Towards comprehensive and quantitative proteomics for diagnosis and therapy of human disease

Paolo Cifani[1] and **Alex Kentsis**[1,2]

[1]Molecular Pharmacology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY

[2]Department of Pediatrics, Weill Cornell College of Cornell University and Memorial Sloan Kettering Cancer Center, New York, NY

Abstract

Given superior analytical features, mass spectrometry proteomics is well suited for the basic investigation and clinical diagnosis of human disease. Modern mass spectrometry enables detailed functional characterization of the pathogenic biochemical processes, as achieved by accurate and comprehensive quantification of proteins and their regulatory chemical modifications. Here, we describe how high-accuracy mass spectrometry in combination with high-resolution chromatographic separations can be leveraged to meet these analytical requirements in a mechanism-focused manner. We review the quantification methods capable of producing accurate measurements of protein abundance and post-translational modification stoichiometries. We then discuss how experimental design and chromatographic resolution can be leveraged to achieve comprehensive functional characterization of biochemical processes in complex biological proteomes. Finally, we describe current approaches for quantitative analysis of a common functional protein modification: reversible phosphorylation. In all, current instrumentation and methods of high-resolution chromatography and mass spectrometry proteomics are poised for immediate translation into improved diagnostic strategies for pediatric and adult diseases.

Keywords

Pediatric disease; functional proteomics; protein quantification; PTM; mass spectrometry

Introduction

Ever since the first discovery of specific proteins associated with human disease [1], the field of protein chemistry and later proteomics sought to identify new and improved markers of disease and targets of therapies. While the instrumentation for analytical chemistry and mass spectrometry has steadily improved, incorporation of this approach into preclinical investigation and clinical care has lagged [2]. With notable exceptions, such as mass spectrometry-based detection of bacterial pathogens [3], and drug and metabolites [4,5],

Corresponding Authors: Paolo Cifani, PhD, cifanip@mskcc.org, Alex Kentsis, MD, PhD, kentsisresearchgroup@gmail.com.

Conflict of Interest

The authors declare no conflict of interest.

recent advances in mass spectrometry remain largely confined to analytical chemistry laboratories [6]. Recently, we and others have sought to apply high-accuracy mass spectrometry [7] approaches for the discovery of improved diagnostic markers and therapeutic targets [8–16]. As a result of these and other studies, several methodological requirements for translational and clinical proteomics have emerged, including the need to balance analytical sensitivity and accuracy with the breadth of analyte detection, as driven by sample throughput. Here, we review the recently developed mass spectrometric methods in their current ability to enable comprehensive and quantitative proteomics, as they relate to the translational and clinical applications.

Biological Mass Spectrometry Proteomics

Protein activities in cells are controlled by multiple factors, including but not limited to protein synthesis and degradation [17], alternative splicing [18], post-translational chemical modification [19], intra-cellular localization [20], and interaction with co-factors and regulators [21]. Understanding differential regulation of all these mechanisms requires accurate quantification of proteins and their proteo- and chemoforms, which is increasingly being achieved by combining mass spectrometry-based proteomics with biochemical techniques and computational analyses [22–25]. These approaches generate data of increasing breadth and depth, as evidenced by the recently established workflows for mass spectrometric detection of post-translationally modified peptides [26, 27]. The general analytical requirement to obtain such biologically meaningful data is the need to accurately and sensitively measure the abundance of all relevant protein chemoforms in a sample. Here, we focus on bottom-up proteomics approaches, which analyze peptides generated by enzymatic or chemical proteolysis instead of the corresponding intact proteins, as this approach remains the most prevalent today [7, 28], though recent improvements in intact protein analysis should lend themselves to large-scale intact proteomics in the foreseeable future [29].

Quantitative Proteomics

High-throughput quantification of proteins and peptides historically relied on dye fluorescence intensity of gel resolved proteins, i.e., DIGE [30], or on correlative measures such as for example the number of fragmentation spectra recorded for a given protein [31]. Nowadays, these methods are used less frequently, because improvements in chromatography, ionization, mass spectrometry instrumentation, and data analysis enable more accurate quantification by direct measure of currents generated by specific peptide ions. The signal produced depends not only on the specific analyte concentration, but also on the efficiency of formation of the relative ions (ionization and fragmentation properties, as applicable). As a result, ion current-based quantification is always a relative and sample-specific measure.

With the exception of methods dependent on reporter ions, discussed later, quantification of peptides by mass spectrometry requires multiple measurements of the mass analyzer current generated by specific ions. These measurements are integrated in the time domain of the corresponding chromatographic peak to calculate the area under the curve (AUC), which is

the complete quantitation metric [32, 33]. This method is more robust than instantaneous ion current measurements, reducing the variability produced by differential chromatographic properties of peptides and variable ionization efficiencies.

Using modern software, specific ion currents can be extracted from any series of mass spectra. For example, signal intensity of un-fragmented peptide ions can be retrieved from full-range high-resolution data-dependent precursor scans [32, 33], a strategy that in principle enables proteome-wide quantification. However, far higher sensitivity, precision, and linear dynamic range are achieved by targeted quantification, which consists of detecting a ions within defined m/z windows selected by mass filters of increasing resolving power (Table 1). The most widespread implementation, still considered the gold-standard for peptide quantitation, is selected reaction monitoring (SRM, also referred to as MRM for multiple reaction monitoring), which uses triple-stage quadrupole instruments to first filter specific m/z range for fragmentation and subsequently filter specific fragment ions produced by collision-induced dissociation before dynode detection [34, 35]. This method benefits from the high sensitivity of dynode detectors, and the robustness conferred by the uninterrupted ion beam, but is limited by the relatively low resolution of current mass filters that hinders the specificity of the assays, which thus require careful validation [36, 37].

