Published in final edited form as:

Anal Methods. 2013 September 21; 5(18): . doi:10.1039/C3AY40853A.

Comparison of bottom-up proteomic approaches for LC-MS analysis of complex proteomes

Leigh A. Weston[§], Kerry M. Bauer[§], and Amanda B. Hummon^{*}

Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, IN 46556, USA

Abstract

Discovery-based proteomic studies aim to answer important biological questions by identifying as many proteins as possible. In order to accomplish this lofty goal, an effort must be placed on determining an optimal workflow that maximizes protein identifications. In this study, we compare protein extraction, digestion and fractionation methods for bottom-up proteomics using a human colon cancer cell line as our model system. Four different buffers for protein extraction, two digestion approaches, as well as three sample fractionation methods were evaluated in order to determine an accessible workflow that gives maximal protein identifications. Samples comparing these workflows were analyzed via UPLC paired with tandem MS on a Q-Exactive mass spectrometer. Our goal is to determine an optimal workflow to enable users to maximize protein identifications. Our results show that an increased number of confident protein identifications are attained with a filter-aided digestion approach as compared to an in-solution digestion. Overall SDS-PAGE fractionation leads to higher numbers of identifications than SCX SpinTip and reverse phased cartridge platforms. The novel aspect of this work is the comparison of two readily available, offline platforms for fractionation in reference to a traditional technique, SDS-PAGE.

Keywords

Q-Exactive; FASP; separation; sample preparation; proteomics

Introduction

Mammalian proteomes are complex and dynamic. Specifically, the human proteome has 21,175 proteins with abundances covering seven orders of magnitude¹. Proteomic analysis of complex systems is further challenged by the dynamic nature of the proteome, which is subject to genomic and environmental influences. The use of functional proteomics to identify biomarkers and enhance our understanding of cellular processes and events is a rapidly growing field and relies heavily on the analysis of large, complex proteomes. The

^{*}Corresponding author: Dr. Amanda B. Hummon, Department of Chemistry and Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, IN 46556, USA. Telephone: 574-631-0583. ahummon@nd.edu, Fax: 574-631-6652.

[§]Authors contributed equally to this work

Electronic Supplementary Material

Electronic Supplementary Information 1 Sample preparations for evaluated extraction, digestion, and fractionation methods. Electronic Supplementary Information 2 Distribution of MW, pI and hydrophobicity for proteins identified from lysis buffer comparison. Electronic Supplementary Information 3 Distribution of MW, pI and hydrophobicity for proteins identified from digestion method comparison. Electronic Supplementary Information 4 Protein identification overlap between fractions for SDS-PAGE, SCX SpinTip and C18 SepPak. Electronic Supplementary Information Protein Identifications provides a list of the proteins identified in this study.

The authors declare no competing financial interest.

desire to analyze the global proteome of complex organisms makes sample preparation particularly important in proteomics experiments. Efficient protein extraction, digestion and effective fractionation of these complex proteomes are critical to increasing peptide and protein identifications in bottom-up proteomics.

Cell lysis buffers for protein extraction most often include detergents or protein denaturing reagents. These agents act to disaggregate large complexes, solubilize membrane bound species and denature larger macromolecules, all to enhance protein extraction. Sodium dodecyl sulfate (SDS), 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and urea are commonly used detergents and denaturing reagents due to the high efficiency with which they solubilize and denature proteins. At high concentrations, SDS and urea can interfere with enzymatic digestion, resulting in reduced proteolytic efficiency. Additionally, these reagents are not known to be compatible with mass spectrometric analysis. Detergents may potentially affect reverse-phase liquid chromatography (RPLC) resolution; while the introduction of these components into the mass spectrometer can lead to a number of problems⁴. These reagents suppress peptide ionization, contaminate the ion source and form adducts with peptides. Protein extraction is an essential step in proteomic workflows and can be accomplished by a number of available solubilizing agents. Therefore it is important to evaluate these common reagents for optimal performance.

