Computational Proteomics and Metabolomics

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WS 11/12

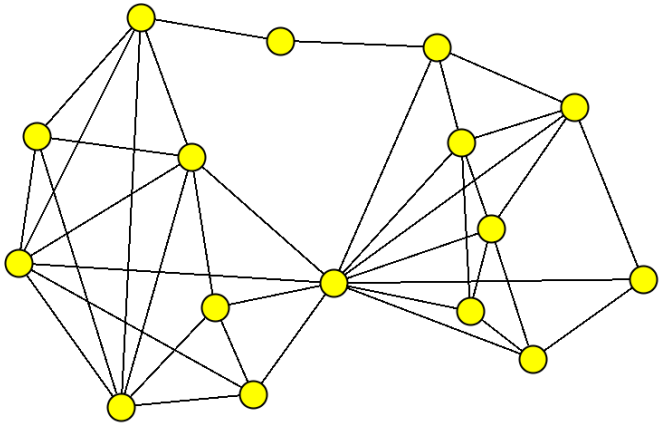
1. Introduction and Overview

Genomics vs. Proteomics

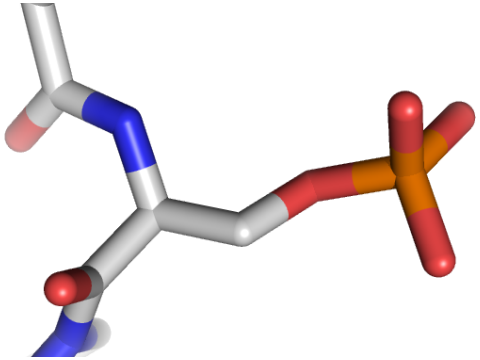
Genomics	Proteomics
Genomes rather **static**	Proteomes are **dynamic**
	(age, tissue, breakfast, …)
~ **20 k** genes	up to **1000 k** proteins
established technology	**emerging** technologies
(capillary sequencer)	(MS, HPLC/MS, protein chips)

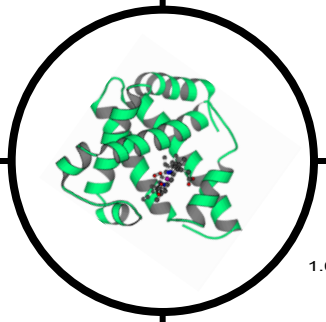
Main fields of proteomics

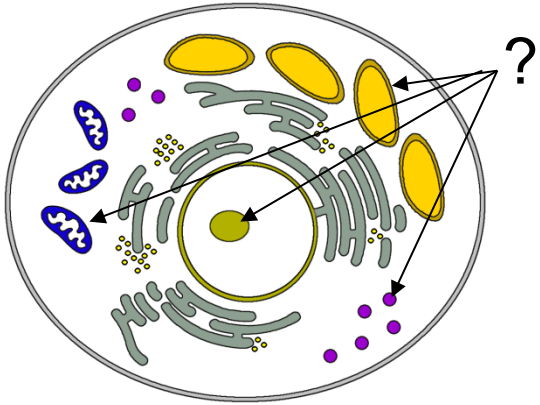
protein interaction



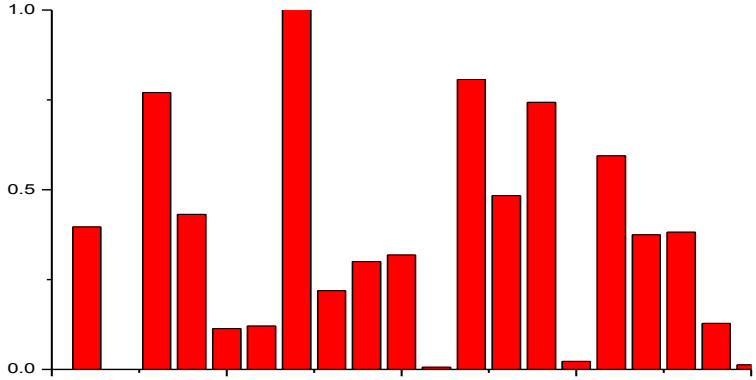
protein characterization
(identification + PTMs)







protein localization



protein expression

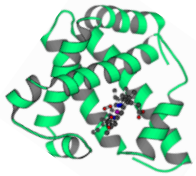
Applications of proteomics

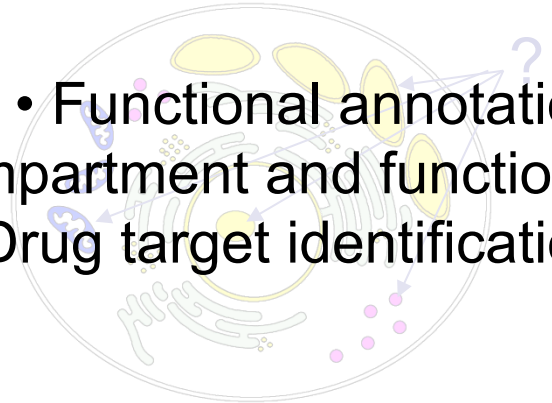
protein interaction

- Drug target identification

protein characterization (identification + PTMs)