Parallel reaction monitoring (PRM) is conceptually similar to SRM in the use of mass filtering of narrow precursor isolation windows (Table 1), but uses high-resolution mass analyzers, such as the Orbitrap, to enable acquisition of complete high-resolution fragment ion spectra [38, 39]. While comparable in sensitivity to SRM, PRM enables potentially complete sequencing of the target peptide, with the consequent improvements in specificity and accuracy of quantitation. However, its higher duty cycle may reduce assay multiplexing, a drawback recently alleviated by the introduction of the internal standard triggered PRM approaches [40]. Both methods enable absolute sensitivity in the attomolar range, and up to five order of magnitude of linear dynamic range, which is still less than the biologic concentration range of proteins in human tissues [41, 42].

On the other hand, data independent acquisition (DIA) in principle can overcome the limited throughput of targeted methods by iteratively selecting portions of the m/z range for fragmentation, prior to high-resolution detection of fragments from all the filtered precursor ions (Table 1). Subsequent deconvolution of these fragmentation spectra permits peptide identification and extraction of chromatographic elution peaks for quantification [43–47]. While recent improvements in the resolution of time-of-flight spectrometers, such as the parallel accumulation-serial fragmentation (PASEF) method [48], promise to increase the instrumental duty cycle to permit data independent analysis of increasing sensitivity and accuracy, recent benchmarking of DIA using existing instruments demonstrated lower accuracy as compared to PRM and SRM [49].

An alternative strategy for peptide quantitation leverages the detection of reporter ions generated by the fragmentation of chemically reactive isobaric tags, such as for example iTRAQ and TMT [50, 51]. Both reagents consist of an isotopically encoded reporter ion, an amine reactive N-hydroxysuccinimidyl moiety, and a normalizing group to ensure that precursors labeled with different isotopologues remain isobaric and are thus co-selected for

fragmentation. These reagents are particularly useful in clinical applications as they enable isotopic labeling of samples derived from human tissues, but require controls for variable labeling efficiency and limited dynamic range [52].

Towards Comprehensive Quantification

While current approaches for quantitative mass spectrometry are sufficiently accurate to permit robust peptide quantification, they have yet to be applied for comprehensive analyses. For example, a typical SRM assay with chromatographic scheduling can monitor on the order of 100 peptides (Table 1). Conversely, DDA experiments, implementing either precursor ion current or reporter ion quantification, permit measuring the abundance of several thousand peptides across multiple samples, although with reduced precision, reproducibility and sensitivity. These observations provided the rationale to consider targeted approaches as a mere validation method for comprehensive DDA surveys. However, it is important to note that the complexity of mammalian tryptic proteomes far exceeds the sequencing duty cycle of current instruments [53], and that DDA is biased towards abundant and readily ionizable peptides that often do not include analytes of interest [54]. As a consequence, these approaches may not be suitable for the analysis of relevant molecular markers.

However, for many human diseases, including childhood diseases, comprehensive proteomic profiling may not be necessary, as relevant molecular markers have been identified using hypothesis-based or other high-throughput approaches such as genomics. For example, numerous childhood and adult cancers exhibit oncogenic activation of kinase signaling [55, 56], and chromatin and gene expression regulatory pathways [57, 58]. Thus, measurements of biologically or pathologically meaningful analytes may not require 'whole-proteome' approaches, and instead may rely on quantification of marker panels defined to probe specific pathways, as for example the PI3K-mTOR/MAPK signaling cascade [59] or the DNA damage response network [60]. This can also involve knowledge-based "sentinel" proteins [61], or other markers of pathway activity, such as those generated by reduced representation approaches [62]. Collections of SRM assays for this purpose have already begun development for cancer and infectious diseases [63–66].

The major determinant of throughput for both analytes and specimens is the duty cycle of targeted mass spectrometric detection in relation to the time scale of analytical chromatographic separation. One obvious solution for this problem involves enhancing chromatographic resolution prior to MS analysis to obtain adequate separation over extended chromatographic gradients [67]. This rationale was indeed successfully applied to increase the number of targeted mass spectrometry assays scheduled in a single experiment [68]. Improved chromatographic resolution can also be achieved by multi-dimensional and orthogonal separation techniques [69, 70], which also provide a means to improve mass spectral sampling, and detection and quantification of low abundance ions, thereby increasing the exposure of specific proteome subsets such as post-translationally modified peptides [7, 71–73]. However, most offline sample fractionation workflows are potentially hindered by sample losses that limit their overall robustness and reproducibility [74]. Online chromatographic fractionation has been successfully applied to DDA experiments,

demonstrating high efficiency and sensitivity due to automation and reduced sample requirements [75–78]. In unpublished results from our laboratory, we observed that automated online fractionation using multi-dimensional chromatography efficiently and reproducibly separated peptides from low-abundance transcription factors from other abundant isobaric ions co-eluting in final chromatographic dimension coupled to nanoelectrospray ionization. This enabled accurate quantification by targeted precursor and fragment ion detection of analytes that were otherwise not detected at all using conventional offline multi-dimensional or online single dimensional chromatographic separations.

Due to the variability of peptide ionization and fragmentation, all quantitative methods based on ion current extraction are inherently relative in nature [32, 33, 79]. Extracted ion chromatograms can be matched to compare the signal produced by the same peptide in different experiments. Such label-free methods have been used for comprehensive analysis of phosphorylation stoichiometry in model cell systems [32], [33, 80]. This strategy was also used in translational and preclinical studies to identify human disease biomarkers [12, 14, 16]. However, far more accurate measurements can be achieved using synthetic external reference peptides by comparing the signals produced by isotopologue peptides undergoing simultaneous chromatographic separation and ionization, thus minimizing technical variability and noise. Such approaches require isotopically encoded reference peptides for all the targeted analytes. Metabolic labeling of cell lines or primary cells *in vitro* has been used to generate reference standards for relative quantification of tumor samples [81–83]. However, it is still unclear whether such standards sufficiently capture the complexity of biologically variable analytes, such as specific post-translational modifications. Moreover, differential protein turn-over rates may lead to uneven proteome labeling [17]. Tissue samples can also be directly labeled using isotopically encoded chemical reagents including cysteine reactive moieties [84], ^{18}O water [85], iTRAQ and TMT reagents [50, 51] as well as other amine reactive groups producing dimethyl [86, 87] or nicotinic acid derivative [88, 89] adducts. While permitting universal labeling for quantitative mass spectrometry, such approaches require controls for variable or non-specific labeling. Alternatively, quantitation can be achieved using isotopologue synthetic peptides, as they can be introduced at known concentrations directly, thus enabling absolute quantification [35, 90].

