A Practical Beginner's Guide to Proteomics

This manuscript (permalink) was automatically generated from jessegmeyerlab/proteomics-tutorial@bfa4090 on January 17, 2022.

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Abstract

Proteomics is the large scale study of protein structure and function from biological systems. “Shotgun proteomics” or “bottom-up proteomics” is the prevailing strategy, in which proteins are hydrolyzed into peptide that are analyzed by mass spectrometry. Proteomics studies can be applied to diverse studies ranging from simple protein identification to studies of protein-protein interactions, post-translational modifications, and protein stability. To enable this range of different experiments, there are diverse strategies for proteome analysis. The nuances of how proteomic workflows differ may be difficult to understand for new practitioners. Here, we provide a comprehensive tutorial of different proteomics methods. Our tutorial covers all necessary steps starting from protein extraction and ending with biological interpretation. We expect that this work will serve as a basic resource for new practitioners of the field of shotgun or bottom-up proteomics.

Introduction

[paragraph about what proteomics means today] Proteomics is the large scale study of protein structure and function. Proteins are translated from mRNAs that are transcribed from the genome. Although the genome encodes potential cellular functions and states, the study of proteins is necessary to truly understand biology.

[history of proteomics? how we got here] How did we get here? Modern proteomics really started around 1990 with the introduction of soft ionization methods that enabled, for the first time, transfer of large biomolecules into the gas phase without destroying them [[1]][2]. Shortly afterward, the first machine algorithm for matching peptides to a database was introduced [3]. Another major milestone that allowed identification of over 1000 proteins were actually improvements to chromatography [4]. As the volume of data exploded, methods for statistical analysis transitioned use from the wild west to modern informatics based on statistical models [5] and the false discovery rate [6].

The wide variety of experimental goals leads to equal diversity in potential proteomics workflows. Even choice is important and every choice will affect the results. In this tutorial, we cover all of the required steps in detail to serve as a tutorial for new proteomics practioners: 1. Types of experiments enabled by proteomics 2. Protein extraction 3. proteolysis 4. Isotopic Labeling 5. Enrichments 6. Peptide purification 7. Mass Spectrometry 8. Peptide Ionization 9. Data Acquisition 10. Basic Data Analysis 11. Biological Interpretation 12. Experimental considerations and design

Types of Experiments

[List of common types of experiments and brief description]

- Protein abundance changes
- Phosphoproteomics
- Glycoproteomics
- Structural techniques (XL-MS, HDX-MS, FPOP, protein-painting, LiP-MS, ...)
- Protein stability measurements (Thermal denaturation)
- PPIs: AP-MS, APEX, BioID
- ...

Protein Extraction

Discussion of methods for protein extraction and solubilizaition.

1. Choice of Lysis buffer

- Urea can cause chemical modifications

2. chemicals to avoid
3. removal of contaminations, Protein Precipitation
4. protein alkylation

- choices of reduction and alkylation reagents, TCEP/DTT/2BME, Chloroacetamide/iodoacetamide, n-ethyl maleimide

Proteolysis

1. discussion of protein sequence coverage is determined by the choice of proteolysis
2. why trypsin is the most common choice (charge and length character)
3. theoretical studies of proteolysis and enzyme [7]
4. Challenges associated with alternative enzyme choices (non-specific and semi-specific enzymes)
5. Alternative enzyme choices (one paragraph each?) - LysC
6. GluC
7. AspN
8. Alpha-lytic protease [8] and how it enables mapping human SUMO sites [9].
9. others?