An alternative to an independent detergent removal step is to combine the processes of contaminant removal and sample digestion. Protocols that use microcentrifuge filter devices to remove detergents and other contaminants are gaining popularity in the proteomics community. These devices allow for protein reduction, alkylation and enzymatic digestion on the filter with isolation of peptides by centrifugation. While protein digestion occurs on the filter, this method combines the advantages of both in-gel and in-solution protein digestion. In-gel digestions are robust for removing contaminating species but are not easily automated. They are also time-consuming and have been previously shown to reduce peptide yields⁵. In-solution digests can be easily automated and minimize sample handling, however interfering substances must be removed prior to digestion or mass spectrometric analysis⁶. The spin filter method is amenable to the presence of detergents and other contaminants and allows for robust sample processing.

Following a highly efficient protein extraction and sample digest, a method to decrease sample complexity and increase resolving power is necessary to enhance proteome coverage. Protein and peptide properties, including size, charge, polarity, and hydrophobicity, are exploited to separate complex samples into discrete fractions which are subsequently analyzed. Most often, a two dimensional strategy is used to capitalize on orthogonal physical properties of the peptides with the second dimension being RPLC⁷. Various fractionation methods have been coupled to RPLC including gel electrophoresis for protein-peptide separations and strong cation exchange (SCX) and RP (at high pH) for peptide-peptide separations.

Although initially HPLC-based, several fractionation methods have been adapted. This has led to an increase in the variety of spin column and cartridge platforms available to perform various stages of sample preparation for mass spectrometry. These tools can be utilized for many applications including detergent removal¹⁰⁻¹², protein digestion^{6,13}, and sample fractionation approaches¹⁴, including SCX and RP. The spin column and cartridge devices are convenient, easy-to-handle and robust. These formats are commercially available and ready-to-use, require little to no additional instrumentation and have high-throughput capabilities for the simultaneous processing of multiple samples. Selecting the optimal fractionation method appropriately reduces the sample complexity, allowing for better resolution and detection of peptides.

In this document, we describe our systematic evaluation of several commercial sample preparation products. Our aim was to determine an easily accessible proteomics platform that maximizes protein identifications. We compared bottom-up proteomics workflows by analyzing tryptic digests of mammalian whole cell lysates using multiple protein extraction buffers, digestion methods and sample fractionation approaches independently. We analyzed cell lysis buffers containing various reagents for protein solubilization including urea, CHAPS and SDS at a low concentration (0.1%) and high concentration (4%) to determine the most effective protein extraction method. An evaluation of protein identifications after in-solution and filter-aided sample digestion was also conducted. Additionally, we investigated SDS-PAGE, SCX spin column and high-pH reverse phase Sep-Pak cartridge fractionation methods to evaluate the efficiencies of various sample fractionation approaches. While previous comparisons have been reported between SDS-PAGE, SCX, and RP fractionations, revealing that SDS-PAGE consistently outperforms the other methods,^{8,9} our study is the first to compare two easily accessible, offline platforms, SCX SpinTips and high pH-Sep-Paks, with reference to SDS-PAGE. For investigators who might not have access to an HPLC for sample preparation or as a first dimension of separation, this study provides valuable information comparing available sample preparation techniques.

Experimental Procedures

Reagents

Fetal bovine serum (FBS) was purchased from Thermo Scientific (Pittsburgh, PA). RPMI 1640 medium and L-glutamine were purchased from Invitrogen (Gaithersburg, MD). Protease inhibitor cocktail and Lysis-M were obtained from Roche (Indianapolis, IN). Trypsin Gold (Mass Spectrometry Grade) was purchased from Promega (Madison, WI). Mass spectrometry solvents including, acetonitrile (ACN) and water with 0.1% formic acid (FA), were obtained from Burdick and Jackson (Muskegon, MI). All reagents not listed were acquired from Sigma Aldrich (St. Louis, MO).

Cell Culture

The cell line SW620 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in RPMI 1640 medium supplemented with 10% FBS and 2mM L-glutamine and grown in 5% CO₂ at 37 °C.

