Proteomics Course

LECTURE-6
Protein Purification and Peptide Isolation using Chromatography

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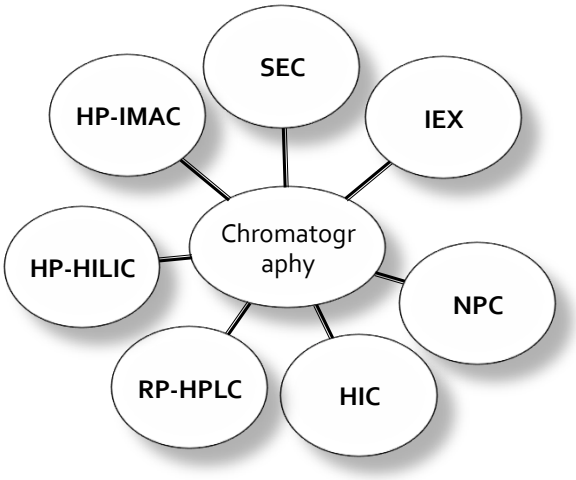
Lecture outline

- 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

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

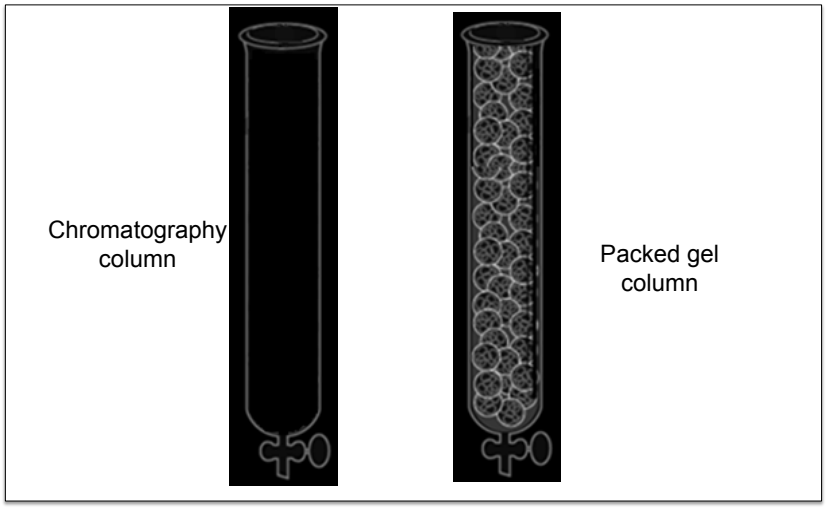