Towards Comprehensive Functional Proteomics

Along with protein abundance, measured by quantification of the corresponding peptides, post-translational protein modifications are biologically important regulatory mechanisms that currently can be analyzed best using quantitative mass spectrometry [91]. In particular, the well-established regulatory functions of protein kinase signaling led to the refinement of methods for enrichment and analysis of phosphorylated peptides. Mass spectrometry is particularly well suited for characterization of protein chemoforms, as specific chemical modifications produce specific diagnostic alterations of peptide molecular mass. However, the sub-stoichiometric nature of protein phosphorylation and the relatively low abundance of many kinases and kinase substrates pose serious challenges for robust measurements of site occupancies and stoichiometries. Instrumental advances that enable robust phospho-proteomics include the development of specific affinity chromatography reagents and chromatographic strategies for the enrichment of phosphorylated peptides [71–72, 92–96].

Such approaches, for example, have recently been used to measure biological kinetic processes [97], and have been successfully coupled to targeted detection for enhanced sensitivity [98].

Enrichment of phosphorylated peptides is most commonly achieved using offline separations, that despite efforts towards miniaturization and automation [62, 99], are still prone to variable adsorptive losses that can potentially confound quantification measurements. To overcome this limitation, online chromatographic enrichment of phosphorylated peptides has been developed [100, 101]. Importantly, the detection of phosphorylated peptides does not appear to be significantly affected by their intrinsic chromatographic and ionization properties [28], suggesting that improved exposure afforded by online multi-dimensional chromatography might enable robust and sensitive quantitative analysis. Consistent with this notion, enhanced detection of phosphorylated peptides was observed using online fractionation by combining alkaline reverse phase and strong-anion exchange chromatography [76, 77]. Importantly, these automated multi-dimensional chromatographic methods might improve the detection and quantitation of other chemically modified, e.g., acetylated, methylated etc, peptides without the need for dedicated affinity enrichment procedures, thus providing a generalized method for quantitative functional proteomics [71].

Future Directions

There is a clear and unmet need for improved strategies for diagnosis, prognostication, and treatment of human disease. Current and emerging methods for high-resolution chromatography and mass spectrometry now enable routine accurate and sensitive quantitation of many biologically and pathologically relevant biomarkers. In particular, modern mass spectrometry satisfies the analytical requirements for comprehensive functional proteomics. Targeted bottom-up proteomics enable accurate quantification over a wide range of analyte concentrations present in clinical tissue specimens. In addition to data-independent approaches, recent advances in mechanism-based analysis of specific cellular processes may permit clinically relevant quantification of biologically or pathologically functional proteome subsets. Specifically, this is empowered by robust and reproducible sample processing and fractionation, which is now achievable using automated online multidimensional chromatography systems. This should enable not only precision functional proteomics by improving targeted detection of chemically modified peptides and proteins, but also provide specific mechanistic insights into biological and disease processes themselves.

Acknowledgments

Abbreviations

DDA   Data Dependent Acquisition

DIA	Data Independent Acquisition

PRM	Parallel Reaction Monitoring

PTM	post-translational modification

SAX	strong anion exchange (chromatography)

SCX	Strong cation exchange (chromatography)

References

1. Ridker PM. C-reactive protein: eighty years from discovery to emergence as a major risk marker for cardiovascular disease. Clin Chem. Feb; 2009 55(2):209–215. [PubMed: 19095723]

2. Lassman ME, McAvoy T, Chappell DL, Lee AY, Zhao XX, Laterza OF. The clinical utility of mass spectrometry based protein assays. Clin Chim Acta. Aug.2016 459:155–161. [PubMed: 27259466]

3. Martiny D, Busson L, Wybo I, El Haj RA, Dediste A, Vandenberg O. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. Apr; 2012 50(4):1313–1325. [PubMed: 22322345]

4. Adaway JE, Keevil BG, Owen LJ. Liquid chromatography tandem mass spectrometry in the clinical laboratory. Ann Clin Biochem. Jan; 2015 52(1):18–38. [PubMed: 25313226]

5. Ombrone D, Giocaliere E, Forni G, Malvagia S, la Marca G. Expanded newborn screening by mass spectrometry: New tests, future perspectives. Mass Spectrom Rev. Jan; 2016 35(1):71–84. [PubMed: 25952022]

6. Diamandis EP. The failure of protein cancer biomarkers to reach the clinic: why, and what can be done to address the problem? BMC Med. 2012; 10:87. [PubMed: 22876833]

7. Aebersold R, Mann M. Mass spectrometry-based proteomics. Nature. Mar; 2003 422(6928):198–207. [PubMed: 12634793]

8. Kentsis A, Monigatti F, Dorff K, Campagne F, Bachur R, Steen H. Urine proteomics for profiling of human disease using high accuracy mass spectrometry. Proteomics Clin Appl. Sep; 2009 3(9):1052–1061. [PubMed: 21127740]

9. Paczesny S, Braun TM, Levine JE, Hogan J, Crawford J, Coffing B, Olsen S, Choi SW, Wang H, Faca V, Pitteri S, Zhang Q, Chin A, Kitko C, Mineishi S, Yanik G, Peres E, Hanauer D, Wang Y, Reddy P, Hanash S, Ferrara JLM. Elafin is a biomarker of graft-versus-host disease of the skin. Sci Transl Med. Jan.2010 2(13):13ra2.

