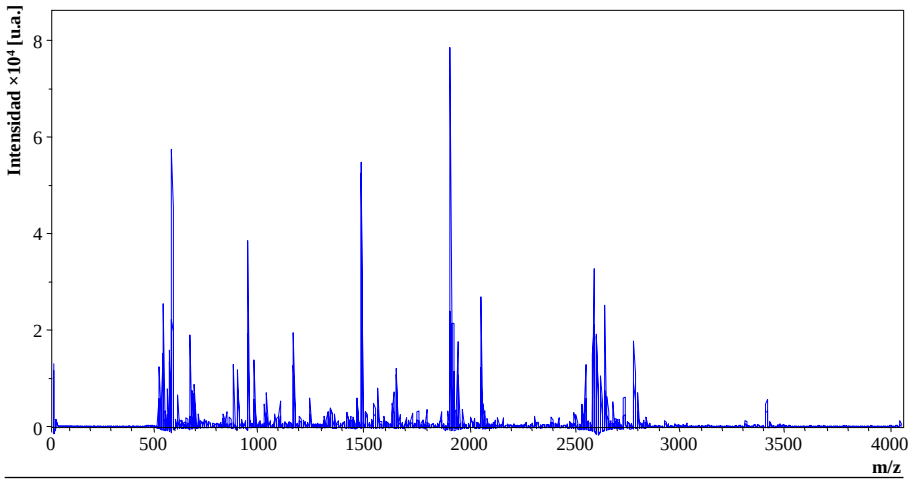
Universidad de Chile
Facultad de Ciencias Químicas y Farmacéuticas
BASES Y APLICACIONES DE LA ESPECTROMETRIA DE MASAS

Preparación de Muestras

22-24 MAYO 2007

Espectro de masas

Muestra

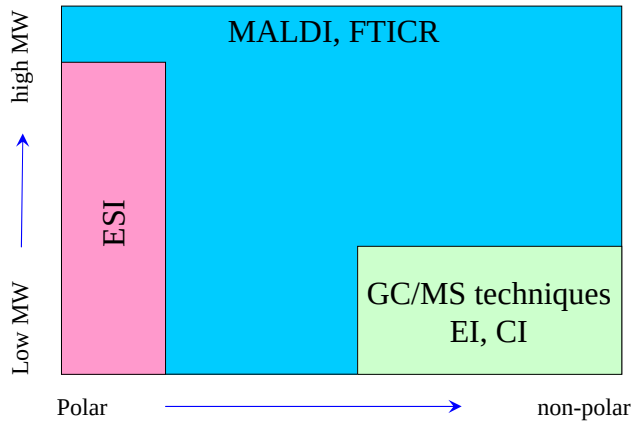


What type of analysis is needed?

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?

What type of analysis is needed?



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

Impurities, contamination & artefacts

Impurity

oxidation in organic solvents, side products not separated after synthesis, additional components after insufficient isolation from biological material

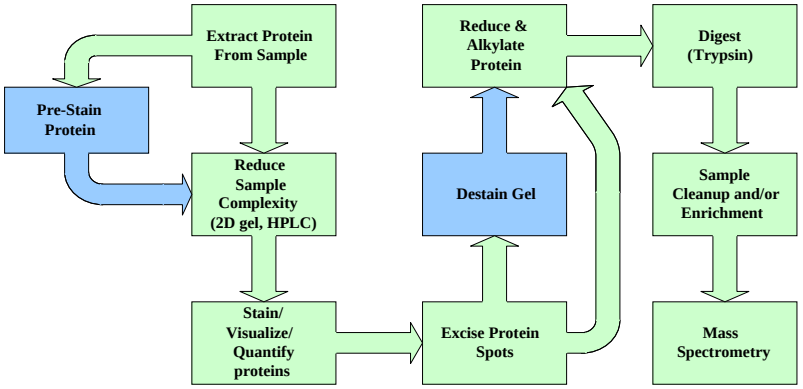
Contamination

Compound which was put into the sample subsequently, *e.g.* through chromatographic column

Artefact

MS-specific key ions, *e.g.* CI with CH4 as ionisation gas:
CH4 + e- → CH4+• (formation of primary ion)
CH4+• → CH3+ + H•
CH3+ + CH4 → C2H5+ + H2 (formation of adducts with m/z +28)