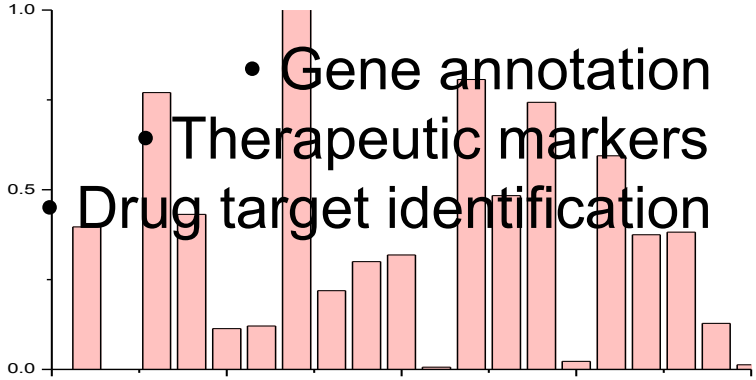
- Determine content of a protein mixture
- Understanding regulation of protein activity





- Functional annotation (compartment and function)
- Drug target identification

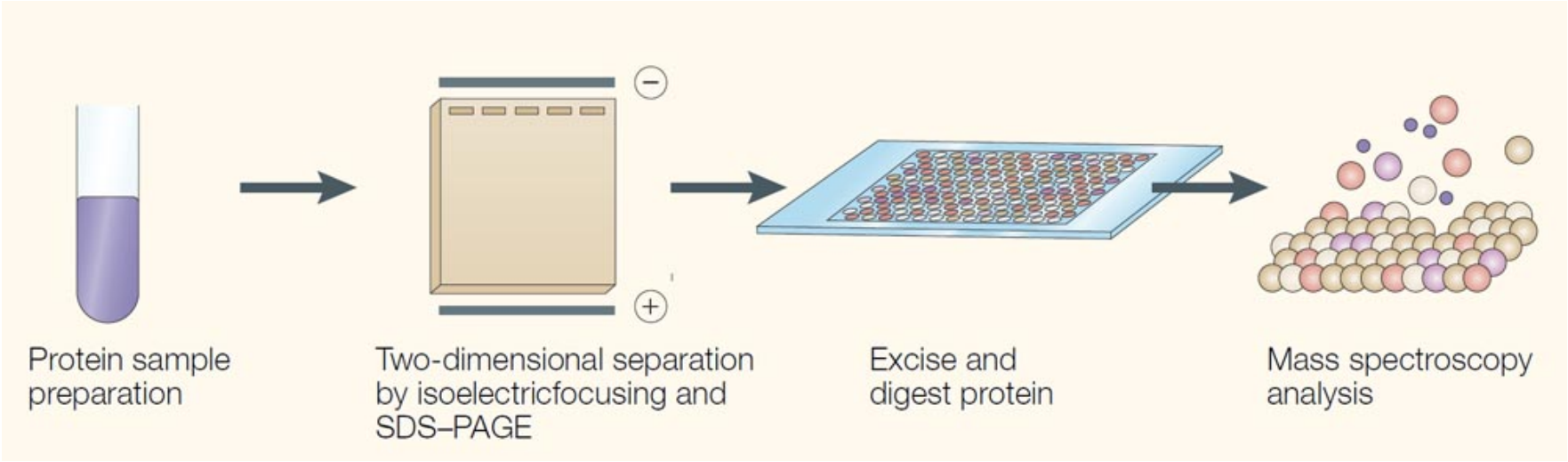
protein localization



- Gene annotation
- Therapeutic markers
- Drug target identification

protein expression

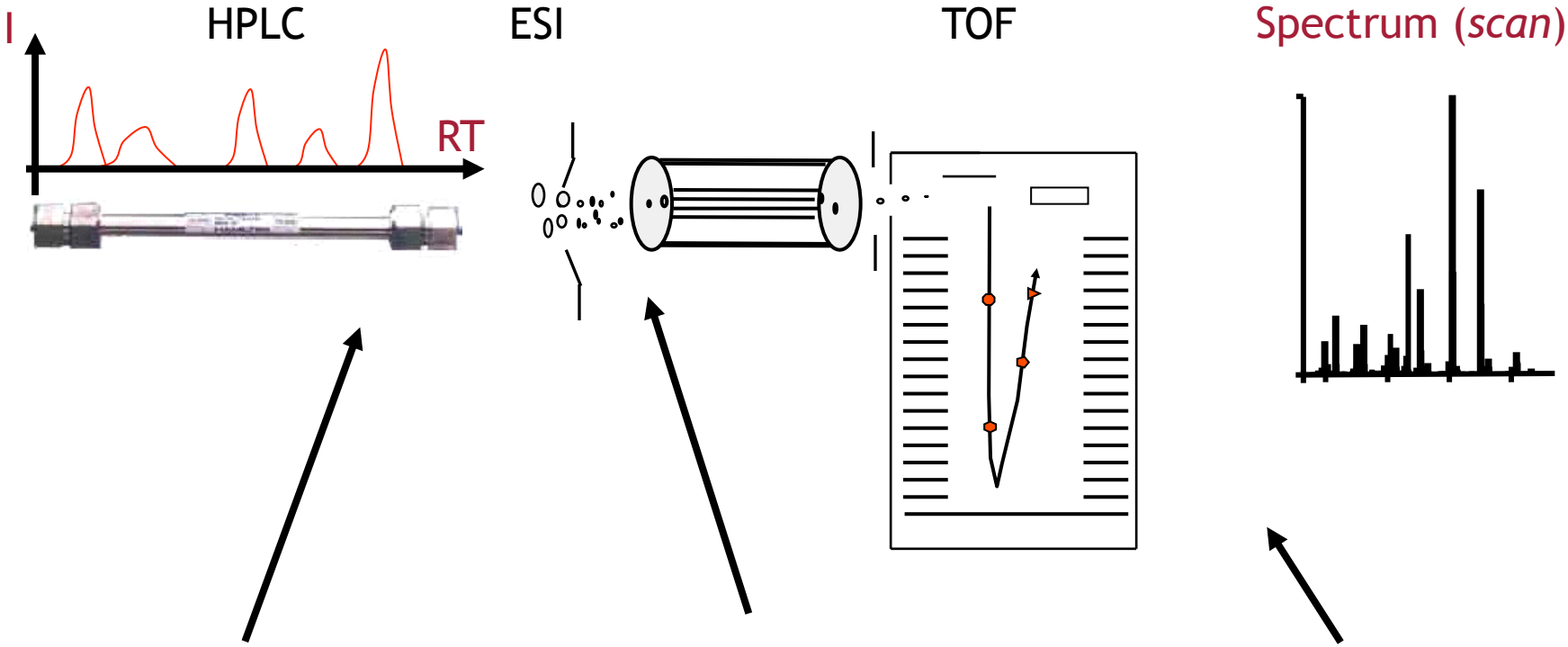
Exp. Methods – Proteomics



- Compare two proteomes (e.g. healthy/diseased)