Gel filtration chromatography

L. Hagel 2001

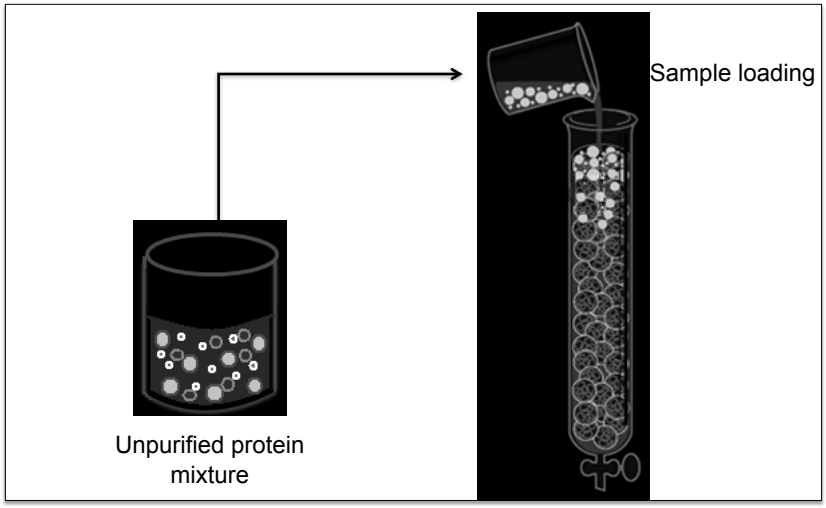
Gel filtration Chromatography

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

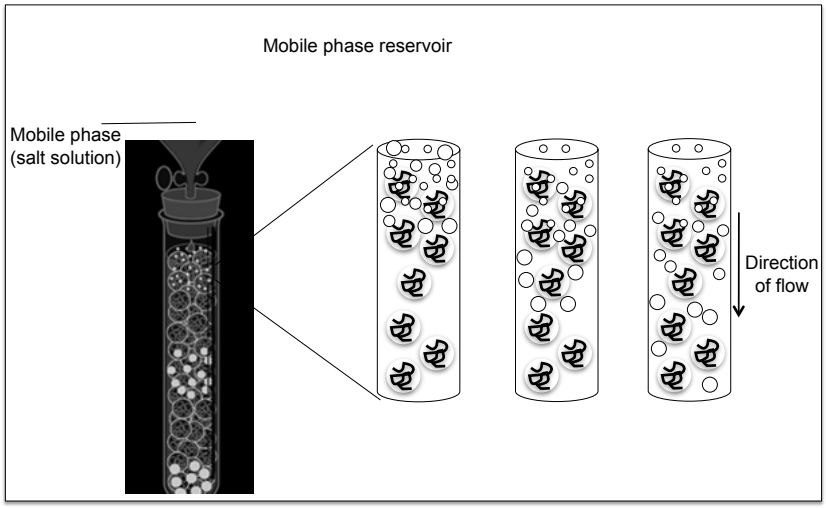
Gel filtration Chromatography



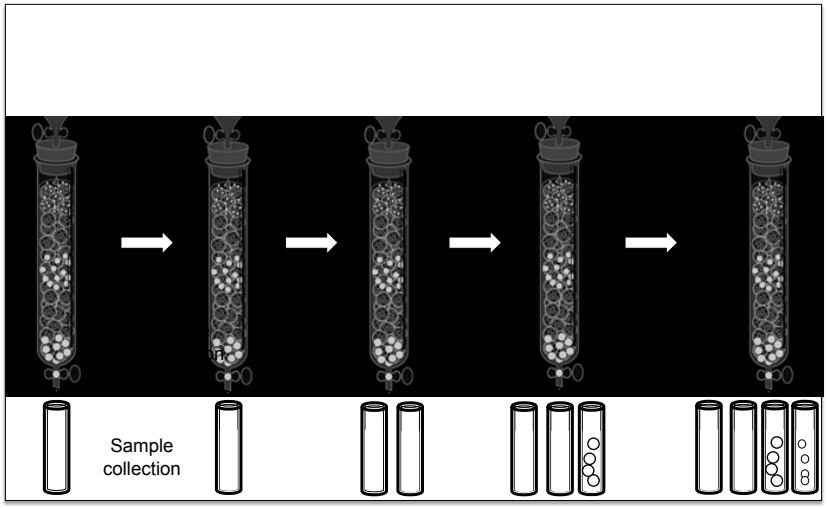
Gel filtration Chromatography



