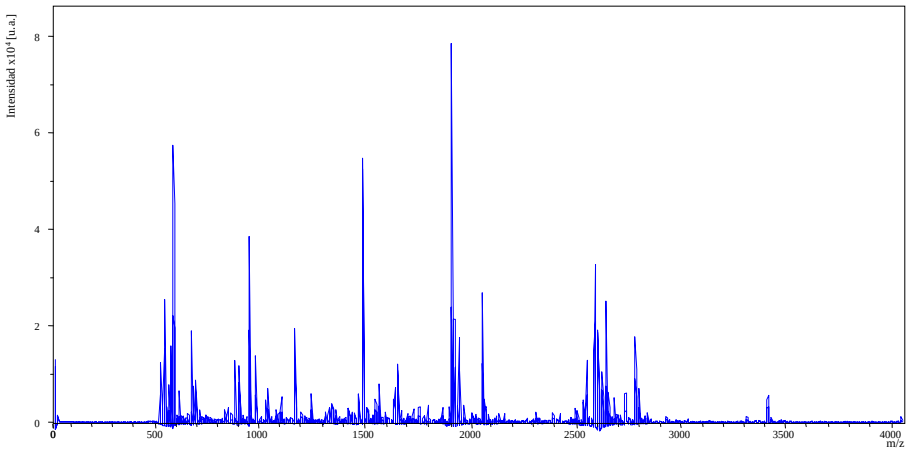
Universidad de Chile
Facultad de Ciencias Químicas y Farmacéuticas
BASES Y APLICACIONES DE TECNICAS DE ESPECTROMETRIA DE MASAS

Preparación de Muestras

2007

Muestra

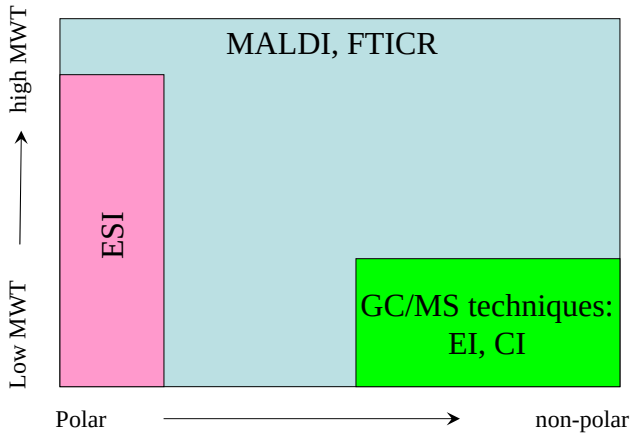


What type of analysis is needed?

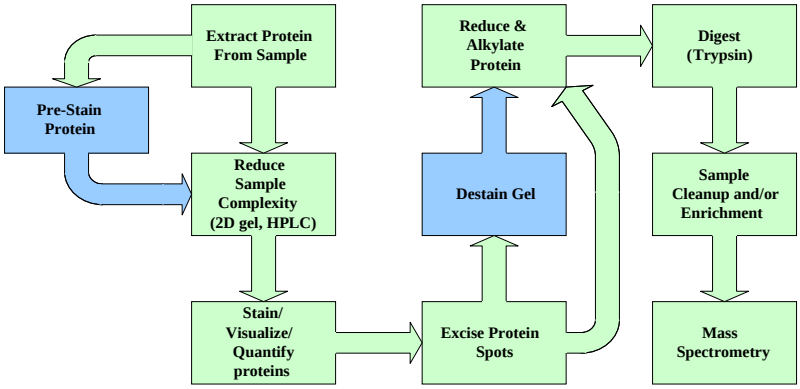
Which MS method is best for the compound I want to analyze ?

- Molecular weigth?
- Solvent & solubility?
- Purity?
- Reactivity?
- Would it distill or sublime under HiVac ?
- One compound or mixture?
- Acidic? Basic?
- Ionic?