- Separate using 2D-PAGE (w.r.t. molecular mass, pI)
- Excise protein spots from the gel
- Tryptic digest of the proteins
- Identify proteins using mass spectrometry and Database search

Lindsay, Nature Rev. Drug Discovery, 2003, 2, 803

HPLC-MS

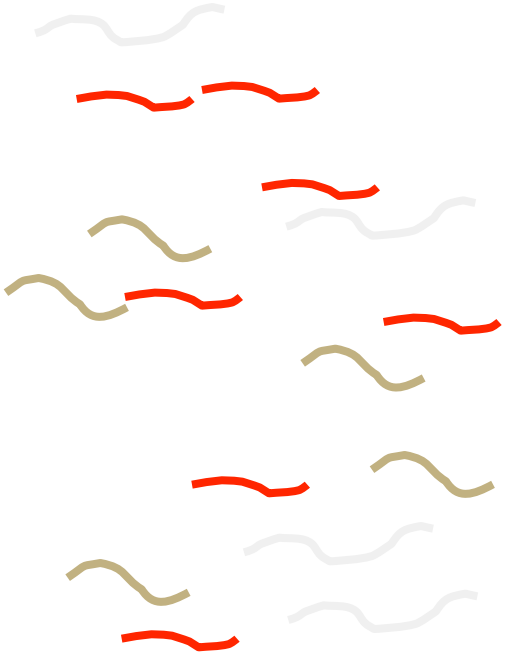


Separation 1
separate peptides by their retention time on column

Ionization
electrospray, transfers charge to the peptides

Separation 2
MS separates by mass-to-charge ratio (m/z)