Gel filtration Chromatography



Gel filtration Chromatography

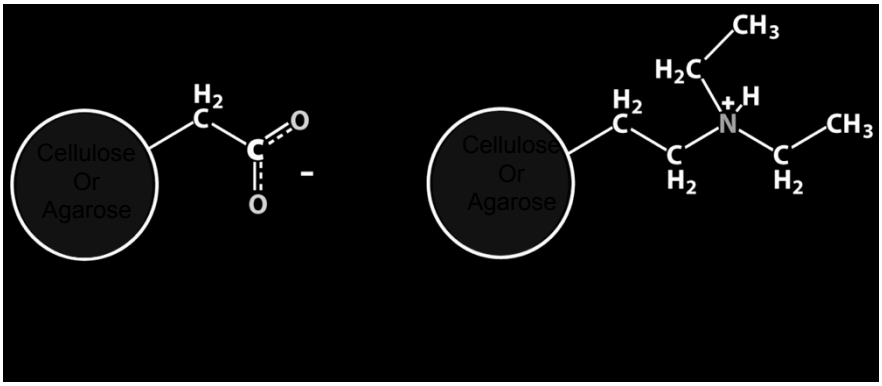


Ion-exchange chromatography

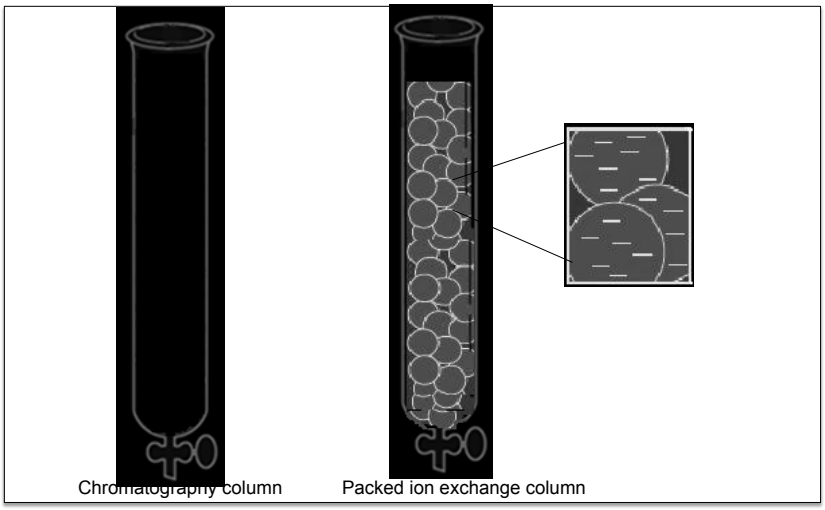
Ion exchange Chromatography

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

Common Ion Exchange Matrices

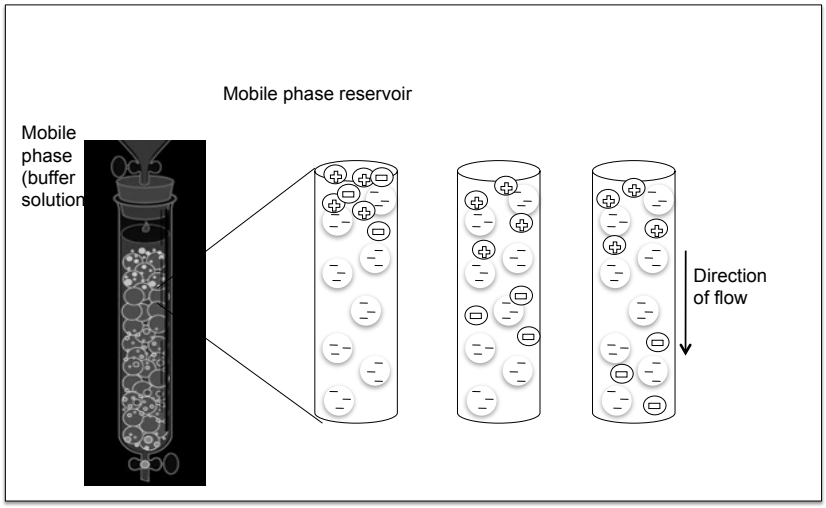