Protein Extraction

The four lysis buffers were prepared according to Table 1. For each of the four equivalent cell populations, 1mL of lysis buffer was applied and cells were collected using a cell scraper. Samples were then sonicated (20% amplitude in 60 s intervals), and the cell lysate was clarified by centrifugation for 10 min (10000 rpm at 4 °C). Protein amounts were quantified using BCA-RAC assay for the SDT lysis buffer and a standard BCA assay for the remaining samples (Pierce, Rockford, IL).

Sample Preparations

In order to maintain systematic comparisons between methods only one variable was altered for a given sample preparation step. To evaluate the lysis buffers, all four protein samples were subjected to SDS-PAGE fractionation and in-gel digestion. For the evaluation of the digestion methods, the same amount of sample from the same lysis buffer protein stock were subjected to in-solution or filter-aided digestion applications. The whole digested proteome was not further fractionated with one of the evaluated methods. Unfractionated whole proteome samples were analyzed using a LC time of 3 h compared to 1.5 h per fraction for all other samples. The fractionation methods were evaluated using samples of the same lysis

buffer and when possible the same digestion method all while keeping the same number of fractions for each of the methods. For sample specifics please see Electronic Supplementary Information 1.

For in-solution digestion, 100 µg aliquots of protein were incubated with 150 µL of 50 mM ammonium biocarbonate (ABC) and 15 µL of 100 mM dithiothreitol (DTT) for 1 h at 65 °C. Reduced cysteine residues were alkylated with 30 µL of 100 mM iodoacetamide (IAA) at ambient temperature for 10 min in the dark. Protein samples were then digested with 1.5 µL trypsin (1 µg/µL) at 37 °C for 2 h followed by incubation with an additional 1.5 µL of trypsin overnight at 30 °C. Protein digestion using the FASP Protein Digestion Kit (Expedeon, San Diego, CA) was performed by following the manufacturer's protocol. All digested protein samples were dried using a vacuum concentrator before being fractionated and analyzed.

SDS-PAGE was performed with samples from each of the four lysis buffers. Aliquots of 50 µg of protein were suspended in 15 µL of 50 mM ABC and reduced by adding 2.5 µL of 500 mM DTT. Samples were vortexed at ambient temperature for 1 h. The reduced proteins were then alkylated with 2.5 µL of 100 mM IAA in the dark for 10 min at ambient temperature. After loading samples, the gel (Invitrogen, Carlsbad, CA) was run and digestion was performed as previously described¹⁵. Each sample was excised into eight sections or fractions prior to in-gel digestion.

SCX fractionation was completed using a SCX SpinTip Sample Preparation Kit (Protea Biosciences, Morgantown, WV). The SCX SpinTips were conditioned and a 100 µg sample of protein stock was loaded according to manufacturer's instructions and the flow-through was retained. The fractions resulting from the flow-through, ammonium formate buffers (20, 50, 80, 150, 250, 500 mM with 10% ACN at pH 3) and the elution solution provided with the SpinTips, divided the sample into eight fractions. After adding 200 µL of the appropriate solution the sample was centrifuged for 4 min at 4,000 rpm and fractions were dried using a vacuum concentrator. The 50 mg C18 SepPak cartridge was also evaluated as a sample separation technique (Waters, Milford, MA). For this procedure, equilibration, wash, and elution solutions are 10 mM ABC at pH 10. The C18 SepPak cartridge was prepared by washing with 3 mL 80% ACN and then equilibrated with 3 mL 1% ACN. Samples were resuspended in 50 µL 0.1% formic acid (FA), loaded onto the cartridge, and then washed with 3 mL 1% ACN. The peptide sample was fractionated using eight elution buffers with concentrations of 5, 10, 15, 20, 23, 27, 30 and 80% ACN.

After all other methods were complete, peptide samples were desalted with C18 ZipTips (Millipore, Billerica, MA). The desalted peptides were dried and resuspended in 0.1% FA. Peptide fractions generated with a C18 SepPak, however, did not require clean-up and were dried and resuspended in 0.1% FA.

