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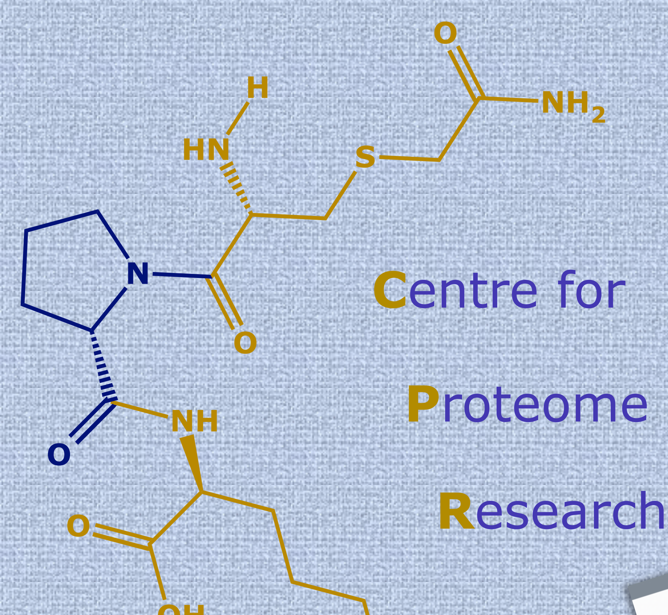

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Elastase levels and activity are increased in dystrophic muscle and impair myoblast cell survival, proliferation and differentiation

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In Duchenne muscular dystrophy, progressive loss of muscle tissue is accompanied by fibrosis, chronic inflammation and reduced muscle regenerative capacity. Although much is known about the development of fibrosis and chronic inflammation in muscular dystrophy, less is known about how they are mechanistically linked to loss of muscle regenerative capacity. We have developed a proteomics method to discover dystrophy-associated changes in the muscle progenitor cell niche, which identified serine proteases, and especially neutrophil elastase, as candidates. We show that elastase activity is increased in dystrophic (mdx^{4cv}) muscle and impairs myoblast survival in culture. While the effect of elastase on C2C12 cell survival correlates with the kinetics of elastase-mediated degradation of the substrate to which the cells adhere, the effect of elastase on satellite cell-derived primary myoblast growth and differentiation is substrate-independent and even more dramatic than the effect on C2C12 cells, suggesting a detrimental role for elastase on myogenesis in vivo. Additionally, elastase impairs differentiation of both primary and C2C12 myoblasts into myotubes. Our findings evidence the importance of neutrophil-mediated inflammation in muscular dystrophy and indicate elastase-mediated regulation of myoblast behaviour as a potential mechanism underlying loss of regenerative capacity in dystrophic muscle.

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KinView: a visual comparative sequence analysis tool for integrated kinome research

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Multiple sequence alignments (MSAs) are a fundamental analysis tool used throughout biology to investigate relationships between protein sequence, structure, function, evolutionary history, and patterns of disease-associated variants. However, their widespread application in systems biology research is currently hindered by the lack of user-friendly tools to simultaneously visualize, manipulate and query the information conceptualized in large sequence alignments, and the challenges in integrating MSAs with multiple orthogonal data such as cancer variants and post-translational modifications, which are often stored in heterogeneous data sources and formats. Here, we present the Multiple Sequence Alignment Ontology (MSAOnt), which represents a profile or consensus alignment in an ontological format. Subsets of the alignment are easily selected through the SPARQL Protocol and RDF Query Language for downstream statistical analysis or visualization. We have also created the Kinome Viewer (KinView), an interactive integrative visualization that places eukaryotic protein kinase cancer variants in the context of natural sequence variation and experimentally determined post-translational modifications, which play central roles in the regulation of cellular signaling pathways. Using KinView, we identified differential phosphorylation patterns between tyrosine and serine/threonine kinases in the activation segment, a major kinase regulatory region that is often mutated in proliferative diseases. We discuss cancer variants that disrupt phosphorylation sites in the activation segment, and show how KinView can be used as a comparative tool to identify differences and similarities in natural variation, cancer variants and post-translational modifications between kinase groups, families and subfamilies. Based on KinView comparisons, we identify and experimentally characterize a regulatory tyrosine ($Y177^{PLK4}$) in the PLK4 C-terminal activation segment region termed the P+1 loop. To further demonstrate the application of KinView in hypothesis generation and testing, we formulate and validate a hypothesis explaining a novel predicted loss-of-function variant ($D523N^{PKC\beta}$) in the regulatory spine of PKCβ, a recently identified tumor suppressor kinase. KinView provides a novel, extensible interface for performing comparative analyses between subsets of kinases and for integrating multiple types of residue specific annotations in user friendly formats.