Ion exchange Chromatography

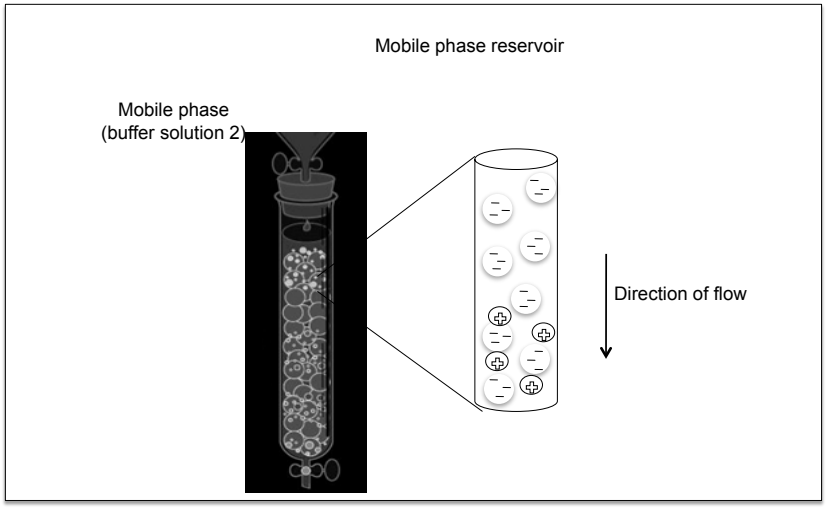


Chromatography column Packed ion exchange column

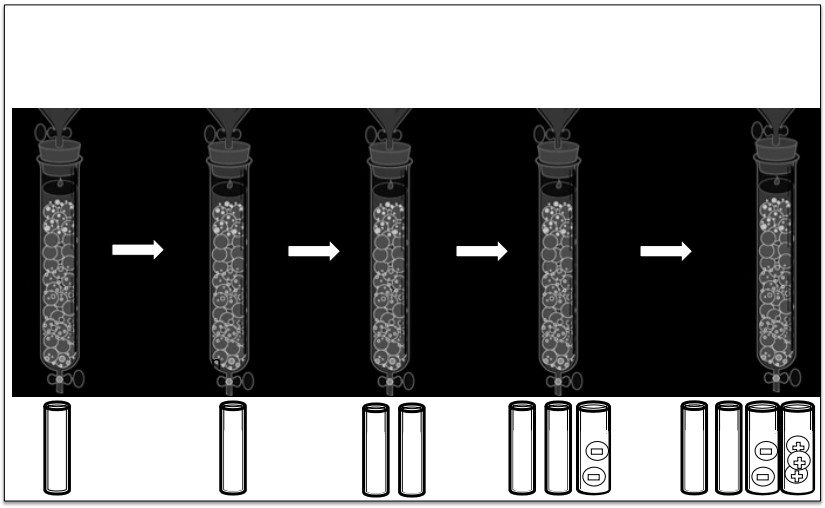
Ion exchange Chromatography



Ion exchange Chromatography



Ion exchange Chromatography



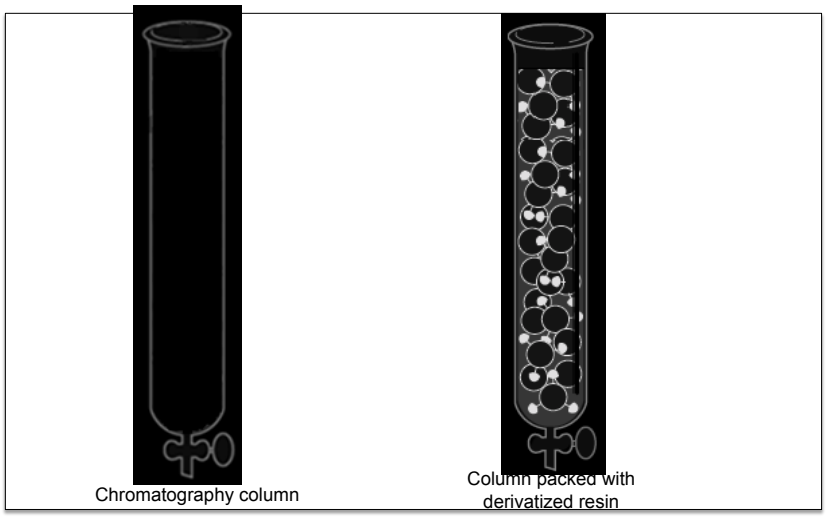
Affinity chromatography

Williams A, Frasca V, 2001.