10. Zhang H, Liu T, Zhang Z, Payne SH, Zhang B, McDermott JE, Zhou J-Y, Petyuk VA, Chen L, Ray D, Sun S, Yang F, Chen L, Wang J, Shah P, Cha SW, Aiyetan P, Woo S, Tian Y, Gritsenko MA, Clauss TR, Choi C, Monroe ME, Thomas S, Nie S, Wu C, Moore RJ, Yu K-H, Tabb DL, Fenyo D, Bafna V, Wang Y, Rodriguez H, Boja ES, Hiltke T, Rivers RC, Sokoll L, Zhu H, Shih I-M, Cope L, Pandey A, Zhang B, Snyder MP, Levine DA, Smith RD, Chan DW, Rodland KD. CPTAC Investigators. Integrated Proteogenomic Characterization of Human High-Grade Serous Ovarian Cancer. Cell. Jul; 2016 166(3):755–765. [PubMed: 27372738]

11. Kentsis A, Lin YY, Kurek K, Calicchio M, Wang YY, Monigatti F, Campagne F, Lee R, Horwitz B, Steen H, Bachur R. Discovery and validation of urine markers of acute pediatric appendicitis using high-accuracy mass spectrometry. Ann Emerg Med. Jan; 2010 55(1):62–70.e4. [PubMed: 19556024]

12. Kentsis A, Shulman A, Ahmed S, Brennan E, Monuteaux MC, Lee YH, Lipsett S, Paulo JA, Dedeoglu F, Fuhlbrigge R, Bachur R, Bradwin G, Arditi M, Sundel RP, Newburger JW, Steen H, Kim S. Urine proteomics for discovery of improved diagnostic markers of Kawasaki disease. EMBO Mol Med. Feb; 2013 5(2):210–220. [PubMed: 23281308]

13. Taguchi A, Politi K, Pitteri SJ, Lockwood WW, Faça VM, Kelly-Spratt K, Wong CH, Zhang Q, Chin A, Park KS, Goodman G, Gazdar AF, Sage J, Dinulescu DM, Kucherlapati R, Depinho RA, Kemp CJ, Varmus HE, Hanash SM. Lung cancer signatures in plasma based on proteome profiling of mouse tumor models. Cancer Cell. Sep; 2011 20(3):289–299. [PubMed: 21907921]

14. Zhang B, Wang J, Wang X, Zhu J, Liu Q, Shi Z, Chambers MC, Zimmerman LJ, Shaddox KF, Kim S, Davies SR, Wang S, Wang P, Kinsinger CR, Rivers RC, Rodriguez H, Townsend RR, Ellis MJC, Carr SA, Tabb DL, Coffey RJ, Slebos RJC, Liebler DC. NCI CPTAC. Proteogenomic characterization of human colon and rectal cancer. Nature. Sep; 2014 513(7518):382–387. [PubMed: 25043054]

15. Mertins P, Mani DR, Ruggles KV, Gillette MA, Clauser KR, Wang P, Wang X, Qiao JW, Cao S, Petralia F, Kawaler E, Mundt F, Krug K, Tu Z, Lei JT, Gatza ML, Wilkerson M, Perou CM, Yellapantula V, Huang K-L, Lin C, McLellan MD, Yan P, Davies SR, Townsend RR, Skates SJ, Wang J, Zhang B, Kinsinger CR, Mesri M, Rodriguez H, Ding L, Paulovich AG, Fenyo D, Ellis MJ, Carr SA. NCI CPTAC. Proteogenomics connects somatic mutations to signalling in breast cancer. Nature. Jun; 2016 534(7605):55–62. [PubMed: 27251275]

16. Geyer PE, Kulak NA, Pichler G, Holdt LM, Teupser D, Mann M. Plasma Proteome Profiling to Assess Human Health and Disease. Cell Syst. Mar; 2016 2(3):185–195. [PubMed: 27135364]

17. Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M. Global quantification of mammalian gene expression control. Nature. May; 2011 473(7347):337–342. [PubMed: 21593866]

18. Graveley BR. Alternative splicing: increasing diversity in the proteomic world. Trends Genet. Feb; 2001 17(2):100–107. [PubMed: 11173120]

19. Seet BT, Dikic I, Zhou MM, Pawson T. Reading protein modifications with interaction domains. Nat Rev Mol Cell Biol. Jul; 2006 7(7):473–483. [PubMed: 16829979]

20. Bauer NC, Doetsch PW, Corbett AH. Mechanisms Regulating Protein Localization. Traffic. Oct; 2015 16(10):1039–1061. [PubMed: 26172624]

21. Robinson CV, Sali A, Baumeister W. The molecular sociology of the cell. Nature. Dec; 2007 450(7172):973–982. [PubMed: 18075576]

22. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell. Nov; 2006 127(3):635–648. [PubMed: 17081983]

23. Mischerikow N, Heck AJR. Targeted large-scale analysis of protein acetylation. Proteomics. Feb; 2011 11(4):571–589. [PubMed: 21246731]

24. Vermeulen M, Eberl HC, Matarese F, Marks H, Denissov S, Butter F, Lee KK, Olsen JV, Hyman AA, Stunnenberg HG, Mann M. Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. Cell. Sep; 2010 142(6):967–980. [PubMed: 20850016]

25. Christoforou A, Mulvey CM, Breckels LM, Geladaki A, Hurrell T, Hayward PC, Naake T, Gatto L, Viner R, Martinez Arias A, Lilley KS. A draft map of the mouse pluripotent stem cell spatial proteome. Nat Commun. 2016; 7:8992. [PubMed: 26754106]

26. Beausoleil SA, Jedrychowski M, Schwartz D, Elias JE, Villén J, Li J, Cohn MA, Cantley LC, Gygi SP. Large-scale characterization of HeLa cell nuclear phosphoproteins. Proc Natl Acad Sci USA. Aug; 2004 101(33):12130–12135. [PubMed: 15302935]

27. Svinkina T, Gu H, Silva JC, Mertins P, Qiao J, Fereshetian S, Jaffe JD, Kuhn E, Udeshi ND, Carr SA. Deep, Quantitative Coverage of the Lysine Acetylome Using Novel Anti-acetyl-lysine Antibodies and an Optimized Proteomic Workflow. Mol Cell Proteomics. Sep; 2015 14(9):2429–2440. [PubMed: 25953088]

