

A Practical Beginner's Guide to Proteomics

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Abstract

Introduction

[paragraph about what proteomics means today]

[history of proteomics? how we got here]

[paragraph about what proteomics can do] A wide range of questions are addressable with proteomics experiments, which translates to a wide range of variations of proteomics workflows. Sometimes identifying what proteins are present is desired, and sometimes the quantities of as many proteins as possible are desired.

Protein Extraction

Discussion of methods for protein extraction and solubilization.

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 [\[1\]](#)
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 [\[2\]](#) and how it enables mapping human SUMO sites [\[3\]](#).
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 [\[4\]](#).
- 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” [[5/](#)].

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.

Analysis of DDA data

Strategies for analysis of DIA data

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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