Relationship between molecular properties and ionization method



Typical Protein ID Workflow



Some important concepts for sample preparation

1. A good sample preparation is the key to good result
2. The protein composition of the cell lysate or tissue must be reflected in the patterns of 2-DE
3. Avoid protein contamination from environment
4. Co-analytical modification (CAM) must be avoided (pre-purification sometimes leads to CAM)
5. Highly selective procedure for tissue analysis

Some important concepts for sample preparation

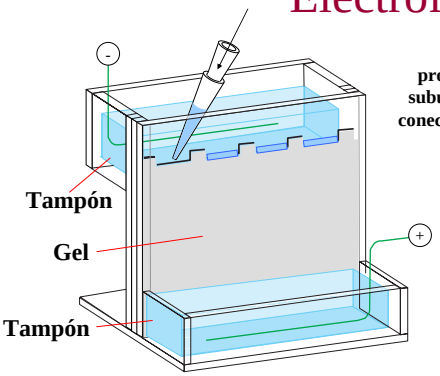
6. Treatment of sample must be kept to a minimum to avoid sample loss
7. Keep sample as cold as possible
8. Shorten processing time as short as possible
9. Removal of salts
10. Minimized the unwanted processing, e.g. proteolytic degradation, chemical modification

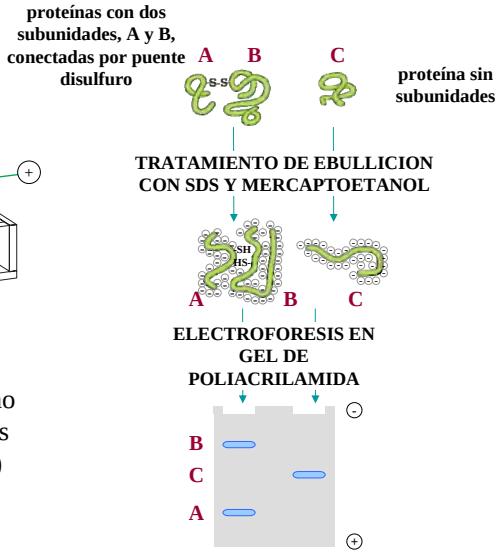
General Sample Handling

Mass spectrometry is a sensitive technique
(for impurities and contamination, too!)

- **Sample Storage**
  – Glass vials can leach salts (Na/K) into sample
  – Ideal storage vial is siliconized polypropylene tubes
- Use Freshly prepared, high purity reagents and water
- Omit high concentrations of buffer salts (NaCl, KH_2PO_4!!!), Detergents (Tween, Triton, SDS) Urea, guanidine salts
  - Cleaning of the sample: dialysis, RP-HPLC, Zip-Tips, ion exchange
  - Use of removable buffer salts (z.B. NH4Ac)
  - Use of removable solvents like water, acetonitrile, methanol

Electroforesis 1D

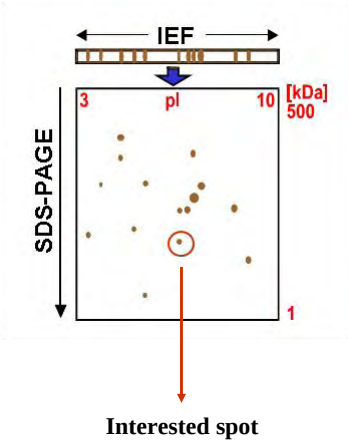




Preparar gel de separación día anterior a la corrida de la electroforesis, la acrilamida que no ha polimerizado puede alquilar las cisteínas (Cys-S-β-propionamida)

Comigración de proteínas

Electroforesis 2D



First dimension:
denaturing isoelectric focusing separation according to the pI

Second dimension:
SDS electrophoresis (SDS-PAGE)
Separation according to the MW

Electroforesis 2D

- The purity of urea is very critical
- Isocyanate impurities and heating will cause carbamylation of the proteins.
- It does not seem to make a difference what grade of urea is used because, urea + heat + protein = carbamylation (N-terminus, Lysine, Arginine, Cysteine)