28. Steen H, Jebanathirajah JA, Rush J, Morrice N, Kirschner MW. Phosphorylation analysis by mass spectrometry: myths, facts, and the consequences for qualitative and quantitative measurements. Mol Cell Proteomics. Jan; 2006 5(1):172–181. [PubMed: 16204703]

29. Ntai I, Kim K, Fellers RT, Skinner OS, Smith AD, Early BP, Savaryn JP, LeDuc RD, Thomas PM, Kelleher NL. Applying label-free quantitation to top down proteomics. Anal Chem. May; 2014 86(10):4961–4968. [PubMed: 24807621]

30. Unlü M, Morgan ME, Minden JS. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. Electrophoresis. Oct; 1997 18(11):2071–2077. [PubMed: 9420172]

31. Liu H, Sadygov RG, Yates JR. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. Anal Chem. Jul; 2004 76(14):4193–4201. [PubMed: 15253663]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

32. MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, Kern R, Tabb DL, Liebler DC, MacCoss MJ. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinformatics. Apr; 2010 26(7):966–968. [PubMed: 20147306]

33. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol. Dec; 2008 26(12): 1367–1372. [PubMed: 19029910]

34. Lange V, Picotti P, Domon B, Aebersold R. Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol. 2008; 4:222. [PubMed: 18854821]

35. Barnidge DR, Dratz EA, Martin T, Bonilla LE, Moran LB, Lindall A. Absolute quantification of the G protein-coupled receptor rhodopsin by LC/MS/MS using proteolysis product peptides and synthetic peptide standards. Anal Chem. Feb; 2003 75(3):445–451. [PubMed: 12585469]

36. Carr SA, Abbatiello SE, Ackermann BL, Borchers C, Domon B, Deutsch EW, Grant RP, Hoofnagle AN, Hüttenhain R, Koomen JM, Liebler DC, Liu T, MacLean B, Mani DR, Mansfield E, Neubert H, Paulovich AG, Reiter L, Vitek O, Aebersold R, Anderson L, Bethem R, Blonder J, Boja E, Botelho J, Boyne M, Bradshaw RA, Burlingame AL, Chan D, Keshishian H, Kuhn E, Kinsinger C, Lee JSH, Lee SW, Moritz R, Oses-Prieto J, Rifai N, Ritchie J, Rodriguez H, Srinivas PR, Townsend RR, Van Eyk J, Whiteley G, Wiita A, Weintraub S. Targeted peptide measurements in biology and medicine: best practices for mass spectrometry-based assay development using a fit-for-purpose approach. presented at the Molecular & cellular proteomics : MCP. 2014; 13(3): 907–917.

37. Abbatiello SE, Mani DR, Keshishian H, Carr SA. Automated detection of inaccurate and imprecise transitions in peptide quantification by multiple reaction monitoring mass spectrometry. Clin Chem. Feb; 2010 56(2):291–305. [PubMed: 20022980]

38. Peterson AC, Russell JD, Bailey DJ, Westphall MS, Coon JJ. Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics. Mol Cell Proteomics. Nov; 2012 11(11):1475–1488. [PubMed: 22865924]

39. Gallien S, Duriez E, Crone C, Kellmann M, Moehring T, Domon B. Targeted proteomic quantification on quadrupole-orbitrap mass spectrometer. Mol Cell Proteomics. Dec; 2012 11(12): 1709–1723. [PubMed: 22962056]

40. Gallien S, Kim SY, Domon B. Large-Scale Targeted Proteomics Using Internal Standard Triggered-Parallel Reaction Monitoring (IS-PRM). Mol Cell Proteomics. Jun; 2015 14(6):1630–1644. [PubMed: 25755295]

41. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics. Nov; 2002 1(11):845–867. [PubMed: 12488461]

42. Wi niewski JR, Hein MY, Cox J, Mann M. A 'proteomic ruler' for protein copy number and concentration estimation without spike-in standards. Mol Cell Proteomics. Dec; 2014 13(12): 3497–3506. [PubMed: 25225357]

43. Purvine S, Eppel JT, Yi EC, Goodlett DR. Shotgun collision-induced dissociation of peptides using a time of flight mass analyzer. Proteomics. Jun; 2003 3(6):847–850. [PubMed: 12833507]

44. Geiger T, Wehner A, Schaab C, Cox J, Mann M. Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. Mol Cell Proteomics. Mar.2012 11(3):M111.014050.

45. Venable JD, Dong MQ, Wohlschlegel J, Dillin A, Yates JR. Automated approach for quantitative analysis of complex peptide mixtures from tandem mass spectra. Nat Methods. Oct; 2004 1(1):39–45. [PubMed: 15782151]

46. Gillet LC, Navarro P, Tate S, Röst H, Selevsek N, Reiter L, Bonner R, Aebersold R. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics. Jun.2012 11(6):O111.016717.

47. Muntel J, Xuan Y, Berger ST, Reiter L, Bachur R, Kentsis A, Steen H. Advancing Urinary Protein Biomarker Discovery by Data-Independent Acquisition on a Quadrupole-Orbitrap Mass Spectrometer. J Proteome Res. Nov; 2015 14(11):4752–4762. [PubMed: 26423119]

48. Meier F, Beck S, Grassl N, Lubeck M, Park MA, Raether O, Mann M. Parallel Accumulation-Serial Fragmentation (PASEF): Multiplying Sequencing Speed and Sensitivity by Synchronized

Scans in a Trapped Ion Mobility Device. J Proteome Res. Dec; 2015 14(12):5378–5387. [PubMed: 26538118]

49. Kockmann T, Trachsel C, Panse C, Wahlander A, Selevsek N, Grossmann J, Wolski WE, Schlapbach R. Targeted proteomics coming of age - SRM, PRM and DIA performance evaluated from a core facility perspective. Proteomics. Apr.2016

50. Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Pappin DJ. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Mol Cell Proteomics. Dec; 2004 3(12):1154–1169. [PubMed: 15385600]