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In-depth proteomic profiling of the uveal melanoma secretome

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ABSTRACT

Uveal melanoma (UM), the most common primary intraocular tumour in adults, is characterised by a high frequency of metastases to the liver, typically with a fatal outcome. Proteins secreted from cancer cells ('secretome') are biologically important molecules thought to contribute to tumour progression. We examined the UM secretome by applying a label-free nanoLCMS/MS proteomic approach to profile proteins secreted into culture media by primary UM tumours with a high– (HR; n = 11) or low– (LR; n = 4) metastatic risk, compared to normal choroidal melanocytes (NCM) from unaffected post-mortem eyes. Across the three groups, 1843 proteins were identified at a 1% false discovery rate; 758 of these by at least 3 unique peptides, and quantified. The majority (539/758, 71%) of proteins were classified as secreted either by classical (144, 19%), non-classical (43, 6%) or exosomal (352, 46%) mechanisms. Bioinformatic analyzes showed that the secretome composition reflects biological differences and similarities of the samples. Ingenuity® pathway analysis of the secreted protein dataset identified abundant proteins involved in cell proliferation-, growth- and movement. Hepatic fibrosis/hepatic stellate cell activation and the mTORC1-S6K signalling axis were among the most differentially regulated biological processes in UM as compared with NCM. Further analysis of proteins upregulated ≥ 2 in HR-UM only, identified exosomal proteins involved in extracellular matrix remodelling and cancer cell migration/invasion; as well as classically secreted proteins, possibly representing novel biomarkers of metastatic disease. In conclusion, UM secretome analysis identifies novel proteins and pathways that may contribute to metastatic development at distant sites, particularly in the liver.

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Human CDK18 promotes replication stress signaling and genome stability

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ABSTRACT

Cyclin-dependent kinases (CDKs) coordinate cell cycle checkpoints with DNA repair mechanisms that together maintain genome stability. However, the myriad mechanisms that can give rise to genome instability are still to be fully elucidated. Here, we identify CDK18 (PCTAIRE 3) as a novel regulator of genome stability, and show that depletion of CDK18 causes an increase in endogenous DNA damage and chromosomal abnormalities. CDK18-depleted cells accumulate in early S-phase, exhibiting retarded replication fork kinetics and reduced ATR kinase signaling in response to replication stress. Mechanistically, CDK18 interacts with RAD9, RAD17 and TOPBP1, and CDK18-deficiency results in a decrease in both RAD17 and RAD9 chromatin retention in response to replication stress. Importantly, we demonstrate that these phenotypes are rescued by exogenous CDK18 in a kinase-dependent manner. Collectively, these data reveal a rate-limiting role for CDK18 in replication stress signalling and establish it as a novel regulator of genome integrity.

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cAMP-dependent protein kinase (PKA) complexes probed by complementary Differential Scanning Fluorimetry and Ion Mobility-Mass Spectrometry

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ABSTRACT: cAMP-dependent protein kinase (PKA) is an archetypal biological signaling module and a model for understanding the regulation of protein kinases. In this study, we combine biochemistry with differential scanning fluorimetry (DSF) and ion mobility-mass spectrometry (IM-MS) to evaluate effects of phosphorylation and structure on the ligand binding, dynamics and stability of components of heteromeric PKA protein complexes in vitro. We uncover dynamic, conformationally-distinct populations of the PKA catalytic subunit with distinct structural stability and susceptibility to the physiological protein inhibitor PKI. Native MS of reconstituted PKA R_2C_2 holoenzymes reveals variable subunit stoichiometry and holoenzyme ablation by PKI binding. Finally, we find that although a 'kinase-dead' PKA catalytic domain cannot bind to ATP in solution, it interacts with several prominent chemical kinase inhibitors. These data demonstrate the combined power of IM-MS and DSF to probe PKA dynamics and regulation, techniques that can be employed to evaluate other complexes, with broad implications for cellular signaling.

