Sample Preparation for Proteomics and Mass Spectrometry Analysis

BMG/PHR 744

Senait Asmellash

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Why is sample preparation so important?

- key step in analysis that affects
  
  • reproducibility, accuracy of results
  
  • durability of instrumentation, columns
  
  • speed of analysis (increased efficiency)
  
  • cost of analysis
Pre-analytical factors:

Sample collection:
• Collection tubes: e.g. serum vs plasma
• Additives: eg. anticoagulants for plasma, antibacterial for urine?
• Centrifugation/filtration

Sample storage:
• Do you need to aliquot?
• Storage temperature?
• Freeze thaw cycles? Temperatures?
What are we interested in determining?

• molecular weight determination
• identifying proteins
• biomarker discovery
• characterizing post-translational modifications (e.g. glycosylation, phosphorylation)
• protein-protein interaction (glycosylation, phosphorylation)
Sample preparation generally involves:

- Extraction or isolation of specific analytes
- Cleaning up – remove interfering substances, contaminants
- Concentration of analytes
- Derivatization
Choice of techniques used in sample preparation depends on:

- starting material
  - simple vs complex matrix
  - volume

- subsequent analysis required

- throughput
  - how many samples need to be analyzed?
Biofluids: Serum, plasma, urine, Saliva, Bronchoalveolar lavage (BALF), Nipple Aspirate

Eknoyan, American Journal of Kidney Diseases, 49, 2007, 865-872
Subsequent analysis?

Urine color wheel

Mass Spectrometer

Starting material: simple or complex

Omenn GS et. al.,

Overview of the HUPO Plasma Proteome Project: results from the pilot phase with 35 collaborating laboratories and multiple analytical groups, generating a core dataset of 3020 proteins and a publicly-available database.

*Genome Biol.* 2006; 7(9): R80.
Adachi J., et al.,

The human urinary proteome contains more than 1500 proteins, including a large proportion of membrane proteins.
General Sample Preparation Methods

- Membrane based
  - Dialysis
  - Ultrafiltration

- Protein precipitation
  (Tricarboxylic acid, NH$_4$SO$_4$, MeOH)

- Liquid-liquid extraction

- Solid phase extraction
Desalting, concentration, buffer exchange

Slide-A-Lyzer™ Dialysis (Pierce)

Microcon centrifugal device (Millipore)


http://www.millipore.com
Micromass MassPREP™ and PE MultiPROBE® compatibility. Integrated device containing an adapter collar press fit into a ZipTipμ-C18 containing a 200 nl bed of C18 allowing elution in minimal volumes.

Additional Automation Protocols:
• ABI Symbiot™
• Bruker MAP™ II and MAP II/8
• Genomic Solution ProMS™
• Tecan Genesis

Automation reduces the labor involved with sample processing and target spotting.

http://www.millipore.com/techpublications/tech1/ps2342enus
Effect of detergents – example of use of nonacid cleavable detergents designed for MS

Liquid-Liquid Extraction

- Separating funnel
- Liquid with the lower density
- Liquid with the higher density
- Funnel tap
- Container

Liquid-Liquid Extraction

Basic Principle: Differential partitioning between two liquid phases

Disadvantages:

- Time consuming
- Low recovery and reproducibility of results
- Large quantities of solvent required
- Not as easy to adapt to high throughput protocols
Basic Principle of SPE

Extraction of target analyte(s) from a liquid sample matrix to a solid sorbent using interactions between the analyte(s) and the sorbent.
Solid Phase extraction – selectivity and efficiency

- widely used

- many advantages
  - versatile: different modes of retention, RP, ion exchange, affinity binding; several combinations of sorbents and solvents, variable sample volumes
  - simple, scalable, easier to automate for increased reproducibility and throughput
SPE - Schematic

A. Matrix:
   Analyte(s):
   Interferences:

   Plasma
   Endothelin(s)
   Unwanted components

B. Inlet
   Sample reservoir
   Polypropylene syringe barrel
   Frit
   Sorbent
   Frit
   Luer tip

C. Conditioning/Activation
   Sample addition
   Wash
   Elution 1
   (50% MeOH)
   Collect CTF sample
   Elution 2
   (80% MeOH)
   Collect ET/big ET sample

Discard
Discard
Discard
Collect ET/big ET sample

http://www.springerimages.com/Images/RSS/1-10.1385_1-59259-289-9_021-0
Different Sorbents based on various functional groups:

- **Ion exchange**: extracting ionic analytes
  - anionic – aminopropyl, quaternary amine
  - cationic – carboxylic acid, benzene sulfonic acid

- **Polar**: “Normal Phase”: extracting polar analyte(s) from non-polar solutions
  - sorbent: silica, alumina, cyano …