51. Thompson A, Schäfer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, Neumann T, Johnstone R, Mohammed AKA, Hamon C. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Anal Chem. Apr; 2003 75(8):1895–1904. [PubMed: 12713048]

52. Savitski MM, Mathieson T, Zinn N, Sweetman G, Doce C, Becher I, Pachl F, Kuster B, Bantscheff M. Measuring and managing ratio compression for accurate iTRAQ/TMT quantification. J Proteome Res. Aug; 2013 12(8):3586–3598. [PubMed: 23768245]

53. Muñoz J, Heck AJR. From the human genome to the human proteome. Angew Chem Int Ed Engl. Oct; 2014 53(41):10864–10866. [PubMed: 25079383]

54. Davis MT, Spahr CS, McGinley MD, Robinson JH, Bures EJ, Beierle J, Mort J, Yu W, Luethy R, Patterson SD. Towards defining the urinary proteome using liquid chromatography-tandem mass spectrometry. II. Limitations of complex mixture analyses. Proteomics. Jan; 2001 1(1):108–117. [PubMed: 11680890]

55. Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, Nakitandwe J, Holmfeldt L, Parker M, Easton J, Huether R, Kriwacki R, Rusch M, Wu G, Li Y, Mulder H, Raimondi S, Pounds S, Kang G, Shi L, Becksfort J, Gupta P, Payne-Turner D, Vadodaria B, Boggs K, Yergeau D, Manne J, Song G, Edmonson M, Nagahawatte P, Wei L, Cheng C, Pei D, Sutton R, Venn NC, Chetcuti A, Rush A, Catchpoole D, Heldrup J, Fioretos T, Lu C, Ding L, Pui C-H, Shurtleff S, Mullighan CG, Mardis ER, Wilson RK, Gruber TA, Zhang J, Downing JR. St. Jude Children's Research Hospital–Washington University Pediatric Cancer Genome Project. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. Apr; 2015 47(4):330–337. [PubMed: 25730765]

56. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang Y-L, Pei D, McCastlain K, Ding L, Lu C, Song G, Ma J, Becksfort J, Rusch M, Chen S-C, Easton J, Cheng J, Boggs K, Santiago-Morales N, Iacobucci I, Fulton RS, Wen J, Valentine M, Cheng C, Paugh SW, Devidas M, Chen I-M, Reshmi S, Smith A, Hedlund E, Gupta P, Nagahawatte P, Wu G, Chen X, Yergeau D, Vadodaria B, Mulder H, Winick NJ, Larsen EC, Carroll WL, Heerema NA, Carroll AJ, Grayson G, Tasian SK, Moore AS, Keller F, Frei-Jones M, Whitlock JA, Raetz EA, White DL, Hughes TP, Guidry Auvil JM, Smith MA, Marcucci G, Bloomfield CD, Mrózek K, Kohlschmidt J, Stock W, Kornblau SM, Konopleva M, Paietta E, Pui C-H, Jeha S, Relling MV, Evans WE, Gerhard DS, Gastier-Foster JM, Mardis E, Wilson RK, Loh ML, Downing JR, Hunger SP, Willman CL, Zhang J, Mullighan CG. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. Sep; 2014 371(11):1005–1015. [PubMed: 25207766]

57. Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, Ranish J, Crabtree GR. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. Nat Genet. Jun; 2013 45(6):592–601. [PubMed: 23644491]

58. Kim KH, Roberts CWM. Targeting EZH2 in cancer. Nat Med. Feb; 2016 22(2):128–134. [PubMed: 26845405]

59. de Graaf EL, Kaplon J, Mohammed S, Vereijken LAM, Duarte DP, Redondo Gallego L, Heck AJR, Peeper DS, Altelaar AFM. Signal Transduction Reaction Monitoring Deciphers Site-Specific PI3K-mTOR/MAPK Pathway Dynamics in Oncogene-Induced Senescence. J Proteome Res. Jul; 2015 14(7):2906–2914. [PubMed: 26011226]

60. Whiteaker JR, Zhao L, Yan P, Ivey RG, Voytovich UJ, Moore HD, Lin C, Paulovich AG. Peptide Immunoaffinity Enrichment and Targeted Mass Spectrometry Enables Multiplex, Quantitative Pharmacodynamic Studies of Phospho-Signaling. Mol Cell Proteomics. Aug; 2015 14(8):2261–2273. [PubMed: 25987412]

61. Soste M, Hrabakova R, Wanka S, Melnik A, Boersema P, Maiolica A, Wernas T, Tognetti M, von Mering C, Picotti P. A sentinel protein assay for simultaneously quantifying cellular processes. Nat Methods. Oct; 2014 11(10):1045–1048. [PubMed: 25194849]

62. Abelin JG, Patel J, Lu X, Feeney CM, Fagbami L, Creech AL, Hu R, Lam D, Davison D, Pino L, Qiao JW, Kuhn E, Officer A, Li J, Abbatiello S, Subramanian A, Sidman R, Snyder E, Carr SA, Jaffe JD. Reduced-representation Phosphosignatures Measured by Quantitative Targeted MS Capture Cellular States and Enable Large-scale Comparison of Drug-induced Phenotypes. Mol Cell Proteomics. May; 2016 15(5):1622–1641. [PubMed: 26912667]

63. Whiteaker JR, Halusa GN, Hoofnagle AN, Sharma V, MacLean B, Yan P, Wrobel JA, Kennedy J, Mani DR, Zimmerman LJ, Meyer MR, Mesri M, Rodriguez H, Paulovich AG. Clinical Proteomic Tumor Analysis Consortium (CPTAC). CPTAC Assay Portal: a repository of targeted proteomic assays. Nat Methods. Jul; 2014 11(7):703–704. [PubMed: 24972168]

64. Karlsson C, Malmström L, Aebersold R, Malmström J. Proteome-wide selected reaction monitoring assays for the human pathogen Streptococcus pyogenes. Nat Commun. 2012; 3:1301. [PubMed: 23250431]