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BBA General Subjects

Applications of ion mobility mass spectrometry for high throughput, high resolution glycan analysis☆

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ABSTRACT

Background: Diverse varieties of often heterogeneous glycans are ubiquitous in nature. They play critical roles in recognition events, act as energy stores and provide structural stability at both molecular and cellular levels. Technologies capable of fully elucidating the structures of glycans are far behind the other '-omic' fields. Liquid chromatography (LC) and mass spectrometry (MS) are currently the most useful techniques for high-throughput analysis of glycans. However, these techniques do not provide full unambiguous structural information and instead the gap in full sequence assignment is frequently filled by a priori knowledge of the biosynthetic pathways and the assumption that these pathways are highly conserved.

Scope of the review: This comprehensive review details the rise of the emerging analytical technique ion mobility spectrometry (IMS) (coupled to MS) to facilitate the determination of three-dimensional shape: the separation and characterization of isobaric glycans, glyco(peptides/proteins), glycolipids, glycosaminoglycans and other polysaccharides; localization of sites of glycosylation; or interpretation of the conformational change to proteins upon glycan binding.

Major conclusions: IMS is a highly promising new analytical route, able to provide rapid isomeric separation (ms timescale) of either precursor or product ions facilitating MS characterization. This additional separation also enables the deconvolution of carbohydrate MS(/MS) information from contaminating ions, improving sensitivity and reducing chemical noise. Derivation of collision cross sections (CCS) from IM-MS(/MS) data and subsequent calculations validate putative structures of carbohydrates from ab initio derived candidates. IM-MS has demonstrated that amounts of specific glycan isomers vary between disease states, which would be challenging to detect using standard analytical approaches.

General significance: IM-MS is a promising technique that fills an important gap within the Glycomics toolbox, namely identifying and differentiating the three-dimensional structure of chemically similar carbohydrates and glycoconjugates. This article is part of a Special Issue entitled "Glycans in personalised medicine" Guest Editor: Professor Gordan Lauc.

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Wound Repair and Regeneration

A selected reaction monitoring-based analysis of acute phase proteins in interstitial fluids from experimental equine wounds healing by secondary intention

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ABSTRACT

In horses, pathological healing with formation of exuberant granulation tissue (EGT) is a particular problem in limb wounds, whereas body wounds tend to heal without complications. Chronic inflammation has been proposed to be central to the pathogenesis of EGT. This study aimed to investigate levels of inflammatory acute phase proteins (APPs) in interstitial fluid from wounds in horses. A novel approach for absolute quantification of proteins, selected reaction monitoring (SRM)-based mass spectrometry in combination with a quantification concatamer (QconCAT), was used for the quantification of five established equine APPs (fibrinogen, serum amyloid A, ceruloplasmin, haptoglobin, and plasminogen) and three proposed equine APPs (prothrombin, α-2-macroglobulin, and α-1-antitrypsin). Wound interstitial fluid was recovered by large pore microdialysis from experimental body and limb wounds from five horses at days 1, 2, 7, and 14 after wounding and healing without (body) and with (limb) the formation of EGT. The QconCAT included proteotypic peptides representing each of the protein targets and was used to direct the design of a gene, which was expressed in Escherichia coli in a media supplemented with stable isotopes for metabolically labeling of standard peptides. Co-analysis of wound interstitial fluid samples with the stable isotope-labeled QconCAT tryptic peptides in known amounts enabled quantification of the APPs in absolute terms. The concentrations of fibrinogen, haptoglobin, ceruloplasmin, prothrombin, and α-1-antitrypsin in dialysate from limb wounds were significantly higher than in dialysate from body wounds. This is the first report of simultaneous analysis of a panel of APPs using the QconCAT-SRM technology. The microdialysis technique in combination with the QconCAT-SRM based approach proved useful for quantification of investigated proteins in the wound interstitial fluid, and the results indicated that there is a state of sustained inflammation in equine wounds healing with formation of EGT.