- **Non-polar**: “Reverse Phase”: extracting non-polar or hydrophobic analyte(s) from polar solutions
  - sorbent: C18, C8, C4, phenyl …

- **Mixed mode**
Choice of solvent in different steps:

- Conditioning – prepare sorbent for effective interaction with target analyte(s)
- Sample loading – retain samples
- Wash – remove weakly bound interfering substances
- Elution – desorb target analyte(s)

Use:
- “Weak” solvent for retention and wash steps
- “Strong” solvent for elution step
Fixed-well Plate

Modular array Plate

C18 Packed Filter Plate

1) Add C18 and Organic to activate
2) Remove Organic
3) Add Sample
4) Adsorb by Shaking
5) Centrifuge off Non-Binding Proteins
6) Elute With Organic
7) Spot to MALDI target Plate
Proteins/peptides of interest are usually of low abundance

For eg. in serum/plasma:

Large dynamic range mg/ml (serum albumin) to pg/ml (interleukins)
difficult to detect and quantify low abundance proteins/peptides

Top 12 most abundant serum proteins:
- HSA (Human Serum Albumin)
- IgG
- Fibrinogen
- Transferrin
- IgA
- IgM
- Haptoglobin
- a2-Macroglobulin
- a1-Acid Glycoprotein
- a1-Antitrypsin
- Apo A-I
- Apo A-II
Depletion techniques to remove most abundant proteins
Example of SepproTip-IgY12 Protein Fractionation Process

Binding
Position 1
Blood Samples
Loading 15 µl
500 µl Unfractionated Sample (1:33 Dilution)

Washing
Wells 1-3

Eluting
Wells 4-7

Neutralizing
Well 8

Regenerating
Wells 9-10

Option to Collect the Washes at Position 2
Collect and Pool Elution Fractions at Positions 3 and 4

Table 1.
Timetable for IgY 14 LC2 column

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Time (minutes)</th>
<th>1x Dilution Buffer (%)</th>
<th>1x Stripping Buffer (%)</th>
<th>1x Neutralization Buffer (%)</th>
<th>Flow Rate (ml/minute)</th>
<th>Maximum Pressure (psi)</th>
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</thead>
<tbody>
<tr>
<td>Injection</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>350</td>
</tr>
<tr>
<td>Wash</td>
<td>17.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>350</td>
</tr>
<tr>
<td>Elution</td>
<td>22.01</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1.5</td>
<td>350</td>
</tr>
<tr>
<td>Neutralization</td>
<td>36.01</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>1.5</td>
<td>350</td>
</tr>
<tr>
<td>Re-equilibration</td>
<td>42.01</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>350</td>
</tr>
<tr>
<td>Stop</td>
<td>50.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Method for 6.4 × 63.0 mm column is optimized for Beckman System Gold HPLC, Pump Module 1 Type: 118, Detector Model: 166.

Results

Figure 1.
Typical Depletion Chromatogram

Peaks A & B are depleted fractions. Peak C is bound fraction.

Seppro IgY 14 LC-2 column
The ProteoPrep 20 Technology depletes the following proteins:

- Albumin
- IgGs
- Transferrin
- Fibrinogen
- IgAs
- α-2-Antitrypsin
- IgMs
- α-1-Antitrypsin
- Complement C3
- Haptoglobin
- Apolipoprotein A1
- Apolipoprotein A2
- Apolipoprotein B
- Acid-1-Glycoprotein
- Ceruloplasmin
- Complement C4
- Complement C1q
- IgDs
- Prealbumin
- Plasminogen
Amylase depletion in whole saliva
Protein Equalizer Technology: the quest for a "democratic proteome".

Proteomics. 2006 Jul;6(14):3980-92
Righetti PG, Boschetti E, Lomas L, Citterio A.
Department of Chemistry, Materials and Chemical Engineering Giulio Natta, Politecnico di Milano, Milano, Italy. piergiorgio.righetti@polimi.it

“This comprises a diverse library of combinatorial ligands coupled to spherical porous beads. When these beads come into contact with complex proteomes (e.g. human urine and serum, egg white, and any cell lysate, for that matter) of widely differing protein composition and relative abundances, they are able to "equalize" the protein population, by sharply reducing the concentration of the most abundant components, while simultaneously enhancing the concentration of the most dilute species.”

• Non-antibody-based
• Uses combinatorial peptide libraries bound to chromatographic beads
Summary – Key points

Design your protocols carefully:
- avoid detergents, salts etc that interfere with ionization and/or detection
- avoid introducing contaminants – such as keratin from skin, hair

Use physicochemical properties of analyte(s) to design effective clean-up, fractionation techniques

Use simplest possible procedure that allows desired result (sample recovery, throughput)
References