Mass Spectrometry Analysis

All samples were analyzed using liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) on a nano-Acquity ultra performance LC system (100x100 C18 BEH column, Waters) coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). LC system solvents were 0.1% FA (A) and 0.1% FA in ACN (B). Peptide sample separations were performed with a linear gradient from 5–50% B for 75 min followed by an organic wash and aqueous re-equilibration at a flow rate of 1000 nL/min with a total run time of 90 minutes. Unfractionated samples were separated in a similar manner, but with a linear gradient from 5–50% B for 160 minutes. The mass spectrometer was operated in a Top 12 data dependent mode with automatic switching between MS and MS/MS. Source ionization parameters were as follows: spray voltage, 2.2

kV; capillary temperature, 280 °C; and s-lens level, 50.0. Full scan MS mode (400–1800 *m/z*) was operated at a resolution of 70,000 with automatic gain control (AGC) target of 1×10^6 ions and a maximum ion transfer (IT) of 20 ms. Ions selected for MS/MS were subjected to the following parameters: resolution 17,500; AGC 1×10^5 ions; maximum IT 80 ms; 2.0 *m/z* isolation window; normalized collision energy 28.0; underfill ratio 1.0%; and dynamic exclusion of 40.0 s. All samples were run with duplicate injections.

Mass Spectrometric Data Analysis

All raw files were analyzed using the Thermo Proteome Discoverer (1.3.0.339) software platform. The files were searched using the Mascot (2.2.04) search engine against the International Protein Index (IPI) human (3.84) and decoy human databases with a strict FDR of 0.01. The following Mascot parameters were used: trypsin was selected as the enzyme; two missed cleavages were allowed; precursor mass tolerance was set to 15 mmu; fragment mass tolerance was set to 20 mmu; dynamic modifications included protein N-terminal acetylation, N-terminal glutamine deamidation, methionine methylation and carbamidomethylation of cysteine was set as a fixed modification. Strict maximum parsimony principle was applied considering only peptide spectrum matches (PSMs) with medium confidence or higher and delta Cn better than 0.15. All generated Venn diagrams were made using the online tool, Venny¹⁶. Subcellular compartments were assigned to proteins using the UniProt database. GRAVY-scores were calculated according to the values from Kyte and Doolittle¹⁷. In addition, proteins with transmembrane regions were predicted using the Trans Membrane Hidden Markov Model (TMHMM)¹⁸.

Results and Discussion

This study independently evaluated several steps in the sample preparation process to determine an optimal set of parameters for maximal protein identifications (Figure 1). Just as the biological questions addressed by bottom-up proteomics vary, so do the different workflows used to prepare samples. Although varied, these approaches share the central steps of extracting proteins from a starting material, digesting these proteins into peptides, and fractionating the complex biological mixture. In addition to evaluating protein identification results, we will address considerations regarding overall experimental design (e.g. quantification strategy).

Protein extraction evaluation

In order to generate protein samples for downstream analysis, an appropriate lysis buffer must be selected to extract proteins. Variety between the evaluated lysis buffers comes mainly from the different identities and concentrations of the detergents present in the buffers. These buffer composition differences are highlighted in Table 1. The SDT lysis buffer (3.15 µg/µL, 1.58 mg) yielded the highest protein concentration based on BCA analysis with comparable protein yields for the label compatible (2.49 µg/µL, 1.25 mg), Lysis-M (2.18 µg/µL, 1.09 mg) and modified RIPA (1.99 µg/µL, 1.00 mg) buffers. Equal amounts of protein samples from the individual buffers were analyzed by SDS-PAGE.

The protein samples in the 1D gel, which differed only by lysis buffer, were excised into eight fractions and digested in-gel. The resulting peptide mixtures were analyzed via UPLC-MS/MS. According to Mascot search results, the different buffers result in similar total numbers of protein group identifications, ranging from 4947 to 5190, comparable protein coverage, 19.8 to 23.2%, and similar identification rates, 40.7 to 45.8% (Figure 2). A considerable number of the identified protein groups were common among the samples indicating no major advantage for any of the evaluated buffers. A broad overview of the mammalian proteome was identified by each of the lysis buffers used in this study as

evidenced by cellular localization and protein properties. Subcellular location assessment of the identified protein groups shows comparable distribution among cellular compartments, including membrane associated proteins (Figure 3). Furthermore, results using the TMHMM prediction algorithm reveal similar relative numbers (percent of total protein groups identified) of transmembrane proteins (data not shown). An evaluation of the distribution of the molecular weight, isoelectric point (pI) and hydrophobicity for the proteins identified with each lysis buffer shows no bias towards any protein class based on these protein properties (Electronic Supplementary Information 2).