PLOS | GENETICS

RESEARCH ARTICLE

Selection on Coding and Regulatory Variation Maintains Individuality in Major Urinary Protein Scent Marks in Wild Mice

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PROTEOMICS

Cross-species proteomics in analysis of mammalian sperm proteins

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ABSTRACT

Many proteomics studies are conducted in model organisms for which fully annotated, detailed, high quality proteomes are available. By contrast, many studies in ecology and evolution are conducted in species which lack high quality proteome data, limiting the perceived value of a proteomic approach for protein discovery and quantification. This is particularly true of rapidly evolving proteins in the reproductive system, such as those that have an immune function or are under sexual selection, and can compromise the potential for cross-species proteomics to yield confident identification. In this investigation we analysed the sperm proteome, from a range of ungulates and rodents, and explored the potential of routine proteomic workflows to yield characterisation and quantification in non-model organisms. We report that database searching is robust to cross-species matching for a mammalian core sperm proteome, comprising 623 proteins that were common to most of the 19 species studied here, suggesting that these proteins are likely to be present and identifiable across many mammalian sperm. Further, label-free quantification reveals a consistent pattern of expression level. Functional analysis of this core proteome suggests consistency with previous studies limited to model organisms and has value as a quantitative reference for analysis of species-specific protein characterisation.

Significance: From analysis of the sperm proteome for diverse species (rodents and ungulates) using LC–MS/MS workflows and standard data processing, we show that it is feasible to obtain cross-species matches for a large number of proteins that can be filtered stringently to yield a highly expressed mammalian sperm core proteome, for which label-free quantitative data are also used to inform protein function and abundance.

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Learning Curve

Quantitative proteomics: it's all about training, of course!

Robert Beynon (University of Liverpool), Victoria Harman (University of Liverpool) and Hannah Russell (Head of Education and Training, Biochemical Society)

The fourth iteration of the Biochemical Society training course on "Quantitative Proteomics" took place from 4–5 April 2016. The first time we delivered the course was to precede a conference in Chester in 2010, supported by the Biochemical Society as an Independent Meeting. Subsequently, the course was delivered as one of the first Biochemical Society training events, held at Charles Darwin House in 2012, attached to a workshop on the same topic. In both instances, these were oversubscribed and hugely successful. We continued to deliver this Biochemical Society course in 2014, and most recently, earlier this year.

"I really enjoyed the training course, it was one of the most useful I have been to. It was really good to keep reinforcing the ideas and I came away feeling like I had learned no end of information", 2016 course delegate.

As in all previous iterations, demand for the 80 places in 2016 considerably exceeded availability. It is easy to see why – real practicing experts as tutors, small group teaching and a friendly environment, all at low cost, particularly for Society student members. Delegates are surrounded by peers who are equally new to the field, creating an environment where questions are tolerated (and positively encouraged). They also receive informal advice and guidance during breaks and the reception, and often in follow up exchanges after the course is over.

The first session comprises an intensive afternoon of talks, providing a grounding in the principles, best practice and pitfalls in quantitative proteomics. The speakers are all experts in the field and establish the overall context of the work that will be further developed in day two.

The second session takes on a very different flavour. Delegates divide into groups of about 10 and over the course of three hours, attend three different round table sessions, each focused on one area of quantitative proteomics data analysis. The attendees express preferences for tables that resonate best with their particular interests. Topics covered included statistics, SILAC (stable isotope labeling by/with amino acids in cell culture), selected reaction monitoring and data analysis and visualisation. These 'round table' tutorials, delivered on specific topics by experienced tutors, are particularly popular.

Speakers and trainers all volunteer their time for the course, and so many of them come back year after year because it is such a rewarding experience. We hope to organize many more courses and maintain our commitment to keep the content cutting edge in this rapid developing field.

Day 1: The lectures

Could you support the Society and provide a training course?

If you are thinking of offering a training course through the Society, here are some of the reasons why you should. Mostly, it gives you, as a member of the Society, the opportunity to give back to the membership in the spirit of collegiality and to help support the next generation of biochemists – the best reason. Additionally, it can be great fun and hugely rewarding, working with a group of delegates who are keen to learn about your specialist area. Lastly, designing and delivering a training course can support your own learning and development, providing valuable experience, for example, to support applications for HEA Fellowship.