Disadvantages of 2D PAGE

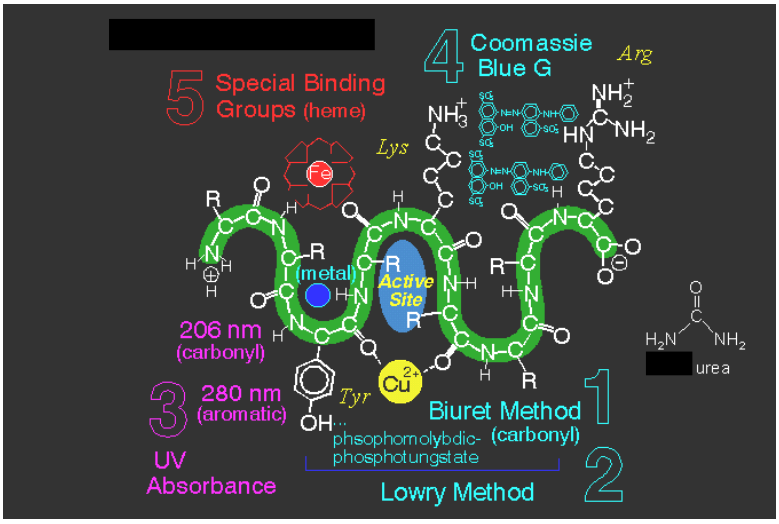
- Restricted to proteins < 10^6 and > 10^4 Da MW
- Cannot detect proteins expressed at low levels
- Limited to 600~800 separate spots
- Gel to gel reproducibility is poor
- Quantitation is poor, ± 50% or worse
- Analysis is not directly coupled to separation

Excisión de Gel

Precaución en excisión de spot/banda del gel, exceso de poliacrilamida aporta señales "contaminantes"

Cortar zona del gel sin proteínas como control de contaminación del gel/determinar señales "contaminantes" de poliacrilamida

Summary of protein quantitation methods



Contaminants

It is currently thought that the presence of contaminants in the sample :

- Prevent proper inclusion of the analyte into the matrix crystal lattice
- Prevent crystallization of the matrix
- Compete for ionization and there by lead to signal suppression

Sample contamination: Detergents, Salts

Detergents:

- ionic and high MW detergents (NP-40, Triton, SDS) impair proper crystallization.
- If the use of detergents is necessary, n-octyl-glucopyranoside (5-10 mM) is the most compatible.

Salts:

- Causes adduct formation.
- Buffer salts affect the pH of the matrix/analyte solution.
- Use volatile salts when possible, acidify the sample.

Other:

- Polymers (PEG, PPG) strongly suppress analyte signals.
- Impure matrix compounds.

Sample contamination: Buffers

No interference:

TFA, formic acid, β-mercaptoethanol, DTT, volatile organic solvents, HCl, NH_4OH, acetic acid

Tolerable: ***(< 50 mM)***

HEPES, MOPS, Tris, NH_4OAc, octyl glucoside

Avoid:

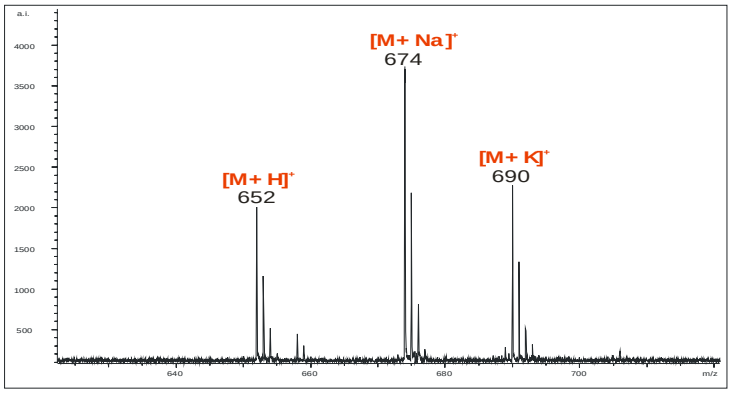
glycerol, sodium azide, DMSO, SDS, phosphate, NaCl, urea 2M, guanidine 2M