Typical protein ID workflow



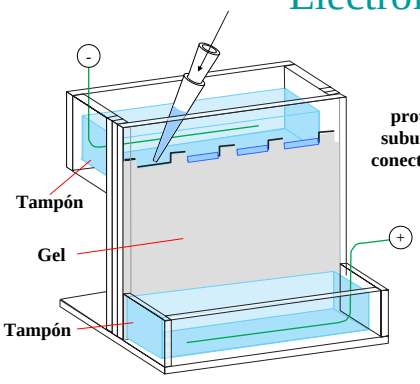
Some important concepts for sample preparation

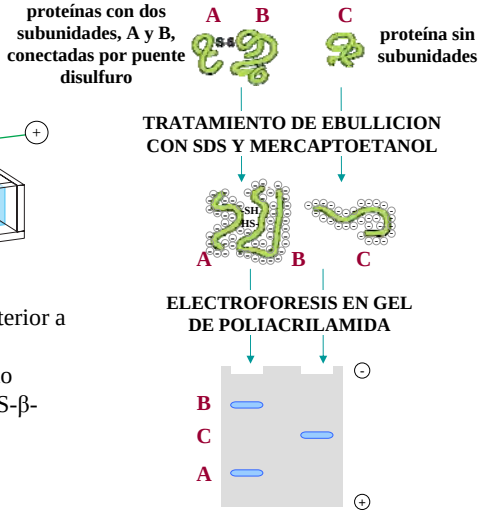
1. A good sample preparation is the key to good result
2. The protein composition of the cell lysate or tissue must be reflected in the patterns of 2-DE
3. Avoid protein contamination from environment
4. Co-analytical modification (CAM) must be avoided (pre-purification sometimes leads to CAM)
5. Highly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

Electroforesis 1D

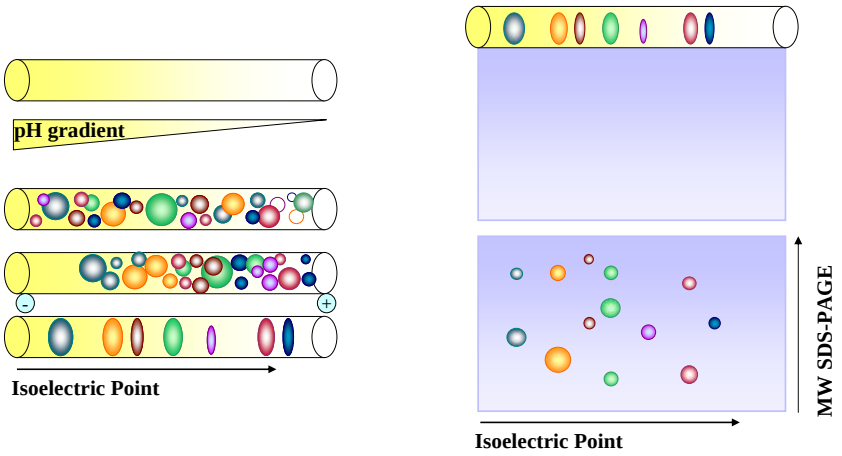




Preparar gel de separación día anterior a la corrida de la electroforesis, la acrilamida que no ha polimerizado puede alquilar las cisteínas (Cys-S-β-propionamida)

Comigración de proteínas

Electroforesis 2D



First dimension: IEF

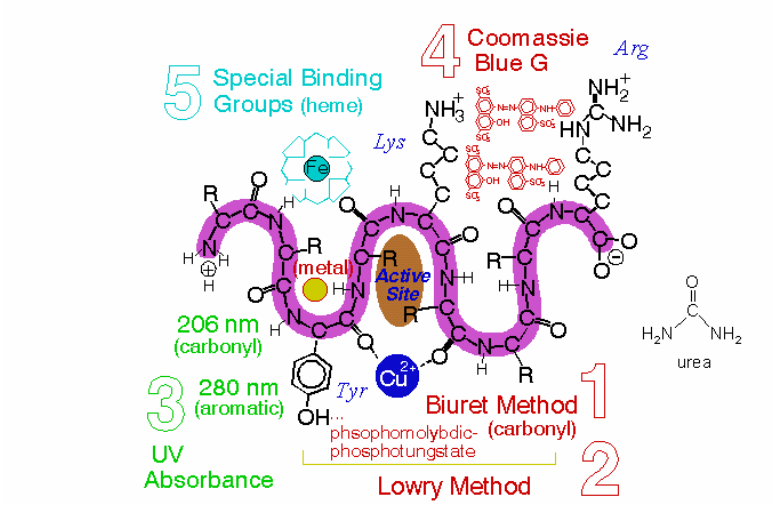
Second dimension: SDS-PAGE

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)