Despite the similarities in protein group identification and cellular location, buffer selection may vary according to application. For example, the Tris in the SDT and modified RIPA buffers would interfere with several amine-reactive proteomic quantification strategies, including reductive dimethylation, iTRAQ and TMT. Furthermore, some buffer components can be removed by sample cleanup procedures, while others are retained in the sample, thus interfering with peptide detection. A contaminant removal step may need to be considered before proceeding with sample preparation based on the selected lysis buffer. One such method is the combination of protein digestion and detergent removal utilized in the filter-aided sample preparation (FASP) approach. The SDT buffer has the highest protein yield, but also requires significant detergent removal and thus is often paired with filter-aided digestion. The label compatible buffer has the least interference with downstream steps, such as chemical labeling for quantification, without sacrificing protein yield and protein identifications.

Protein digestion evaluation

For bottom-up proteomic workflows, protein digestion is a key step. The most commonly used digestion schemes are in-gel, solution and filter based. The in-solution and filter-aided methods are most comparable since each has the ability to digest complex cell proteomes without prior fractionation. They are also automatable methods. A direct comparison to the in-gel digestion approach was not in the scope of this work as defined gel sections are usually digested individually as fractions instead of as a whole proteome. The use of SDS-PAGE as a sample fractionation method is discussed in a subsequent section.

Comparing the results for in-solution and filter-aided digestion methods shows that the filter approach leads to higher numbers of identified protein groups (Figure 4). Using equal amounts of material from the same cell lysate sample, we identified 3715 protein groups with the filter-aided preparation compared to 2113 identifications for the in-solution preparation. The filter-aided and in-solution digestion methods also differed in proteolytic efficiency and protein identification rate. The filter-aided approach yields a higher percentage of fully tryptic peptides and fewer peptides with missed cleavages. The protein identification rate for the filter-aided method is 66%, which is enhanced compared to the in-solution digestion, 48%. The filter-aided method includes a protein solubilization step with the denaturing agent urea, which possibly leads to these differences. Despite the difference in number of proteins identified, proteolytic efficiency and protein identification rate, there was no apparent bias towards a particular protein class based on similar molecular weight, pI and hydrophobicity distributions (Electronic Supplementary Information 3). Based on the improved protein identifications and identification rate, the filter-aided digestion approach offers enhanced performance compared to in-solution digestion.

Sample fractionation evaluation

Whole mammalian proteomes are complex with upwards of 20,000 proteins leading to an even greater number of peptides after digestion. In order to maximize protein identifications, sample fractionation is usually necessary prior to mass spectrometric analysis. Equal

amounts (in micrograms) of the same stock protein sample were fractionated using SDS-PAGE, a SCX SpinTip and a C18 SepPak. Each individual method resulted in eight fractions. MS analysis was conducted with equivalent method parameters and instrument time. SDS-PAGE easily outperforms the SCX SpinTip and C18 SepPak fractionation methods with 4947 identified protein groups compared to 2922 and 3360 groups, respectively (Figure 5). The enhanced degree of identified protein groups is further shown with the 2444 protein groups solely identified through SDS-PAGE fractionation. According to our results, the increased total identifications by SDS-PAGE are due to improved separation of the complex sample.