Concentration of sample contaminants tolerated by MALDI of peptides and proteins

Contaminant	*Maximum Recommended Concentration*
» Urea	0.5 M
» Guanidine-HC	10.5 M
» Glycerol	1%
» Alkali metal salts	0.1 M
» Tris buffer	0.05 M
» NH_4HCO_3	0.05 M
» Phosphate buffer	0.01 M
» Detergents (not SDS)	0.1%
» SDS	0.01%

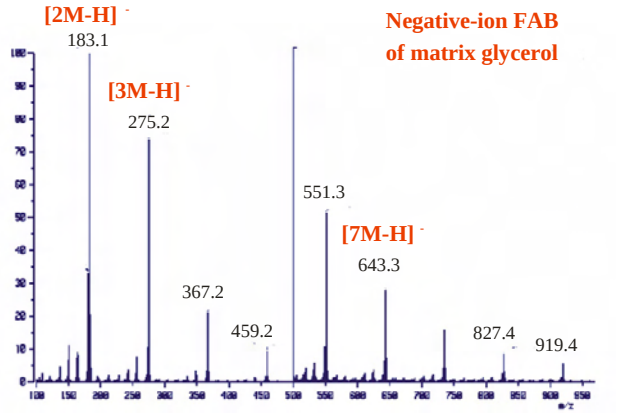
Adduct ions

An ion formed by interaction of two species, usually an ion and a molecule, and often within an ion source, to form an ion containing all the constituent atoms of one species as well as an additional atom



Cluster ion

An ion formed by the combination of two or more atoms, ions or molecules of a chemical species, often in association with a second species



Impurities - Contamination - Artefacts

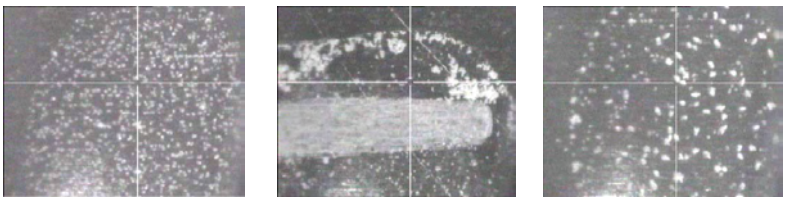
- **Impurity**
  e.g. antioxidantia in organic solvents, side products not separated after synthesis, additional components after insufficient isolation from biological material

- **Contamination**
  Compound which was putinto the sample subsequently, e.g. through chromatographic column

- **Artefact**
  MS-specific key ions, e.g. CI with CH4 as ionisation gas:
  $CH_4 + e^- \rightarrow CH_4^{+\bullet}$ (formation of primary ion)
  $CH_4^{+\bullet} \rightarrow CH_3^+ + H^\bullet$
  $CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$ formation of adducts with m/z +28

Preparación de muestra para MALDI-TOF

Mezcla de matriz más analito, matriz presenta un patrón característico de cristalización



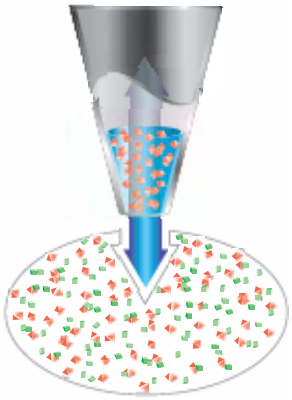
Presencia de contaminantes distorsiona patrón de cristalización

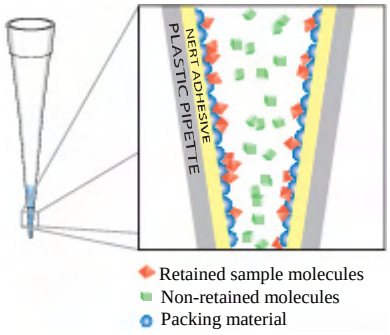
Sample clean-up

Removal of buffer salts, urea, guanidine, EDTA, glycerol, DMSO, detergents, etc.