- 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

Protein quantitation methods

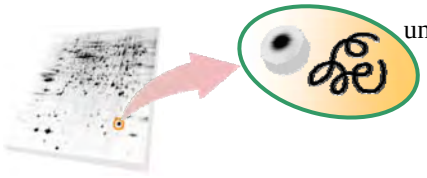


Protein quantitation methods

Staining method	Detection mode	Detection limit (ng)	MS compatibility
Post-electrophoretic stains			
CBB-R	Colorimetry	8-10 (50-100)	+
CBB-G (colloidal)	Colorimetry	8-10 (10-20, 30-100)	+
Silver nitrate (acidic methods)	Colorimetry	1 (3-5)	+
Silver ammonia (alkaline methods)	Colorimetry	1 (5-10)	-
Zinc imidazole	Colorimetry	1 (10)	+
SYPRO® Ruby	Fluorescence	1	+
SYPRO® Orange, Red, Tangerine	Fluorescence	4-8 (30)	+
Epicoccone (Lightning Fast®, Deep Purple®)	Fluorescence	1	+

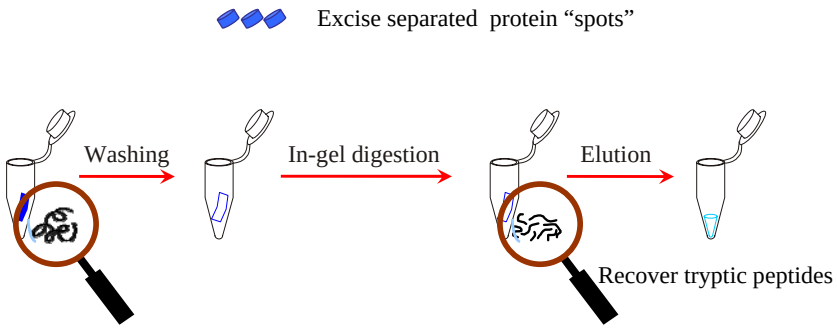
Pre-electrophoretic stains			
CyDyes® minimal labeling	Fluorescence	0.1-0.2 (2)	+
CyDyes® saturation labeling	Fluorescence	0.005-0.01	+
FlaSHPro® Dyes	Fluorescence	2–3	+

Excisión



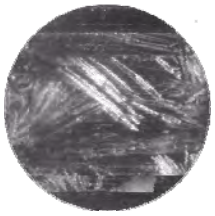
- Precaución en excisión de spot/banda del gel, exceso de poliacrilamida aporta señales “contaminantes”
- Cortar zona del gel sin proteínas como control de contaminación del gel/determinar señales “contaminantes” de poliacrilamida

Digestión

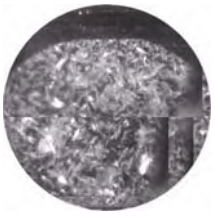


MALDI sample preparation

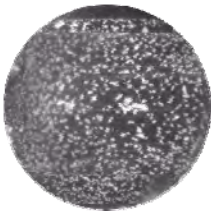
Matrix	Application
2,5-Dihydroxybenzoic acid (DHB)	Peptides, proteins, lipids, and oligosaccarides
Sinapinic acid (SA)	Peptides, proteins, and glycoproteins
α-Cyano-4-hydroxycinnamic acid (CHCA)	Peptides, proteins, lipids, and oligonucleotides



DHB



SA

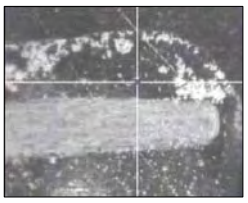


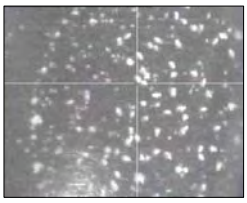
CHCA

MALDI sample preparation

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





MALDI sample preparation

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.