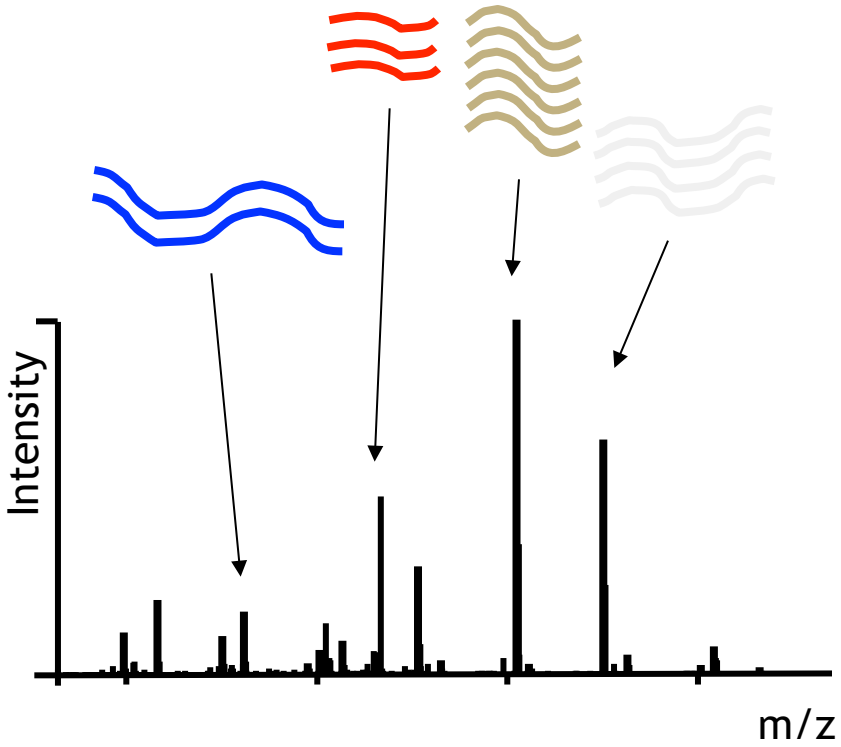
Mass Spectrometry



mass spectrometry

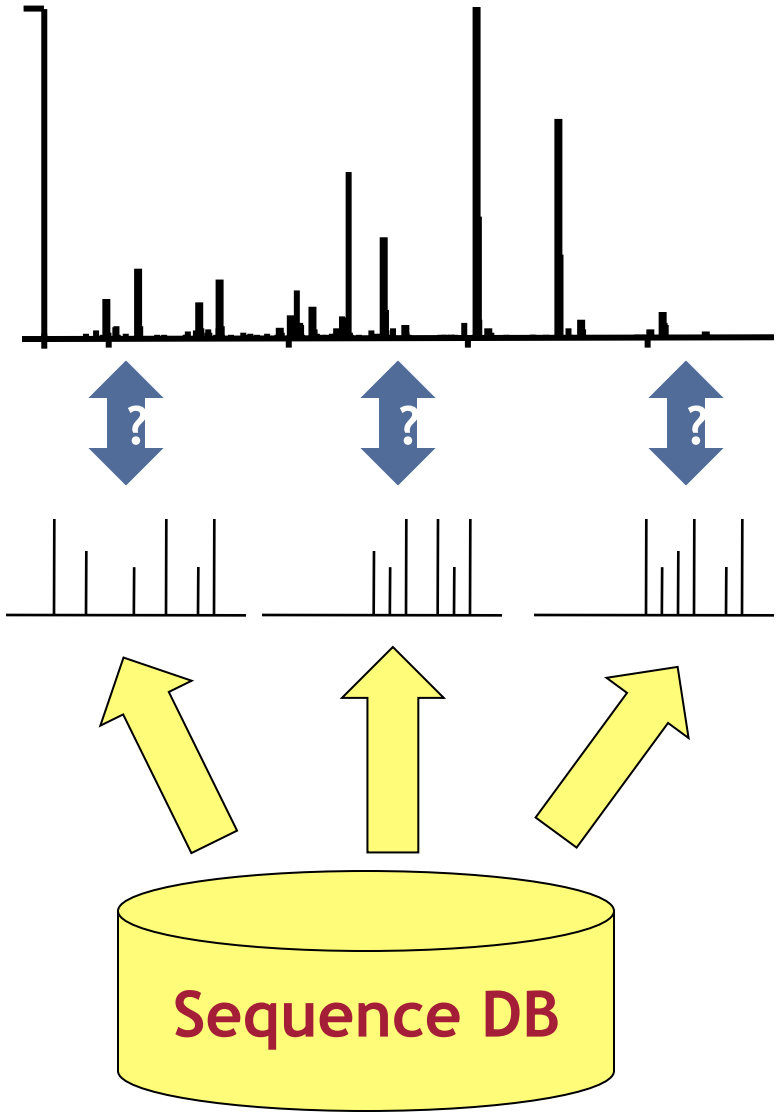
measure a peptide's mass-to-charge ratio