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Research

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Dynamic phosphorylation of RelA on Ser42 and Ser45 in response to TNFα stimulation regulates DNA binding and transcription

Francesco Lanucara[1], Connie Lam[2], Jelena Mann[3], Tom P. Monie[4], Stefano A. P. Colombo[2], Stephen W. Holman[1], James Boyd[2], Manohar C. Dange[1], Derek A. Mann[3], Michael R. H. White[2] and Claire E. Eyers[1]

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The NF-κB signalling module controls transcription through a network of protein kinases such as the IKKs, as well as inhibitory proteins (IκBs) and transcription factors including RelA/p65. Phosphorylation of the NF-κB subunits is critical for dictating system dynamics. Using both non-targeted discovery and quantitative selected reaction monitoring-targeted proteomics, we show that the cytokine TNFα induces dynamic multisite phosphorylation of RelA at a number of previously unidentified residues. Putative roles for many of these phosphorylation sites on RelA were predicted by modelling of various crystal structures. Stoichiometry of phosphorylation determination of Ser45 and Ser42 revealed preferential early phosphorylation of Ser45 in response to TNFα. Quantitative analyses subsequently confirmed differential roles for pSer42 and pSer45 in promoter-specific DNA binding and a role for both of these phosphosites in regulating transcription from the IL-6 promoter. These temporal dynamics suggest that RelA-mediated transcription is likely to be controlled by functionally distinct NF-κB proteoforms carrying different combinations of modifications, rather than a simple 'one modification, one effect' system.

Protein turnover measurement using selected reaction monitoring-mass spectrometry (SRM-MS)

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Protein turnover represents an important mechanism in the functioning of cells, with deregulated synthesis and degradation of proteins implicated in many diseased states. Therefore, proteomics strategies to measure turnover rates with high confidence are of vital importance to understanding many biological processes. In this study, the more widely used approach of non-targeted precursor ion signal intensity (MS1) quantification is compared with selected reaction monitoring (SRM), a data acquisition strategy that records data for specific peptides, to determine if improved quantitative data would be obtained using a targeted quantification approach. Using mouse liver as a model system, turnover measurement of four tricarboxylic acid cycle proteins was performed using both MS1 and SRM quantification strategies. SRM outperformed MS1 in terms of sensitivity and selectivity of measurement, allowing more confident determination of

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× Author's Choice

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This paper is available on line at http://www.mcponline.org

Research

Direct and Absolute Quantification of over 1800 Yeast Proteins via Selected Reaction Monitoring*

Craig Lawless‡||, Stephen W. Holman§||, Philip Brownridge§||, Karin Lanthaler‡, Victoria M. Harman§, Rachel Watkins‡, Dean E. Hammond§, Rebecca L. Miller§, Paul F. G. Sims‡, Christopher M. Grant‡, Claire E. Eyers§, Robert J. Beynon§, and Simon J. Hubbard‡¶

Defining intracellular protein concentration is critical in molecular systems biology. Although strategies for determining relative protein changes are available, defining robust absolute values in copies per cell has proven significantly more challenging. Here we present a reference data set quantifying over 1800 Saccharomyces cerevisiae proteins by direct means using protein-specific stable-isotope labeled internal standards and selected reaction monitoring (SRM) mass spectrometry, far exceeding any previous study. This was achieved by careful design of over 100 QconCAT recombinant proteins as standards, defining 1167 proteins in terms of copies per cell and upper limits on a further 668, with robust CVs routinely less than 20%. The selected reaction monitoring-derived proteome is compared with existing quantitative data sets, highlighting the disparities between methodologies. Coupled with a quantification of the transcriptome by RNA-seq taken from the same cells, these data support revised estimates of several fundamental molecular parameters: a total protein count of ~100 million molecules-per-cell, a median of ~1000 proteins-per-transcript, and a linear model of protein translation explaining 70% of the variance in translation rate. This work contributes a "gold-standard" reference yeast proteome (including 532 values based on high quality, dual peptide quantification) that can be widely used in systems models and for other comparative studies. Molecular & Cellular Proteomics 15: 10.1074/mcp.M115.054288, 1–14, 2016.