Even though the terms fractionation and separation are often used interchangeably, a true sample separation would result in discrete subpopulations with minimal overlap. Not all fractionation methods achieve defined sample separation. Using unique protein identifications by fraction as a measure of separation efficiency, SDS-PAGE is superior (Figure 6B). On average, 67% of the protein identifications in each SDS-PAGE fraction are not common to any other fraction. Whereas, the SCX SpinTip and C18 SepPak fractions contain 16% and 23% unique proteins, respectively. This trend also continues on the peptide level. Similar percentages of the identified peptides in each fraction are not found in any other fraction with an average of 67%, 27% and 34% unique peptides for SDS-PAGE, SCX SpinTips and C18 SepPaks (Figure 6A). The limitation of identifying the same protein in multiple fractions using SCX and reverse phase methods could result from the presence of identical peptides in multiple fractions, a consequence of poor resolution. Due to separation at the peptide level, this limitation could also occur from the identification of distinct peptides in multiple fractions that ultimately correspond to the same protein (Electronic Supplementary Information 4). Overall, SDS-PAGE separation paired with in-gel digestion yields a higher number of total protein groups identified, approximately 5000.

Conclusions

An ideal proteomics strategy hinges upon the overall biological question, specific experimental design and available resources for sample preparation. In this study, we have examined easily accessible, commercially available, offline platforms for bottom-up proteomics. Our goal was to identify strategies that maximize protein identifications. Based on our results, SDS-PAGE and in-gel digestion leads to the greatest number of protein identifications for bottom-up proteomic experiments when protein level separation is compatible with the experimental design. When peptide separation is desired, the filter-aided digestion paired with the C18 SepPak fractionation gives the highest number of protein identifications. The novel comparison of SCX SpinTip and high-pH RP SepPak fractionation to SDS-PAGE demonstrates that these newer platforms do not outperform the traditional technique. However, these two alternative fractionation platforms offer additional options for investigators preferring approaches with limited instrumentation. Using the results provided by this study, researchers can make more informed decisions regarding bottom-up proteomic workflows.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

K.M.B. was supported by the Notre Dame Chemistry Biochemistry Biology Interface (CBBi) program and NIH training grant T32GM075762 and by the Indiana Clinical and Translational Sciences Institute (CTSI) Program and NIH grant TL1 TR000162. We also thank the Mass Spectrometry and Proteomics Facility at the University of Notre Dame, and in particular Dr. Michelle Joyce, for helpful discussions.

References

1. Beck M, Schmidt A, Malmstroem J, Claassen M, Ori A, Szymborska A, Herzog F, Rinner O, Ellenberg J, Aebersold R. *Molecular systems biology*. 2011; 7:549. [PubMed: 22068332]
2. Fagerberg L, Jonasson K, von Heijne G, Uhlen M, Berglund L. *Proteomics*. 2010; 10:1141–1149. [PubMed: 20175080]
3. Almen MS, Nordstrom KJ, Fredriksson R, Schioth HB. *BMC biology*. 2009; 7:50. [PubMed: 19678920]
4. Ogorzalek Loo, Rachele R.; Dales, Natalie; Andrews, PC. *Protein and Peptide analysis by mass spectrometry methods in molecular biology*. 1996; vol. 61:141–160.
5. Yeung YG, Nieves E, Angeletti RH, Stanley ER. *Analytical biochemistry*. 2008; 382:135–137. [PubMed: 18713617]
6. Wisniewski JR, Zougman A, Nagaraj N, Mann M. *Nature methods*. 2009; 6:359–362. [PubMed: 19377485]
7. Issaq HJ, Conrads TP, Janini GM, Veenstra TD. *Electrophoresis*. 2002; 23:3048–3061. [PubMed: 12207315]
8. Fang Y, Robinson DP, Foster LJ. *Journal of proteome research*. 2010; 9:1902–1912. [PubMed: 20078137]
9. Piersma SR, Fiedler U, Span S, Lingnau A, Pham TV, Hoffmann S, Kubbutat MH, Jimenez CR. *Journal of proteome research*. 2010; 9:1913–1922. [PubMed: 20085282]
10. Antharavally BS, Mallia KA, Rosenblatt MM, Salunkhe AM, Rogers JC, Haney P, Haghdoust N. *Analytical biochemistry*. 2011; 416:39–44. [PubMed: 21640699]
11. Hengel SM, Floyd E, Baker ES, Zhao R, Wu S, Pasa-Tolic L. *Proteomics*. 2012; 12:3138–3142. [PubMed: 22936678]
12. Sharma R, Dill BD, Chourey K, Shah M, Verberkmoes NC, Hettich RL. *Journal of proteome research*. 2012; 11:6008–6018. [PubMed: 23126408]
13. Manza LL, Stamer SL, Ham AJ, Codreanu SG, Liebler DC. *Proteomics*. 2005; 5:1742–1745. [PubMed: 15761957]
14. Baseler WA, Dabkowski ER, Williamson CL, Croston TL, Thapa D, Powell MJ, Razunguzwa TT, Hollander JM. *American journal of physiology. Regulatory, integrative and comparative physiology*. 2011; 300:R186–R200.
15. Bauer KM, Hummon AB. *Journal of proteome research*. 2012; 11:4744–4754. [PubMed: 22897626]
16. Oliveros JC. 2007
17. Kyte J, Doolittle RF. *Journal of molecular biology*. 1982; 157:105–132. [PubMed: 7108955]
18. Sonnhammer EL, von Heijne G, Krogh A. *Proceedings / ... International Conference on Intelligent Systems for Molecular Biology ; ISMB. International Conference on Intelligent Systems for Molecular Biology*. 1998; 6:175–182.