Peak area proportional to peptide concentration



Proteomics: Database Search

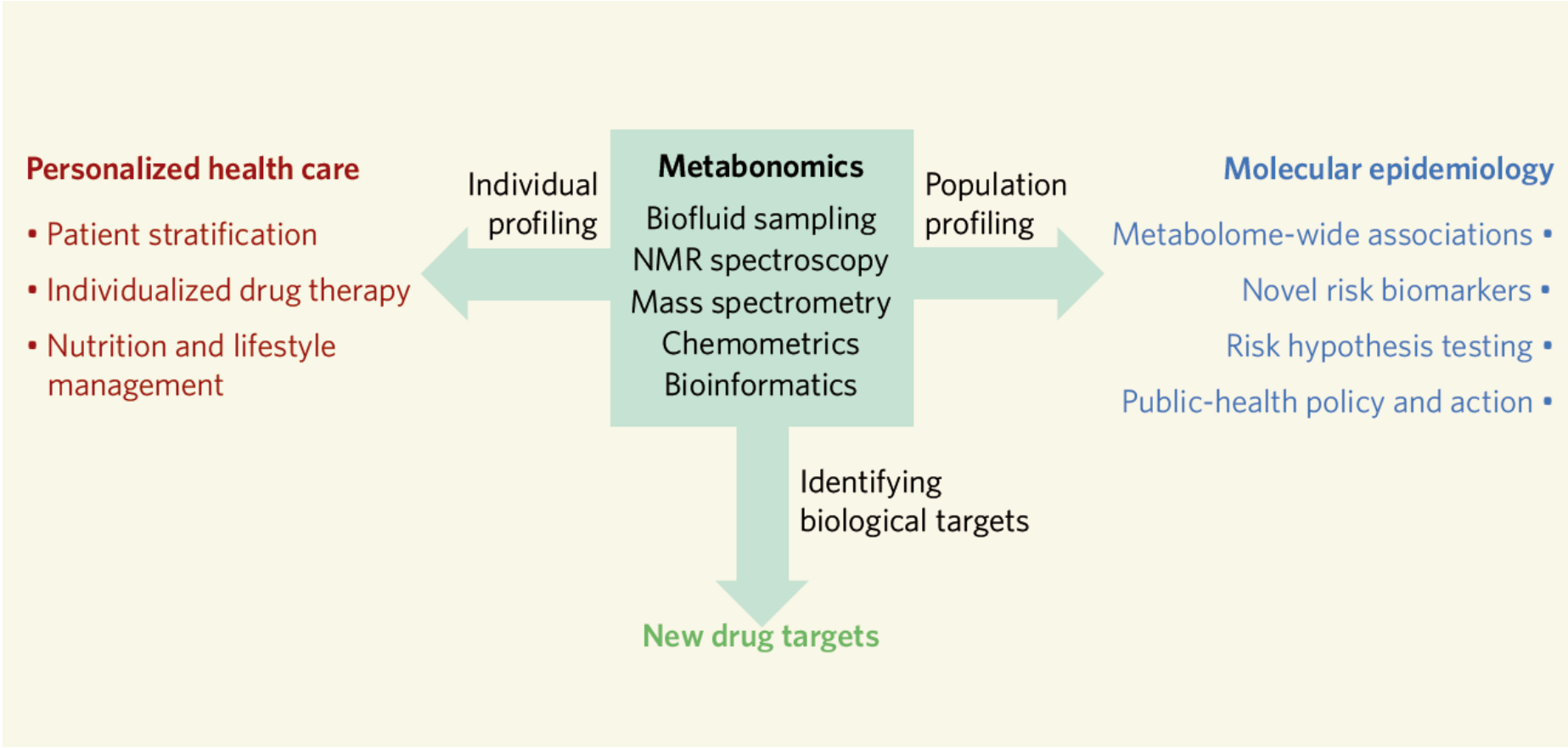
- Identification of mass spectra is easily done through database search
  - Search all peptides of matching mass from a database
  - Construct a theoretical mass spectrum for these peptide candidates
  - Score against the experimental spectrum