Journal of proteome research

RePLiCal: A QconCAT Protein for Retention Time Standardization in Proteomics Studies

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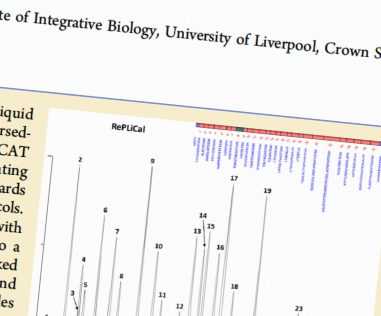
Supporting Information

ABSTRACT: This study introduces a new reversed-phase liquid chromatography retention time (RT) standard, RePLiCal (Reversed-phase liquid chromatography calibrant), produced using QconCAT technology. The synthetic protein contains 27 lysine-terminating calibrant peptides, meaning that the same complement of standards can be generated using either Lys-C or trypsin-based digestion protocols. RePLiCal was designed such that each constituent peptide is unique with respect to all eukaryotic proteomes, thereby enabling integration into a wide range of proteomic analyses. RePLiCal has been benchmarked against three commercially available peptide RT standard kits and outperforms all in terms of LC gradient coverage. RePLiCal also provides a higher number of calibrant points for chromatographic retention time standardization and normalization. The standard provides stable RTs over long analysis times and can be readily transferred between different LC gradients and nUHPLC instruments. Moreover, RePLiCal can be used to predict RTs for other peptides in a timely manner. Furthermore, it is shown that RePLiCal can be used effectively to evaluate trapping column performance for nUHPLC instruments using trap-elute configurations, to optimize gradients to maximize peptide and protein identification rates, and to recalibrate the m/z scale of mass spectrometry data post-acquisition.



KEYWORDS: retention time, LC, standardization, SRM, proteomics, QconCAT, mass spectrometry, calibration, quality control

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RESEARCH ARTICLE 1

Absolute protein quantification of the yeast chaperome under conditions of heat shock

Rebecca J. Mackenzie[1,2], Craig Lawless[2], Stephen W. Holman[1], Karin Lanthaler[2], Robert J. Beynon[1], Chris M. Grant[2], Simon J. Hubbard[2]* and Claire E. Eyers[1]

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Chaperones are fundamental to regulating the heat shock response, mediating protein recovery from thermal-induced misfolding and aggregation. Using the QconCAT strategy and selected reaction monitoring (SRM) for absolute protein quantification, we have determined copy per cell values for 49 key chaperones in Saccharomyces cerevisiae under conditions of normal growth and heat shock. This work extends a previous chemostat quantification study by including up to five Q-peptides per protein to improve confidence in protein quantification. In contrast to the global proteome profile of S. cerevisiae in response to heat shock, which remains largely unchanged as determined by label-free quantification, many of the chaperones are upregulated with an average two-fold increase in protein abundance. Interestingly, eight of the significantly upregulated chaperones are direct gene targets of heat shock transcription factor-1. By performing absolute quantification of chaperones under heat stress for the first time, we were able to evaluate the individual protein-level response. Furthermore, this SRM data was used to calibrate label-free quantification values for the proteome in absolute terms, thus improving relative quantification between the two conditions. This study significantly enhances the largely transcriptomic data available in the field and illustrates a more nuanced response at the protein level.

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Anatomical heterogeneity of tendon: Fascicular and interfascicular tendon compartments have distinct proteomic composition

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Tendon is a simple aligned fibre composite, consisting of collagen-rich fascicles surrounded by a softer interfascicular matrix (IFM). The composition and interactions between these material phases are fundamental in ensuring tissue mechanics meet functional requirements. However the IFM is poorly defined, therefore tendon structure-function relationships are incompletely understood. We hypothesised that the IFM has a more complex proteome, with faster turnover than the fascicular matrix (FM). Using laser-capture microdissection and mass spectrometry, we demonstrate that the IFM contains more proteins, and that many proteins show differential abundance between matrix phases. The IFM contained more protein fragments (neopeptides), indicating greater matrix degradation in this compartment, which may act to maintain healthy tendon structure. Protein abundance did not alter with ageing, but neopeptide numbers decreased in the aged IFM, indicating decreased turnover which may contribute to age-related tendon injury. These data provide important insights into how differences in tendon composition and turnover contribute to tendon structure-function relationships and the effects of ageing.