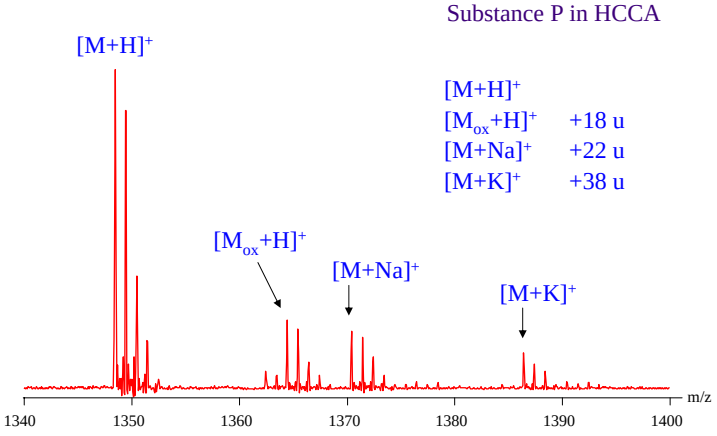
Others

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

Efecto de sales inorgánicas

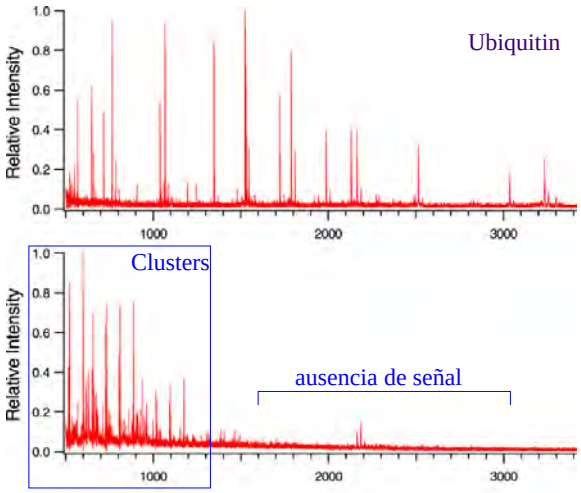
Adducts

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



Efecto de SDS (1%)

Clusters
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



MALDI sample preparation

Contaminant	Maximum Concentration
NaCl	50 mM
Fosfato	10 mM
Tris, HEPES, MOS	50 mM
Urea	1 M
Guanidina	1 M
Glicerol	1 % v/v
PEG	0.1% v/v
Triton X-100, NP-40, Tween	0.1% v/v
SDS, CHAPS	0.01% v/v
N-octil-β-glucopiranósido	1% v/v

No interference
TFA, formic acid, volatile organic solvents, HCl, NH_4OH, acetic acid

ESI sample preparation

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

ESI sample preparation

Salts

- NaCl, K_2HPO_4, etc., decrease electrospray signal, form adducts complicating spectra

Buffers

- TRIS, MES, MOPS: same reason as salts

Reducing agents

- BME, DTT

Stabilizers

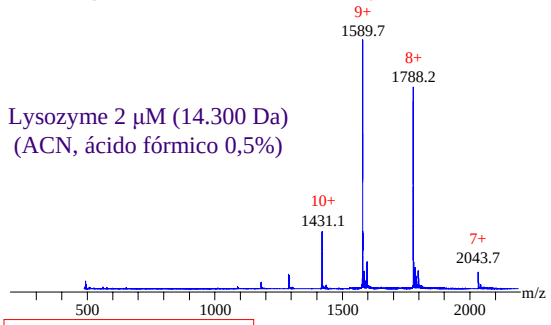
- Glycerol, PEG: overwhelm spectra, difficult to rinse