Exp. Metabolomics

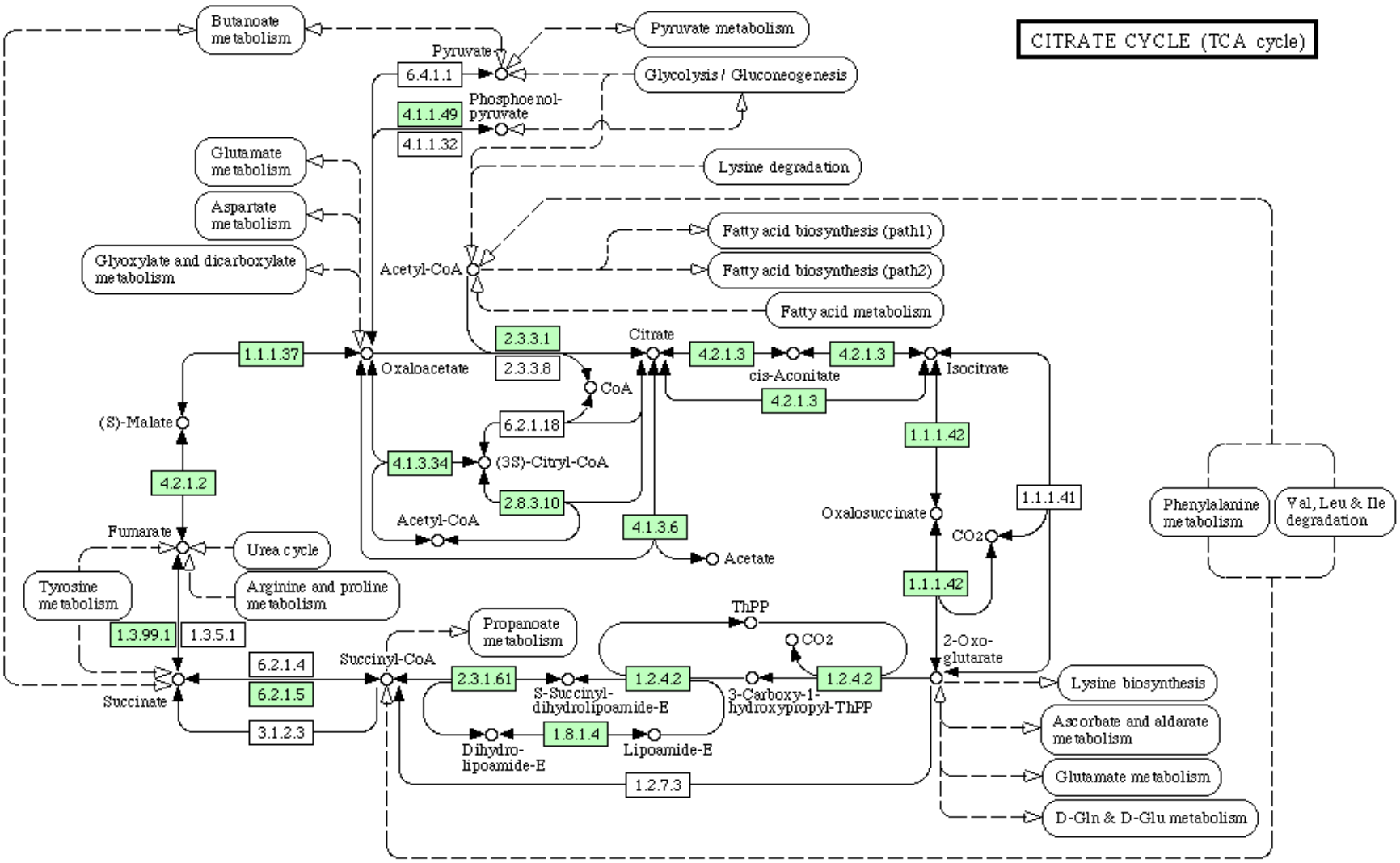
- Extract all metabolites/ small molecules, usually < 800 Da
- Separate homogenous collection of analytes (lipids, di- or tripeptides, phospholipids, sugars, etc.)
- Identify and quantify the analytes

Exptl. Metabolomics



Nicholson and Lindon. Nature 2008, 455, 1054-1056

Metabolic Networks



http://www.genome.jp/dbget-bin/www_bget?pathway+ecj00020

Proteomics

- Studying the proteome
  Proteome:=

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  Proteome:= All proteins that are expressed in a given organism, tissue or cell at a given state and time