65. Whiteaker JR, Halusa GN, Hoofnagle AN, Sharma V, MacLean B, Yan P, Wrobel JA, Kennedy J, Mani DR, Zimmerman LJ, Meyer MR, Mesri M, Boja E, Carr SA, Chan DW, Chen X, Chen J, Davies SR, Ellis MJC, Fenyo D, Hiltke T, Ketchum KA, Kinsinger C, Kuhn E, Liebler DC, Liu T, Loss M, MacCoss MJ, Qian W-J, Rivers R, Rodland KD, Ruggles KV, Scott MG, Smith RD, Thomas S, Townsend RR, Whiteley G, Wu C, Zhang H, Zhang Z, Rodriguez H, Paulovich AG. Using the CPTAC Assay Portal to Identify and Implement Highly Characterized Targeted Proteomics Assays. Methods Mol Biol. 2016; 1410:223–236. [PubMed: 26867747]

66. Kusebauch U, Campbell DS, Deutsch EW, Chu CS, Spicer DA, Brusniak MY, Slagel J, Sun Z, Stevens J, Grimes B, Shteynberg D, Hoopmann MR, Blattmann P, Ratushny AV, Rinner O, Picotti P, Carapito C, Huang CY, Kapousouz M, Lam H, Tran T, Demir E, Aitchison JD, Sander C, Hood L, Aebersold R, Moritz RL. Human SRMAtlas: A Resource of Targeted Assays to Quantify the Complete Human Proteome. Cell. Jul; 2016 166(3):766–778. [PubMed: 27453469]

67. Shen Y, Zhao R, Belov ME, Conrads TP, Anderson GA, Tang K, Pasa-Toli L, Veenstra TD, Lipton MS, Udseth HR, Smith RD. Packed capillary reversed-phase liquid chromatography with high-performance electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry for proteomics. Anal Chem. Apr; 2001 73(8):1766–1775. [PubMed: 11338590]

68. Shi T, Fillmore TL, Gao Y, Zhao R, He J, Schepmoes AA, Nicora CD, Wu C, Chambers JL, Moore RJ, Kagan J, Srivastava S, Liu AY, Rodland KD, Liu T, Camp DG, Smith RD, Qian WJ. Long-gradient separations coupled with selected reaction monitoring for highly sensitive, large scale targeted protein quantification in a single analysis. Anal Chem. Oct; 2013 85(19):9196–9203. [PubMed: 24004026]

69. Antberg L, Cifani P, Sandin M, Levander F, James P. Critical comparison of multidimensional separation methods for increasing protein expression coverage. J Proteome Res. May; 2012 11(5): 2644–2652. [PubMed: 22449141]

70. Percy AJ, Simon R, Chambers AG, Borchers CH. Enhanced sensitivity and multiplexing with 2D LC/MRM-MS and labeled standards for deeper and more comprehensive protein quantitation. J Proteomics. Jun.2014 106:113–124. [PubMed: 24769237]

71. Mohammed S, Heck A. Strong cation exchange (SCX) based analytical methods for the targeted analysis of protein post-translational modifications. Curr Opin Biotechnol. Feb; 2011 22(1):9–16. [PubMed: 20926283]

72. Alpert AJ, Hudecz O, Mechtler K. Anion-exchange chromatography of phosphopeptides: weak anion exchange versus strong anion exchange and anion-exchange chromatography versus electrostatic repulsion-hydrophilic interaction chromatography. Anal Chem. 2015; 87(9):4704–4711. [PubMed: 25827581]

73. Alpert AJ. Electrostatic repulsion hydrophilic interaction chromatography for isocratic separation of charged solutes and selective isolation of phosphopeptides. Anal Chem. Jan; 2008 80(1):62–76. [PubMed: 18027909]

74. Magdeldin S, Moresco JJ, Yamamoto T, Yates JR. Off-Line Multidimensional Liquid Chromatography and Auto Sampling Result in Sample Loss in LC/LC-MS/MS. J Proteome Res. Aug; 2014 13(8):3826–3836. [PubMed: 25040086]

75. Wolters DA, Washburn MP, Yates JR. An automated multidimensional protein identification technology for shotgun proteomics. Anal Chem. Dec; 2001 73(23):5683–5690. [PubMed: 11774908]

76. Ficarro SB, Zhang Y, Carrasco-Alfonso MJ, Garg B, Adelmant G, Webber JT, Luckey CJ, Marto JA. Online nanoflow multidimensional fractionation for high efficiency phosphopeptide analysis. Mol Cell Proteomics. Nov.2011 10(11):O111.011064.

77. Zhou F, Lu Y, Ficarro SB, Adelmant G, Jiang W, Luckey CJ, Marto JA. Genome-scale proteome quantification by DEEP SEQ mass spectrometry. Nat Commun. 2013; 4:2171. [PubMed: 23863870]

78. Liu H, Finch JW, Luongo JA, Li G-Z, Gebler JC. Development of an online two-dimensional nano-scale liquid chromatography/mass spectrometry method for improved chromatographic performance and hydrophobic peptide recovery. J Chromatogr A. Nov; 2006 1135(1):43–51. [PubMed: 17027011]

79. Steen H, Pandey A. Proteomics goes quantitative: measuring protein abundance. Trends Biotechnol. Sep; 2002 20(9):361–364. [PubMed: 12175758]

80. Sharma K, D'Souza RCJ, Tyanova S, Schaab C, Wiśniewski JR, Cox J, Mann M. Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling. Cell Rep. Sep; 2014 8(5):1583–1594. [PubMed: 25159151]

81. Ong S-E, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics. May; 2002 1(5):376–386. [PubMed: 12118079]

82. Geiger T, Cox J, Ostasiewicz P, Wiśniewski JR, Mann M. Super-SILAC mix for quantitative proteomics of human tumor tissue. Nat Methods. May; 2010 7(5):383–385. [PubMed: 20364148]