Detergents

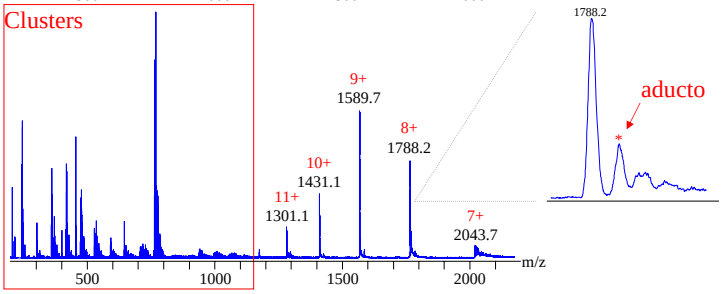
- Includes ionic (SDS) and non-ionic (Triton X-100): decrease electrospray signal. Avoid during sample preparation, difficult to get rid of

Efecto de sales inorgánicas

Lysozyme 2 µM (14.300 Da)
(ACN, ácido fórmico 0,5%)

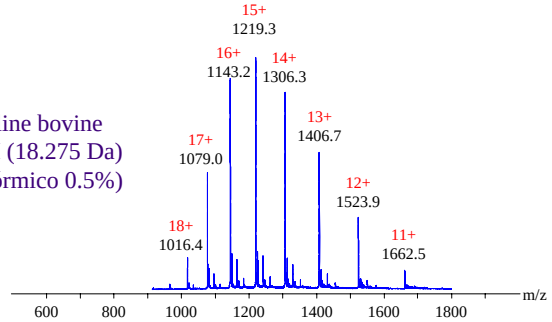


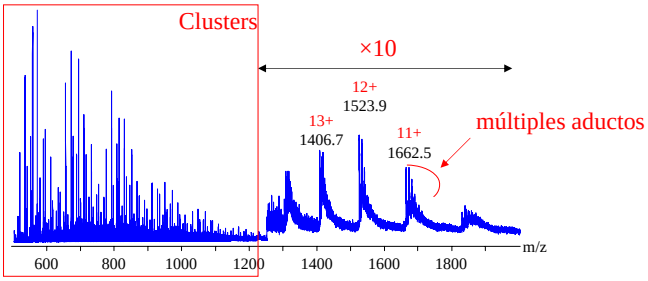
+ 5 mM de NaCl



Efecto de tampón (Tris, KH_2PO_4 50 mM)

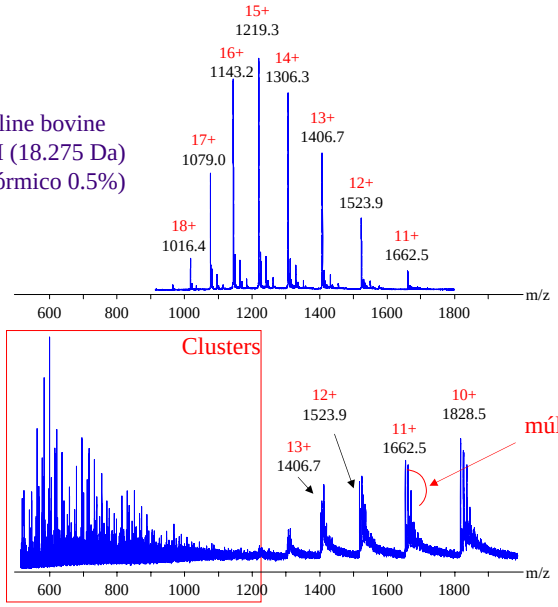
β-lactoglobuline bovine
variant B 2 µM (18.275 Da)
(ACN, ácido fórmico 0.5%)





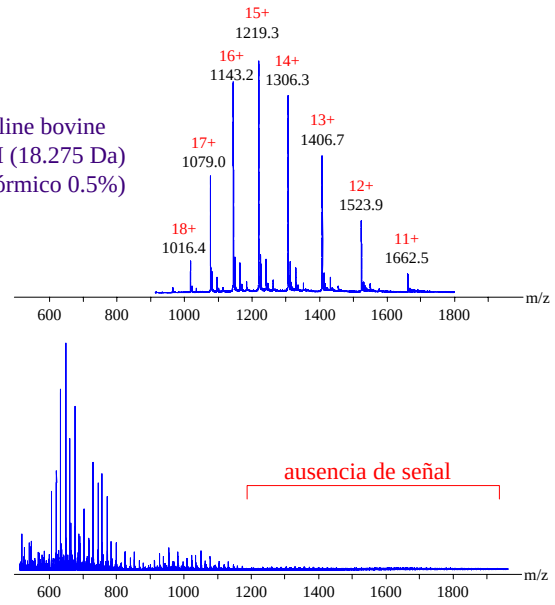
Efecto de urea (< 1 M)