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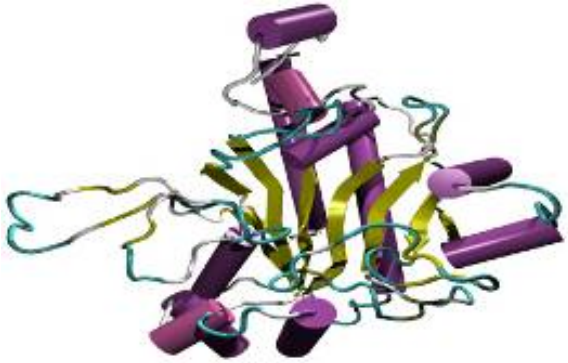
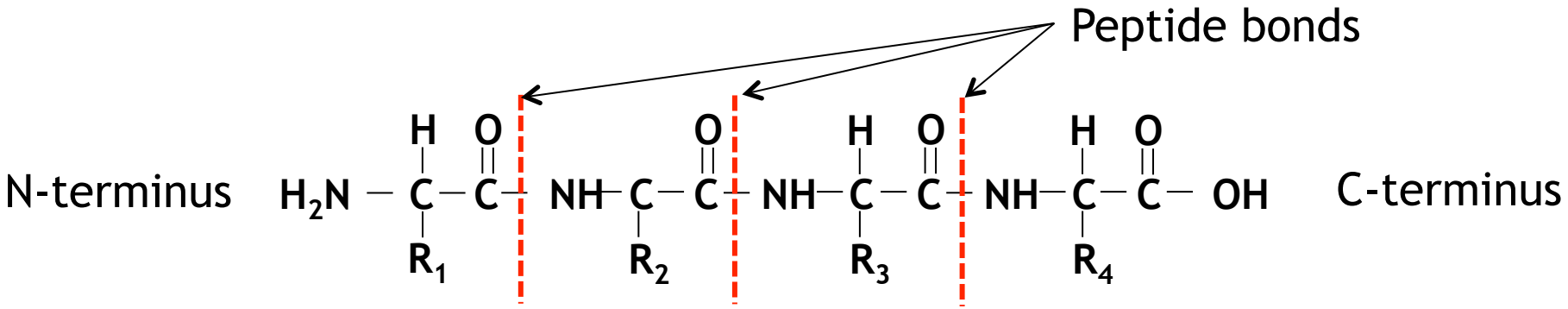
- Studying the proteome
  Proteome:= All proteins that are expressed in a given organism, tissue or cell at a given state and time
- Goal of studying proteomes: understand the function of all proteins in a biological system
- Large databases have been established, e.g., the Gene Ontology Consortium (www.geneontology.org) catalogues all proteins by their molecular function, biological process and cellular compartment

Protein

- A protein or polypeptide consists of a linear chain of amino acids that build 3-dimensional structures



- Amino acids are connected via peptide bonds



Protein

- There are some problematic issues on defining a protein
  - Protein identity: unique amino acid sequence and single source of origin?
    - There may be different genes encoding the identical amino acid sequence
    - Different organisms may encode identical proteins
  - Splice variants: A gene can give rise to different mRNAs
  - Polymorphisms: many genes occur in allelic variants encoding sequence variations
  - Posttranslational modifications: PTMs are very hetero-geneous and significantly alter the function of the protein

Metabolomics

- Studying the metabolome
  Metabolome:=

Metabolomics

- Studying the metabolome
  Metabolome:= The metabolome refers to the complete set of small-molecule metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism. (http://en.wikipedia.org/wiki/Metabolome)

Technologies

Modern Proteomics and Metabolomics studies are based on

Liquid chromatography (LC)

-

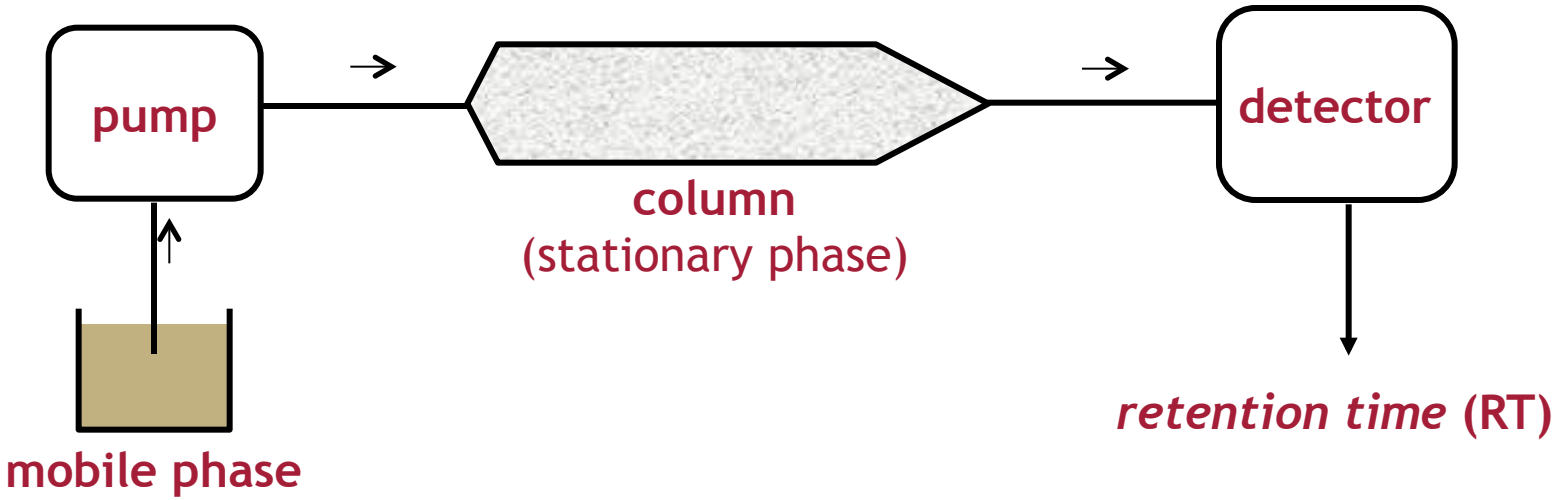
Mass spectrometry (MS)