- Dilution
- Washing
- Drop dialysis (remove low molecular weight contaminants)
- Cation exchange (removal of alkali metal ions)
- Pipette tip column chromatography (ZipTips)

ZipTips





Sample-Matrix Preparation

Dried droplet method

Mix acid, the analyte sample and the matrix either on the target or in solution

- Generates larger crystals with some crystals containing more analyte molecules than others

-Tolerance towards low-molecular weight contaminants (e.g. salts) depend on how readily the crystals can be washed. (water soluble or not!)

Fast evaporation methods

The matrix in a highly volatile solvent (acetone) is applied onto the target. The solvent will evaporate fast leaving a thin even layer of crystals. The sample is applied onto the top of the crystals and allowed to dry

- Higher tolerance towards low-molecular weight contaminants - easy to wash
- More sensitive then the dried droplet method
- Completely decouples the matrix handling from the sample handling

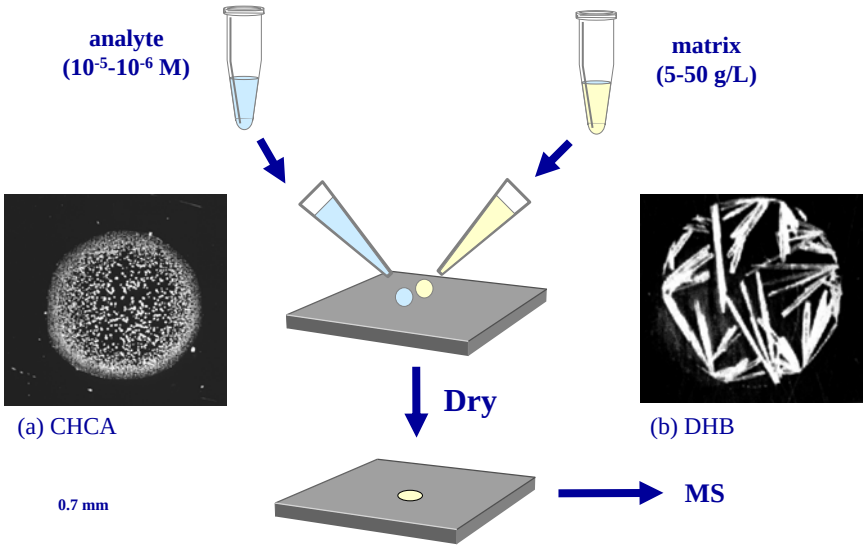
-Inclusion of Nitrocellulose improve data quality and signal intensity

Sandwich method

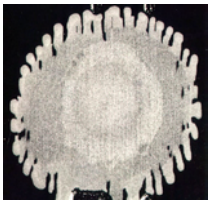
Prepare a thin layer matrix and make the dried droplet preparation on top of it

- Higher tolerance towards contaminants
- More sensitive then the dried droplet method