83. Staal JA, Lau LS, Zhang H, Ingram WJ, Hallahan AR, Northcott PA, Pfister SM, Wechsler-Reya RJ, Rusert JM, Taylor MD, Cho Y-J, Packer RJ, Brown KJ, Rood BR. Proteomic profiling of high risk medulloblastoma reveals functional biology. Oncotarget. Jun; 2015 6(16):14584–14595. [PubMed: 25970789]

84. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. Nat Biotechnol. Oct; 1999 17(10):994–999. [PubMed: 10504701]

85. Schnölzer M, Jedrzejewski P, Lehmann WD. Protease-catalyzed incorporation of 18O into peptide fragments and its application for protein sequencing by electrospray and matrix-assisted laser desorption/ionization mass spectrometry. Electrophoresis. May; 1996 17(5):945–953. [PubMed: 8783021]

86. Boersema PJ, Aye TT, van Veen TAB, Heck AJR, Mohammed S. Triplex protein quantification based on stable isotope labeling by peptide dimethylation applied to cell and tissue lysates. Proteomics. Nov; 2008 8(22):4624–4632. [PubMed: 18850632]

87. Hsu J-L, Huang S-Y, Chow N-H, Chen S-H. Stable-isotope dimethyl labeling for quantitative proteomics. Anal Chem. Dec; 2003 75(24):6843–6852. [PubMed: 14670044]

88. Schmidt A, Kellermann J, Lottspeich F. A novel strategy for quantitative proteomics using isotope-coded protein labels. Proteomics. Jan; 2005 5(1):4–15. [PubMed: 15602776]

89. Münchbach M, Quadroni M, Miotto G, James P. Quantitation and facilitated de novo sequencing of proteins by isotopic N-terminal labeling of peptides with a fragmentation-directing moiety. Anal Chem. Sep; 2000 72(17):4047–4057. [PubMed: 10994964]

90. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proc Natl Acad Sci USA. Jun; 2003 100(12): 6940–6945. [PubMed: 12771378]

91. Wu R, Dephoure N, Haas W, Huttlin EL, Zhai B, Sowa ME, Gygi SP. Correct interpretation of comprehensive phosphorylation dynamics requires normalization by protein expression changes. Mol Cell Proteomics. Aug.2011 10(8):M111.009654.

92. Zoumaro-Djayoon AD, Heck AJR, Muñoz J. Targeted analysis of tyrosine phosphorylation by immuno-affinity enrichment of tyrosine phosphorylated peptides prior to mass spectrometric analysis. Methods. Feb; 2012 56(2):268–274. [PubMed: 21945579]

93. Pinkse MWH, Uitto PM, Hilhorst MJ, Ooms B, Heck AJR. Selective isolation at the femtomole level of phosphopeptides from proteolytic digests using 2D-NanoLC-ESI-MS/MS and titanium oxide precolumns. Anal Chem. Jul; 2004 76(14):3935–3943. [PubMed: 15253627]

94. Guerrera IC, Predic-Atkinson J, Kleiner O, Soskic V, Godovac-Zimmermann J. Enrichment of phosphoproteins for proteomic analysis using immobilized Fe(III)-affinity adsorption chromatography. J Proteome Res. Sep; 2005 4(5):1545–1553. [PubMed: 16212405]

95. Posewitz MC, Tempst P. Immobilized gallium(III) affinity chromatography of phosphopeptides. Anal Chem. Jul; 1999 71(14):2883–2892. [PubMed: 10424175]

96. Ritorto MS, Cook K, Tyagi K, Pedrioli PGA, Trost M. Hydrophilic strong anion exchange (hSAX) chromatography for highly orthogonal peptide separation of complex proteomes. J Proteome Res. Jun; 2013 12(6):2449–2457. [PubMed: 23294059]

97. Emdal KB, Pedersen A-K, Bekker-Jensen DB, Tsafou KP, Horn H, Lindner S, Schulte JH, Eggert A, Jensen LJ, Francavilla C, Olsen JV. Temporal proteomics of NGF-TrkA signaling identifies an inhibitory role for the E3 ligase Cbl-b in neuroblastoma cell differentiation. Sci Signal. Apr.2015 8(374):ra40. [PubMed: 25921289]

98. Kennedy JJ, Yan P, Zhao L, Ivey RG, Voytovich UJ, Moore HD, Lin C, Pogosova-Agadjanyan EL, Stirewalt DL, Reding KW, Whiteaker JR, Paulovich AG. Immobilized Metal Affinity Chromatography Coupled to Multiple Reaction Monitoring Enables Reproducible Quantification of Phospho-signaling. Mol Cell Proteomics. Feb; 2016 15(2):726–739. [PubMed: 26621847]

99. Humphrey SJ, Azimifar SB, Mann M. High-throughput phosphoproteomics reveals in vivo insulin signaling dynamics. Nat Biotechnol. Sep; 2015 33(9):990–995. [PubMed: 26280412]

100. Pinkse MWH, Mohammed S, Gouw JW, van Breukelen B, Vos HR, Heck AJR. Highly robust, automated, and sensitive online TiO2-based phosphoproteomics applied to study endogenous phosphorylation in Drosophila melanogaster. J Proteome Res. Feb; 2008 7(2):687–697. [PubMed: 18034456]

101. Lemeer S, Pinkse MWH, Mohammed S, van Breukelen B, den Hertog J, Slijper M, Heck AJR. Online automated in vivo zebrafish phosphoproteomics: from large-scale analysis down to a single embryo. J Proteome Res. Apr; 2008 7(4):1555–1564. [PubMed: 18307296]

Table 1

Analytical features of quantitative mass spectrometry methods.

	DDA	SRM	PRM	DIA
Sensitivity	Medium	High	High	High
Specificity	Medium	High	High	Medium
Throughput	5,000	150	150 (600[*])	2,000

DDA: data-dependent acquisition; SRM: selected reaction monitoring; PRM: parallel reaction monitoring; DIA: data-independent acquisition. Throughput describes the maximum number of analytes currently accessible per experiment using a single liquid chromatography separation with typical conditions, and assay scheduling for targeted methods.

[*] Internal standard triggered-parallel reaction monitoring (IS-PRM).