β-lactoglobuline bovine
variant B 2 µM (18.275 Da)
(ACN, ácido fórmico 0.5%)



Efecto de SDS (0,1%)

β-lactoglobuline bovine
variant B 2 µM (18.275 Da)
(ACN, ácido fórmico 0.5%)



Suppression du signal en ESI par les détergents

Detergent	Signal
Sodium dodecylsulfate (SDS)	< 10%
Sodium taurocholate	< 10%
Sodium cholate	21-30%
CTAB	< 10%
LDAO	< 10%
CHAPS	31-60%
Tween-20	10-20%
Thesit	< 10%
Triton X-100	< 10%
NP40	< 10%
n-octyl sucrose	10-20%
n-dodecyl sucrose	10-20%
n-dodecyl maltoside	21-30%
Octyl glucoside	21-30%
Octyl thioglucoside	21-30%
n-hexyl glucoside	30-60%
n-dodecyl glucpside	> 60%

Signal avec 0.1% de détergent comparé au signal sans détergent

Sample clean-up

Les solutions?

1. Remplacer les tampons par des sels d'ammonium plus volatils (acétate d'ammonium ou bicarbonate d'ammonium). Il faut rester à des concentrations < 20 mM

2. Si la solubilité de la protéine requiert la présence de détergent, préférer des octyl-glucosides

3. Dessaler ou purifier l'échantillon

Sample clean-up

- HPLC
- Solid phase extraction cartridges (SPE)
- Solid phase extraction tips (such as zip tip)
- Dialysis (µL volume methods available)
- Protein precipitation

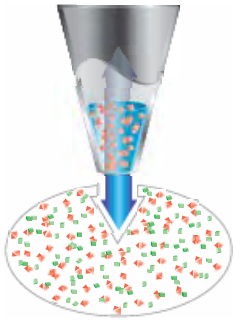
Membranes d'ultrafiltration

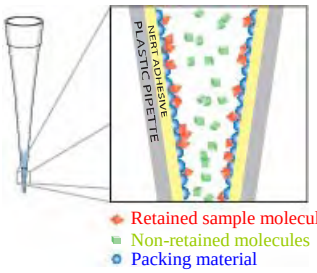
- Il s'agit de membranes semi-perméables qui permettent de séparer les composants d'un mélange selon leur taille
- La séparation s'effectue par ultracentrifugation à travers la membrane
- Le seuil de coupure est choisi de sorte que la protéine reste dans le rétentat et que les contaminants de petite taille soient éliminés dans le filtrat

Elimination des détergents			
	3 kD MWCO	**10 kD MWCO**	**100 kD MWCO**
SDS	> 90%	> 90%	> 90%
CHAPS	> 90%	> 90%	> 90%
Tween-20	< 40%	< 40%	> 90%
Triton X-100	< 40%	< 40%	> 90%
Valeurs données pour des membranes Millipore après 1 cycle de filtration. Détergent présent à 0.1%			

Tween et Triton forment des micelles pouvant atteindre 70-90 kDa lorsque leur concentration est supérieure à 0.1% (vrai aussi pour SDS ou CHAPS > 1%)

Zip tips



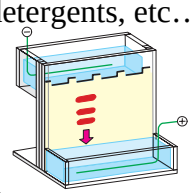


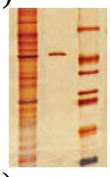
Resin	Application
SCX	Dilute peptide capture and nonionic detergent removal
C18/µC18	Desalting and concentration of peptides, proteins, or oligonucleotides Step-fractionation of complex peptide or protein mixtures for increased MALDI-TOF MS sensitivity and resolution
C4	Desalting and concentration of peptides
MC	Enrichment of phosphopeptides

Electrophoresis

1D SDS-PAGE

- Great clean-up tool (rid of salts, detergents, etc…)
- Great concentration tool





2D Electrophoresis

- Great clean-up tool (rid of salts, detergents, etc…)
- 2D gels are very much sample related (sample may require further clean-up prior to 2D gel)
- Avoid excess salts in sample (not focus, IPGs burn, 30-40 mM max salt)