Figure 1.

Schematic diagram of experimental design. Proteins were extracted from human colon cancer cells with the buffers outlined. Extracted proteins were digested and fractionated by the approaches shown followed by analysis via UPLC-MS/MS. The results were compared to evaluate the lysis buffers, digestion methods and fractionation schemes.

Lysis Buffer	Protein Groups	Peptides	Peptide Spectrum Matches (PSMs)	MS/MS Spectrum	Identification Rate (%)	Average Sequence Coverage (%)
Label Compatible	4947	52352	90573	218294	41.5	20.3
SDT	5039	57644	86745	213065	40.7	19.8
Lysis M	5190	60306	93050	220017	42.3	21.0
Modified RIPA	5019	59325	102985	224811	45.8	23.2

Figure 2.

Identification results from analysis of lysis buffers by UPLC-MS/MS. In terms of number of protein groups identified, identification rate and average sequence coverage, all four lysis buffers performed similarly. The Venn diagram below compares the number of unique and common proteins identified between the lysis buffers.

Figure 3. Subcellular localization of proteins identified by UPLC-MS/MS based on UniProt annotation. Cellular locations are shown in relative percent and absolute protein number. Use of various lysis buffers result in comparable relative amounts of proteins from each cellular compartment.

Figure 4. Identification results and evaluation of proteolytic efficiency for sample digestion schemes. Note the increased number of overall and unique protein group identifications seen with the filter-aided digestion scheme.

Fractionation Approach	Protein Groups	Peptides	Average # Peptides/ Protein
SCX SpinTip	2922	28496	6.6
C18 SepPak	3360	33241	7.2
SDS-PAGE	4947	52352	8.1

Figure 5.

Identification results for comparison of fractionation approaches shows increased protein and peptide identifications when using SDS-PAGE. A similar number of protein and peptide identifications are seen between C18 SepPaks and SCX SpinTips.

Figure 6.

(A) Unique peptide identifications across sample fractionation methods. (B) Unique protein identifications across sample fractionation methods. Data points represent the percent of unique proteins in an individual fraction for the given method. Overall the SDS-PAGE fractionation approach results in the greatest percent of unique peptides and proteins in each individual fraction.

Table 1

Composition of evaluated lysis buffers

Lysis Buffer	Composition
Label Compatible	0.01% SDS, 500 mM TEAB, 1 mM NaF, 1 mM -glycerophosphate, 1 mM Na ₃ VO ₄ , Protease Inhibitor Cocktail
SDT	4% SDS, 100 mM DTT, 100 mM Tris, Protease Inhibitor Cocktail
Lysis M ^a	CHAPS, 25 mM bicine
Modified RIPA	8 M Urea, 75 mM NaCl, 50 mM Tris, 1 mM - glycerophosphate, 1 mM Na ₃ VO ₄ , 10 mM Na ₄ P ₂ O ₇ , 1 mM PMSF, Protease Inhibitor Cocktail

^aLysis-M (Roche) is a commercially available product and therefore complete contents are not publically available