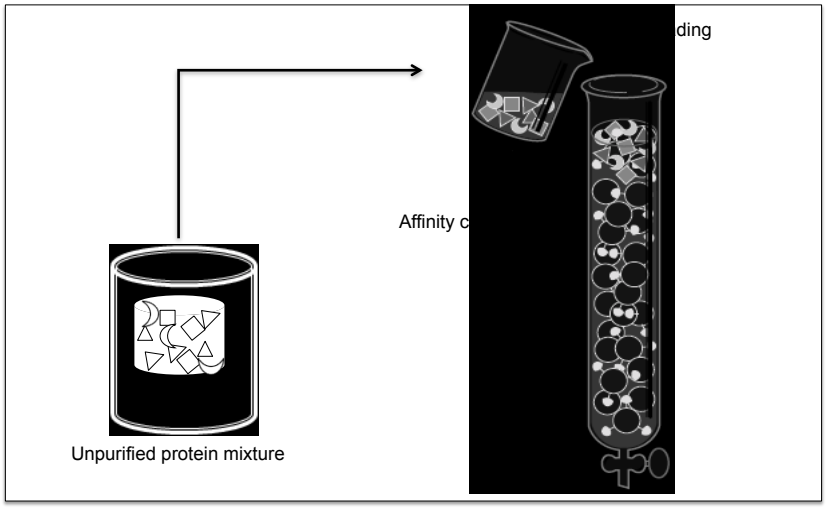
Affinity Chromatography

- 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

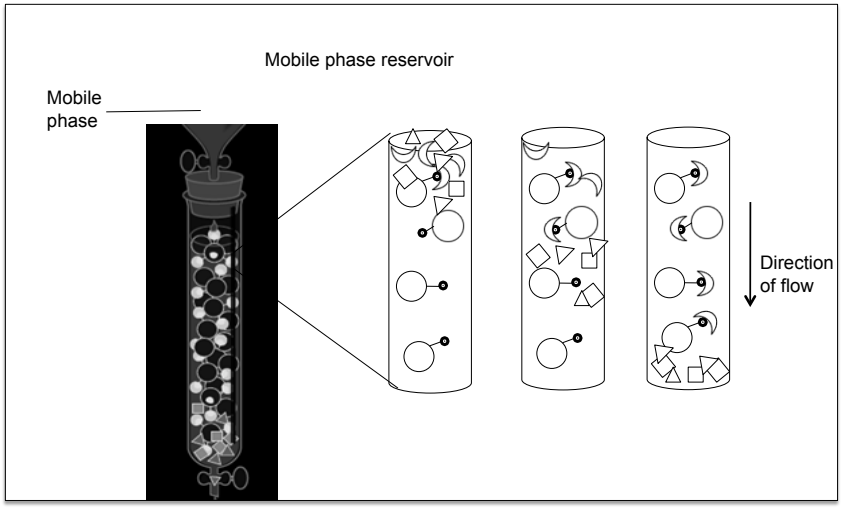
Affinity Chromatography



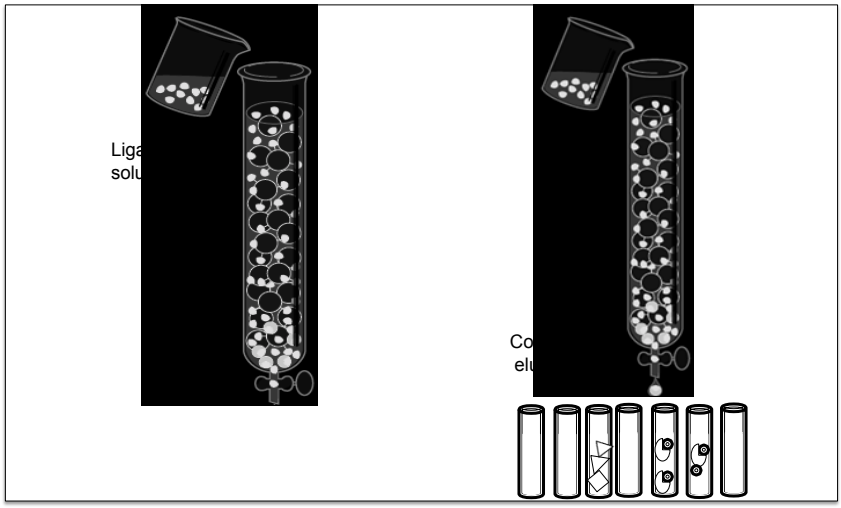
Affinity Chromatography



Affinity Chromatography



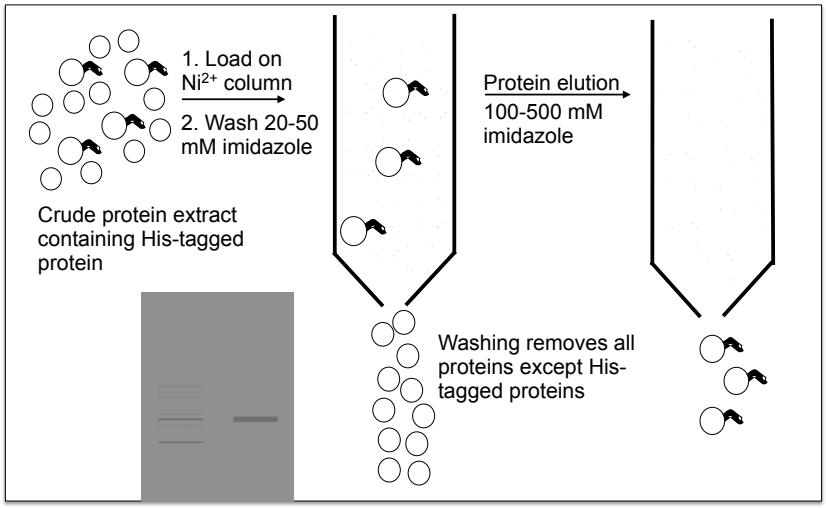
Affinity Chromatography



Affinity chromatography: examples

Fusion partner	Ligand	Elution
Protein A	IgG	Low pH
ABP	HSA	Low pH