× Author's Choice

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MOLECULAR & CELLULAR PROTEOMICS

Proteome Dynamics: Tissue Variation in the Kinetics of Proteostasis in Intact Animals*

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Understanding the role of protein turnover in the maintenance of proteostasis requires accurate measurements of the rates of replacement of proteins in complex systems, such as intact animals. Moreover, any investigation of allometric scaling of protein turnover is likely to include species for which fully annotated proteomes are not available. We have used dietary administration of stable isotope labeled lysine to assess protein turnover rates for proteins from four tissues in the bank vole, Myodes glareolus. The annotated genome for this species is not available, so protein identification was attained through cross-species matching to the mouse. For proteins for which confident identifications were derived, the pattern of lysine incorporation over 40 days was used to define the rate of synthesis of individual proteins in the four tissues. The data were heavily filtered to retain a very high quality dataset of turnover rates for 1088 proteins. Comparative analysis of the four tissues revealed different median rates of degradation (kidney: 0.099 $days^{-1}$; liver 0.136 $days^{-1}$; heart, 0.054 $days^{-1}$, and skeletal muscle, 0.035 $days^{-1}$). These data were compared with protein degradation rates from other studies on intact animals or from cells in culture and indicate that both cell type and analytical methodology may contribute to variance in turnover data between different studies. These differences were not only due to tissue-specific proteins but were reflected in gene products common to all tissues. All data are available via ProteomeXchange with identifier PXD002054. Molecular & Cellular Proteomics 15: 10.1074/mcp.M115.053488, 1204–1219, 2016.

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REVIEW ARTICLE 2573

Targeting protein function: the expanding toolkit for conditional disruption

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A major objective in biological research is to understand spatial and temporal requirements for any given gene, especially in dynamic processes acting over short periods, such as catalytically driven reactions, subcellular transport, cell division, cell rearrangement and cell migration. The interrogation of such processes requires the use of rapid and flexible methods of interfering with gene function. However, many of the most widely used interventional approaches, such as RNAi or CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated 9), operate at the level of the gene or its transcripts, meaning that the effects of gene perturbation are exhibited over longer time frames than the process under investigation. There has been much activity over the last few years to address this fundamental problem. In the present review, we describe recent advances in disruption technologies acting at the level of the expressed protein, involving inducible methods of protein cleavage, (in)activation, protein sequestration or degradation. Drawing on examples from model organisms we illustrate the utility of fast-acting techniques and discuss how different components of the molecular toolkit can be employed to dissect previously intractable biochemical processes and cellular behaviours.

Key words: biochemical techniques and resources, cellular targeting, chemical biology, protein dynamics, optogenetics.

PLOS | GENETICS

RESEARCH ARTICLE

Hydrophobic Core Variations Provide a Structural Framework for Tyrosine Kinase Evolution and Functional Specialization

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Analysis of Intrinsic Peptide Detectability via Integrated Label-Free and SRM-Based Absolute Quantitative Proteomics

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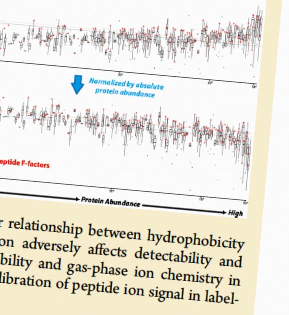
Supporting Information

ABSTRACT: Quantitative mass spectrometry-based proteomics of complex biological samples remains challenging in part due to the variability and charge competition arising during electrospray ionization (ESI) of peptides and the subsequent transfer and detection of ions. These issues preclude direct quantification from signal intensity alone in the absence of a standard. A deeper understanding of the governing principles of peptide ionization and exploitation of the inherent ionization and detection parameters of individual peptides is thus of great value. Here, using the yeast proteome as a model system, we establish the concept of peptide F-factor as a measure of detectability, closely related to ionization efficiency. F-factor is calculated by normalizing peptide precursor ion intensity by absolute abundance of the parent protein. We investigated F-factor characteristics in different shotgun proteomics experiments, including across multiple ESI-based LC–MS platforms. We show that F-factors mirror previously observed physicochemical predictors as peptide detectability but demonstrate a nonlinear relationship between hydrophobicity and peptide detectability. Similarly, we use F-factors to show how peptide ion coelution adversely affects detectability and ionization. We suggest that F-factors have great utility for understanding peptide detectability and gas-phase ion chemistry in complex peptide mixtures, selection of surrogate peptides in targeted MS studies, and for calibration of peptide ion signal in label-free workflows. Data are available via ProteomeXchange with identifier PXD003472.



KEYWORDS: quantitative proteomics, peptide ionization, peptide detectability, hydrophobicity, coelution

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