

# A Practical Beginner's Guide to Proteomics

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# Abstract

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# Introduction

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[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

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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

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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

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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

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

- coIP
- APEX
- bioID
- bioplex

### Peptide enrichment

- antibody enrichments of modifications, e.g. lysine acetylation [\[4\]](#).
- TiO<sub>2</sub> and Fe enrichment of phosphorylation
- Glycosylation
- SISCAPA

# Methods for Peptide Purification

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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

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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

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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

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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**

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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

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

## Experiment Design

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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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