His6	Ni (Metal chelator)	Imidazole/ low pH
GST	Glutathione	Glutathione (reduced)
MBP	Amylose	Maltose
FLAG	M1/M2 Ab	EDTA/ Low pH

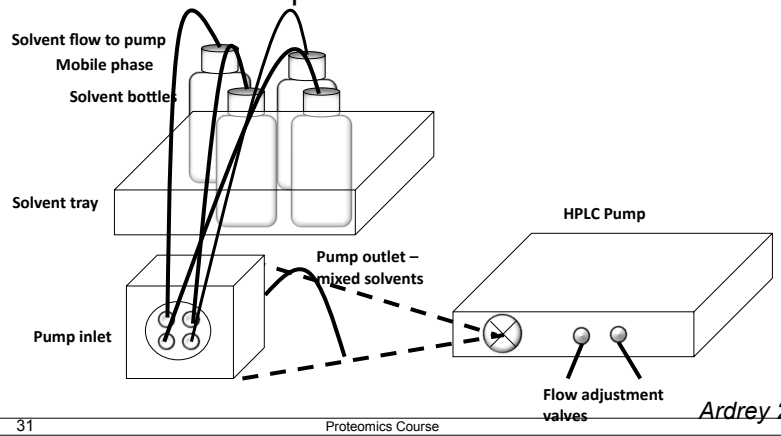
Affinity chromatography: His-tag protein purification



High Performance Liquid Chromatography

Liquid chromatography

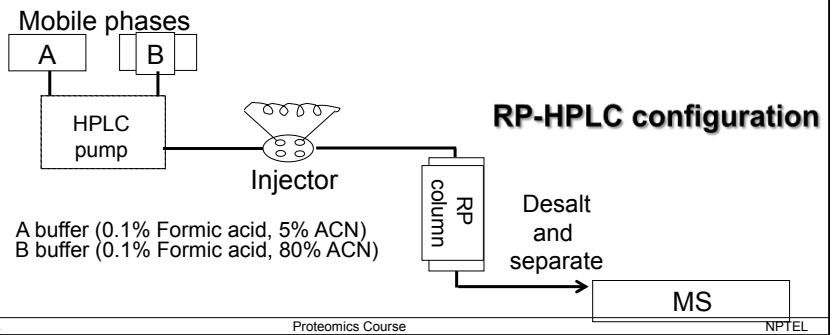
- Separate mixture components on basis of differences in affinity for stationary & mobile phase
- Removes undesired impurities & concentrates diluted samples



