Proteomics Course

LECTURE-6
Protein Purification and Peptide Isolation using Chromatography

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Lecture outline

- Gel filtration chromatography
- Ion exchange chromatography
- Affinity chromatography
- HPLC
  - SCX and RP chromatography
Protein Purification, Peptide Isolation and Analysis

Protein purification

- Techniques which separate proteins rely on:
  - Differential solubility of proteins
  - Size of proteins
  - Charge on proteins
  - Affinity for ligands
Chromatography

- Separation of proteins over a bed of appropriate material
- The material used to pack a column is called matrix/resin, which is usually beads
Chromatography

- Chromatography involves four major components:
  - sample introduction, mobile phase, stationary phase, detector
- Chromatography requires selection of matrix
  - based on bead shape, size, porosity, charge etc.

Chromatography matrix

- Matrix/resin: usually beads
- With/without attached chemical groups
- Binding/interaction of proteins with column matrix is an important feature of chromatography
Gel filtration chromatography

• Size exclusion chromatography
  • according to size
• Small size molecules retained longer by gel filtration systems
• Larger protein molecules elute first
Gel filtration Chromatography

Chromatography column

Packed gel column

Unpurified protein mixture

Sample loading
Gel filtration Chromatography

Mobile phase reservoir

Mobile phase (salt solution)

Direction of flow

Sample collection
Ion-exchange chromatography

- Proteins separated based on charge difference
- Varying amounts of positive/ negative amino acids
- pH influences net charge on proteins
Common Ion Exchange Matrices

Chromatography column
Packed ion exchange column
Ion exchange Chromatography

Mobile phase reservoir

Mobile phase
(buffer solution 1)

Direction of flow

Mobile phase reservoir

Mobile phase
(buffer solution 2)

Direction of flow
Ion exchange Chromatography

Affinity chromatography

Affinity Chromatography

- Based on affinity of protein to other molecules
- Metal chelation widely used in purification of recombinant proteins
- Substrates, products, cofactors, antibodies, metal
- Matrix beads are chemically coupled to ligand

Affinity Chromatography

Chromatography column

Column packed with derivatized resin
Affinity Chromatography

Unpurified protein mixture

Affinity column

Direction of flow

Mobile phase reservoir

Mobile phase
Affinity Chromatography

Affinity chromatography: examples

<table>
<thead>
<tr>
<th>Fusion partner</th>
<th>Ligand</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A</td>
<td>IgG</td>
<td>Low pH</td>
</tr>
<tr>
<td>ABP</td>
<td>HSA</td>
<td>Low pH</td>
</tr>
<tr>
<td>His6</td>
<td>Ni (Metal chelator)</td>
<td>Imidazole/ low pH</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione</td>
<td>Glutathione (reduced)</td>
</tr>
<tr>
<td>MBP</td>
<td>Amylose</td>
<td>Maltose</td>
</tr>
<tr>
<td>FLAG</td>
<td>M1/M2 Ab</td>
<td>EDTA/ Low pH</td>
</tr>
</tbody>
</table>
1. Load on Ni$^{2+}$ column
2. Wash 20-50 mM imidazole

Crude protein extract containing His-tagged protein

Protein elution
100-500 mM imidazole

Washing removes all proteins except His-tagged proteins

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High Performance Liquid Chromatography
Liquid chromatography

- Separate mixture components on basis of differences in affinity for stationary & mobile phase
- Removes undesired impurities & concentrates diluted samples

Reversed Phase Chromatography

- Based upon hydrophobic binding interaction:
  - peptides/proteins (mobile phase)
  - immobilized hydrophobic ligand (stationary phase)
- RP is used with ESI
Strong cation exchange (SCX) resin

- Silica based cation exchange stationary phase
- Sulfonic acid cation-based exchange ligand
- Ligand covalently bound to polymer coated silica

Multi-dimensional Protein Identification Technology (MudPIT)
Multi-dimensional Protein Identification Technology (MudPIT)

Strong cation exchanger (SCX)  
Separation by charge

Reverse phase column (RPC)  
Separation by hydrophobicity

Multidimensional approaches coupled with MS

- SEC → RP
- RP → CE
- SEC → CE
- IMAC → RP
- Avidin → RP
- SCX → RP
Summary

- Gel filtration chromatography
- Ion exchange chromatography
- Affinity chromatography
- SCX and RP chromatography

REFERENCES