Peptide and Protein Labeling

Discussion of methods to isotopically label peptides or proteins that enable quantification

1. SILAC/SILAM
2. iTRAQ
3. TMT
4. dimethyl labeling

Peptide or Protein Enrichment

Protein enrichment (e.g. for protein protein interactions)

- coIP
- APEX
- bioID
- bioplex

Peptide enrichment

- antibody enrichments of modifications, e.g. lysine acetylation [10].
- TiO2 and Fe enrichment of phosphorylation
- Glycosylation
- SISCAPA

Methods for Peptide Purification

1. Reverse phase including tips and cartridges
2. stage tips
3. in stage tip (iST)
4. SP2, SP3
5. s traps

Types of Mass Spectrometers used for Proteomics

1. QQQ
2. Q-TOF
3. Q-Orbitrap
4. LTQ-Orbitrap
5. TOF/TOF
6. FT-ICR
7. types of ion mobility

- SLIM
- FAIMS
- traveling wave
- tims

Peptide Ionization

The 2002 Nobel Prize in Chemistry was awarded to partially to John Fenn and Koichi Tanaka “for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules” [11/].

MALDI

Electrospray Ionization

Data Acquisition

Data acquisition strategies for proteomics fall generally within targeted or untargeted, and they can depend on the data (data dependent acquisition or DDA) or be data independent (data-independent acquisition or DIA).

DDA

Targeted DDA

Untargeted DIA

DIA

Targeted DIA

Untargeted DIA

Analysis of Raw Data

The goal of basic data analysis is to convert raw spectral data into identities and quantities of peptides and proteins that can be used for biologically-focused analysis. This step may often include measures of quality control, cross-run data normalization, quantification on different levels (precursor, peptide, protein), protein inference, PTM (post translational modification) localization and also first steps of data analysis, such as statistical hypothesis tests.

In typical bottom-up proteomics experiments, proteins are digested into peptides and further analyzed with LC-MS/MS systems. Peptides can have different PTMs and ionize differently depending on their length and amino acid distributions. Therefore, mass spectrometers often record different charge and modification states of one single peptide. The entity that is recorded on a mass spectrometer is usually referred to as a precursor ion (peptide with its modification and charge state). This precursor ion is fragmented and the precursor or peptide sequences are obtained though spectral matching. The quantity of a precursor is estimated with various methods. The measured precursor quantities are combined to generate a peptide quantity. Peptides are also often combined into a protein group through protein inference, which combines multiple peptide identifications into a single protein identification [12] [13]. Protein inference is still a challenge in bottom-up proteomics.

Due to the inherent differences in the data structures of DDA and DIA measurements, there exist different types of software that can facilitate the steps mentioned above. The existing software for DDA and DIA analysis can be further divided into freeware and non-freeware:

DDA freeware

Name	Publication	Website
MaxQuant	Cox and Mann, 2008[14]	MaxQuant
MSFragger	Kong et al., 2017[15]	MSFragger
Mascot	Perkins et al., 1999[16]	Mascot
MS-GF+	Kim et al., [17]	MS-GF+

DIA freeware:

Name	Publication	Website
MaxDIA	Cox and Mann, 2008[14]	MaxQuant
Skyline	MacLean et al., 2010[18]	Skyline
DIA-NN	Demichev et al., 2019[19]	DIA-NN

Targeted proteomics freeware:

Name	Publication	Website
Skyline	MacLean et al., 2010[18]	Skyline

DDA non-freeware:

Name	Publication	Website
ProteomeDiscoverer		ProteomeDiscoverer
Mascot	Perkins et al., 1999[16]	Mascot
Spectromine		Spectromine
PEAKS	Tran et al., 2018[20]	PEAKS

DIA non-freeware:

Name	Publication	Website
Spectronaut	Bruderer et al., 2015[21]	ProteomeDiscoverer
PEAKS	Tran et al., 2018[20]	PEAKS

Analysis of DDA data

Strategies for analysis of DIA data

Targeted proteomics data analysis

Quality control

Statistical hypothesis testing

Biological Interpretation

1. term enrichment analysis (KEGG, GO)
2. network analysis methods
3. structure analysis
4. isoform analysis
5. follow-up experiments

Experiment Design

This section should discuss trade offs and balancing them to design an experiment. 1. constraints: Each experiment will have different constraints, which may include the number of samples needed for analysis, or desire to quantify a specific subset of proteins within a sample. 2. sample size 3. statistics 4. costs

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