Ardrey 2003

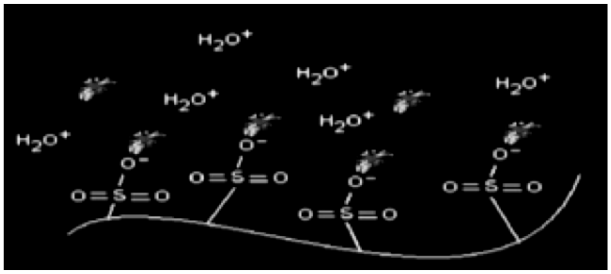
Reversed Phase Chromatography

- 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)

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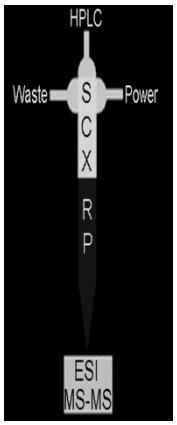
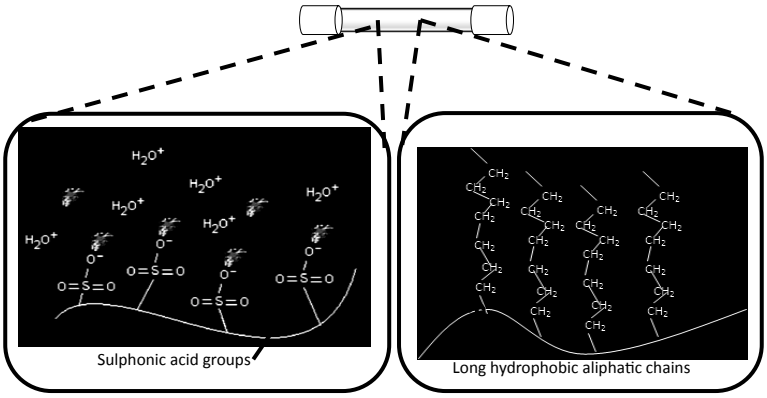
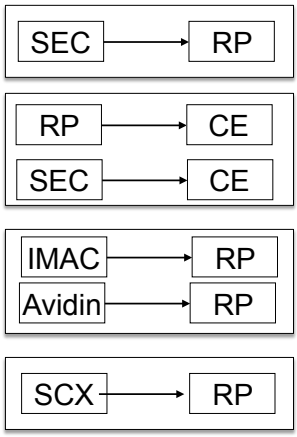




Strong cation exchanger (SCX)
Separation by charge

Reverse phase column (RPC)
Separation by hydrophobicity

Multidimensional approaches coupled with MS



Summary

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

REFERENCES

• Hagel L. 2001. Current Protocols in Molecular Biology. UNIT 10.9 Gel-Filtration Chromatography. DOI: 10.1002/0471142727.mb1009s44

• Ardrey R E. 2003. Liquid Chromatography - Mass Spectrometry: An Introduction. Chapter 2. Liquid Chromatography. DOI: 10.1002/0470867299.ch2.

• Williams A, Frasca V, 2001. UNIT 8.2 Ion-Exchange Chromatography. Current Protocols in Protein Science

• Cravatt BF, Simon GM, Yates JR, The biological impact of mass-spectrometry-based proteomics. Nature 450, 991-1000 (13 December 2007)

• Berg J., Tmyoczko J. & Stryer L., Biochemitry fifth ed., W. H. Freeman & company, 2002. ISBN: 0716746840.

•Nelson D. & Cox M.,Lehninger, Principles of Biochemistry fourth ed., W. H. Freeman and company, 2004. ISBN: 023022699X.

•Voet D. & Voet J., Biochemistry fourth ed., Wiley, 2000. ISBN: 047158651X.

• Issaq et al. Methods for fractionation, separation and profiling of proteins and peptides. Electrophoresis. Volume 23, Issue 17, pages 3048–3061, No. 17 September 2002