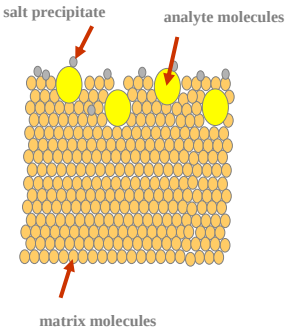
Dried Droplet Method

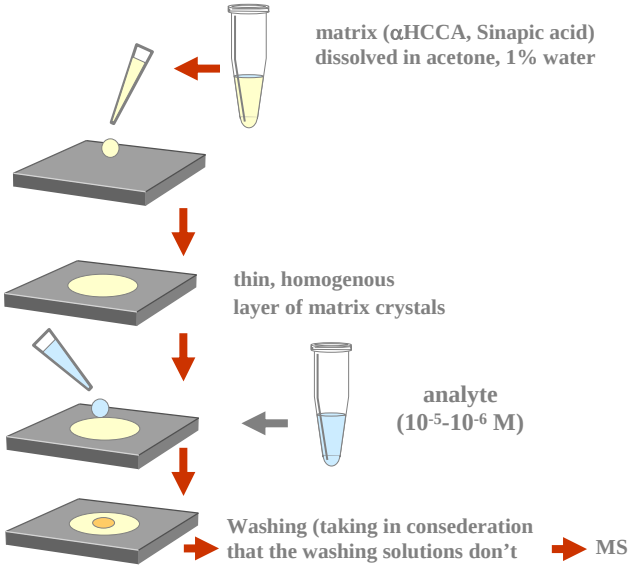


Thin-Layer Method



CHCA





matrix (αHCCA, Sinapic acid) dissolved in acetone, 1% water

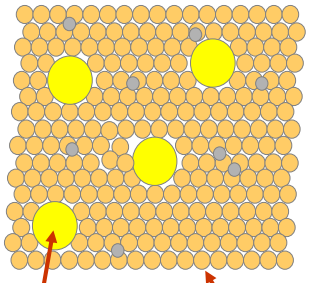
thin, homogenous layer of matrix crystals

analyte (10^{-5}-10^{-6} M)

Washing (taking in consederation that the washing solutions don't solubilise the matrix e.g. DHB) → MS

Dried droplet vs. Thin layer

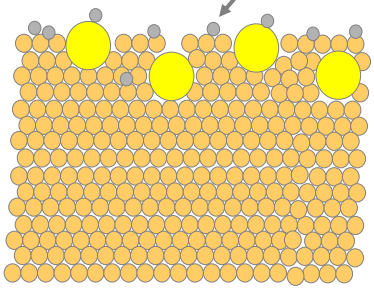
Dried droplet sample



analyte molecules

matrix molecules

Thin-layer sample



MALDI Sample Preparation

Matrix	Application
2,5-Dihydroxybenzoic acid (DHB)	Peptides, proteins, lipids, and oligosaccarides
3,5-Dimethoxy-4-hydroxycinnamic acid (sinapinic acid)	Peptides, proteins, and glycoproteins
α-Cyano-4-hydroxycinnamic acid (CHCA)	Peptides, proteins, lipids, and oligonucleotides

On-Plate Washing

Buffer and Salt Removal
- Dry sample and matrix
- Deposit 1-2 mL cold 0.1% TFA
- Leave on for 5-10 sec., then remove

Detergent contamination
- Use 5% Isopropanol

Cell Extract Contamination
- Use 100% Isopropanol

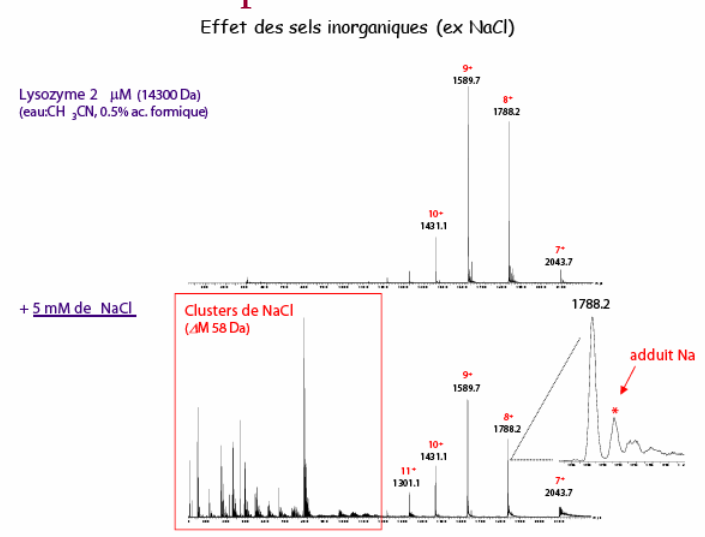
Sample preparation for ESI MS

The presence of contaminants in the sample is more detrimental for ESI than MALDI:

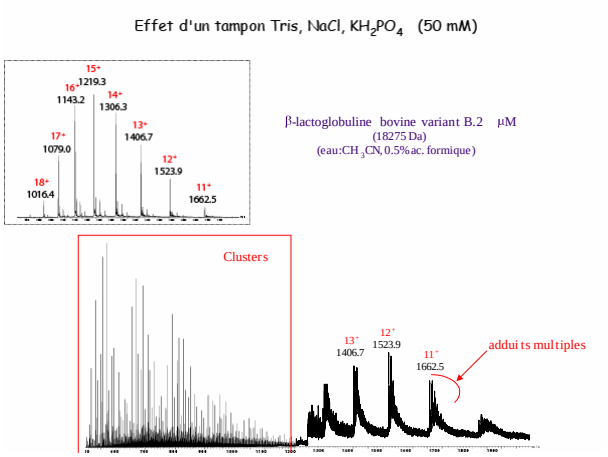
Therefore:

- Pre-purification often needed to obtain ionization and removal of contaminants
- Sample purification for MALDI can also be used prior to ESI
- On-line integration with de-salting device (HPLC) is an advantage

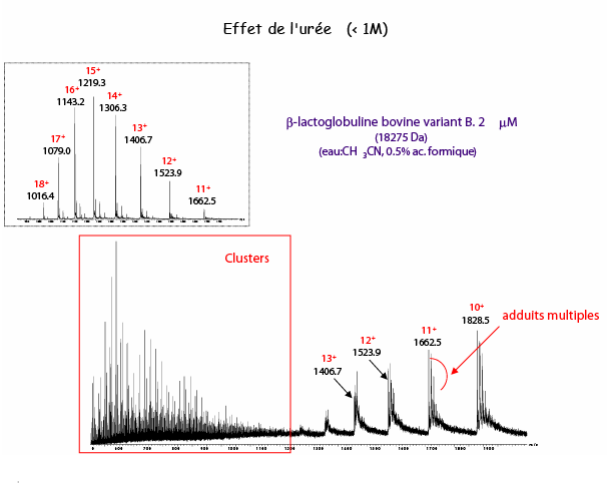
Sample contamination



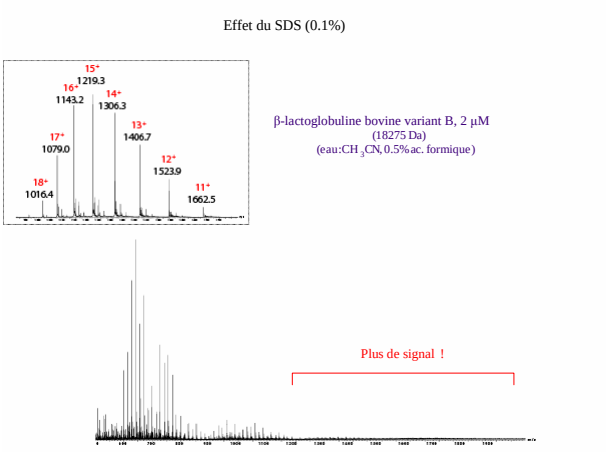
Sample contamination



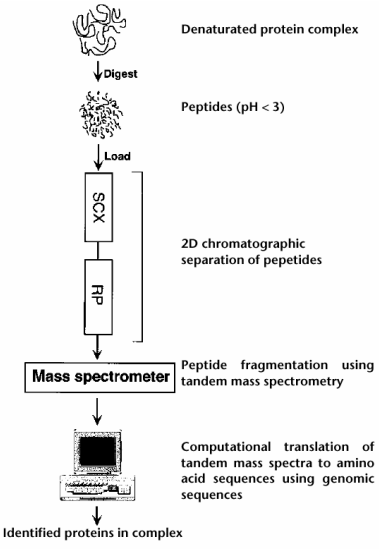
Sample contamination



Sample contamination



Direct identification of proteins using MS



- Removes the requirement to separate proteins by electrophoresis, etc
- MudPIT: multidimensional protein identification technology, or “Shotgun” approach
- Protein lysate is digested with trypsin
- The peptide mixture is loaded onto a strong cation exchange (SCX) column (to separate on the basis of charge). A discrete fraction of peptides is displaced from the SCX column using a salt step gradient to a reversed-phase (RP) column (to separate on the basis of hydrophobicity).
- This fraction is eluted from the RP column into the MS. This iterative process is repeated, obtaining the fragmentation patterns of peptides in the original peptide mixture.
- MS/MS spectra are used to identify the proteins in the original protein complex.