse-phase pre-column. Proteomic analysis was applied to stage I and II melanosomes purified from pigmented (MNT1) or from non-pigmented (SK-MEL-28) human melanoma cells, and to stage IV melanosomes from MNT1 cells. An average of 600 distinct proteins were detected in each preparation. Proteins identified were grouped into 12 subsets according to stage and cell type. Proteins identified in various maturation stages by LC/MS were organized into families or sub-groups based on functional classifications such as Gene Ontology (GO). A combination of immunoblotting, immunofluorescence microscopy and bioinformatics analysis was used to characterize the expression profiles of melanosomal proteins at various developmental stages. The stage-related proteins provide direct evidence of protein sorting and trafficking to this organelle and provide information about their biogenesis as lysosome-related organelles. About 25% of these proteins are potential transmembrane proteins with various functions, including ion/solute transporters, receptors and membrane trafficking proteins. Over 33% are enzymes with heterogeneous and broad catalytic activities (oxidoreductases, transferases, hydrolases, lyases and isomerases). Other proteins include molecular motor and cytoskeleton proteins, and potential signaling molecules. Melanosomal proteins also include some known to be associated with the plasma membrane (e.g. Na,K-ATPase subunits) and other organelles, such as the endoplasmic reticulum (e.g. ribophorin I, GRP 78/BiP and calnexin) and lysosomes (e.g.cathepsin D, B, -Glu-X carboxypeptidase). The presence of vacuolar proton ATPases (e.g. VATPases A, B, H, and the clathrin coated vesicle/synaptic vesicle proton pump) in both early and late melanosomes is consistent with the critical importance of pH in regulating the physiological functions of melanosomes. In addition, the presence of sulfhydryl enzymes (e.g. glutathione S-transferase pi, protein disulfide isomerases and quinone reductase) indicates their likely importance in regulating melanin synthesis, since pheomelanins, a major subclass of melanins, contain sulfur. Proteins that participate in membrane dynamics also represent a major component of the melanosome proteome and emphasize the critical nature of melanosome interactions with other subcellular components. Further, 17 of the 63 human homologs of mouse pigment gene products were identified in various melanosome stages, many of which are associated with different inherited pigmentary diseases in humans.

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Poster ***Abstract 83***

A standard nomenclature for modifications encountered in the analysis of proteins by mass spectrometry

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Authors: Sean L. Seymour,[1] Ronald C. Beavis,[2] Pierre-Alain Binz,[3,4] David M. Horn,[5] Robert J. Chalkley,[6] Detlev Suckau,[7] Luisa Montecchi-Palazzi,[8] John S. Garavelli,[8] Jim Shofstahl,[9] David M. Creasy,[10] and John S. Cottrell[10]

Content : In the identification of proteins and peptides using mass spectrometry, artifactual and biological modifications are indicated as differential moieties on the polypeptide sequence. Because there are many different software applications that conduct identification analyses on this type of data, and there has been no standard for the nomenclature of modifications, the reconciliation of results originating from different tools can be challenging. There are currently multiple sources of modifications names, including the major public reference sites UniMod, RESID, and Delta Mass, and also software-specific catalogs. In order to remove this unnecessary complication, these authors formed a small working group that collectively represents the interests of a significant number of databases, search engines, and other software tools. By the arduous alignment of these multiple resources and point by point debate of specific names and the general principles, we arrived at a proposal for standard names of approximately 300 modifications and also a set of nomenclature principles to guide the addition of new modifications in subsequent versions. This was presented at HUPO Proteomics Standards Initiative (PSI) working group meeting in San Francisco, CA in April of this year and has since been adopted as Version 1. These standard names have been integrated as synonyms into the PSI-MOD modification ontology created by the PSI sub-group of the same name. This will allow reconciliation of modifications with other domains such as the study of molecular interactions.

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