(Liquid) chromatography

- Mobile phase liquid, stationary phase is usually solid
- Analytes are held back on a column
- “Mobile phase” is pumped over the column
- Analytes continously separate and elute from the column according to specific properties (e.g. hydrophobicity)

- Other chromatography (e.g. gas chromatography) techniques will also be mentioned

HPLC (High Performance Liquid Chromatography)



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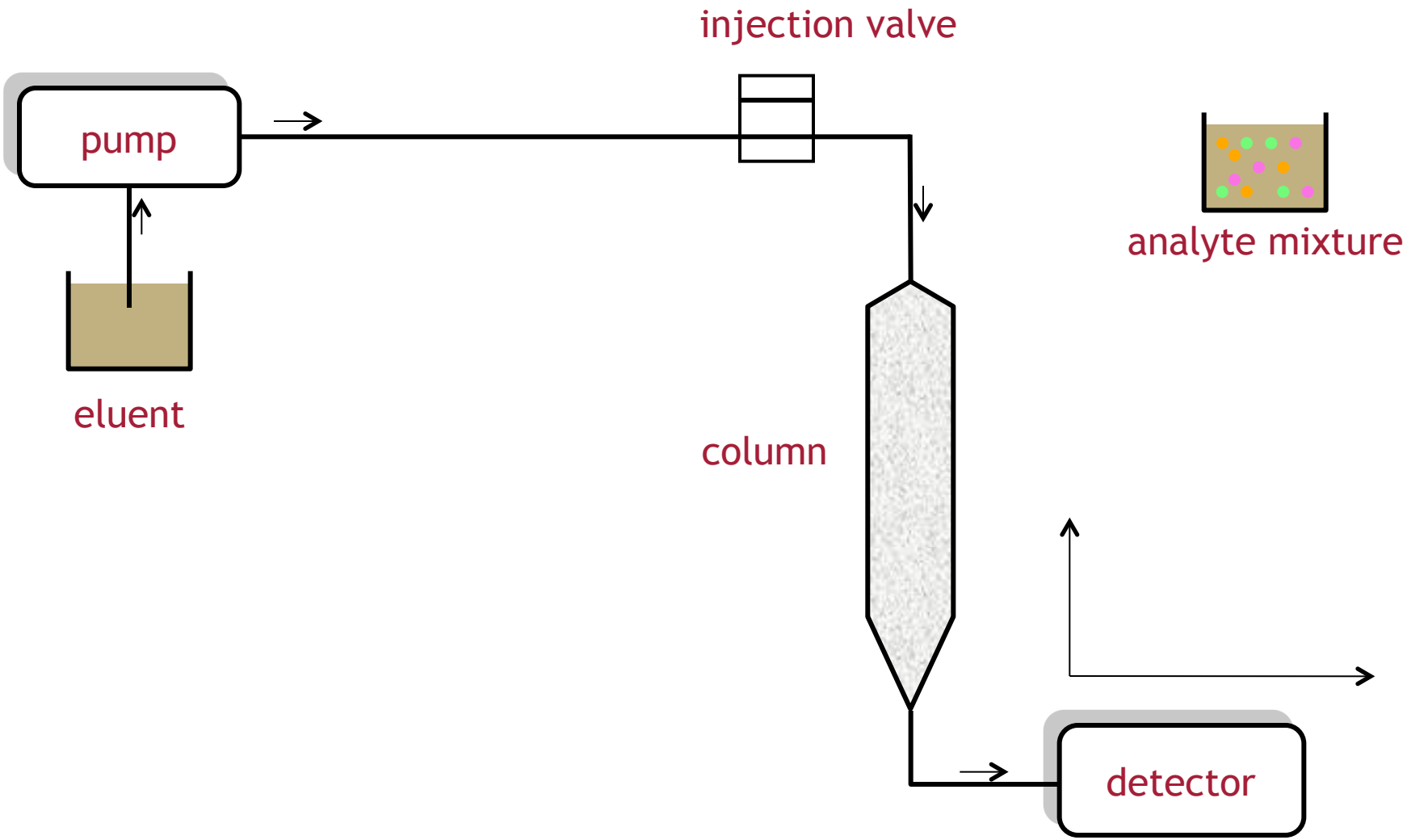

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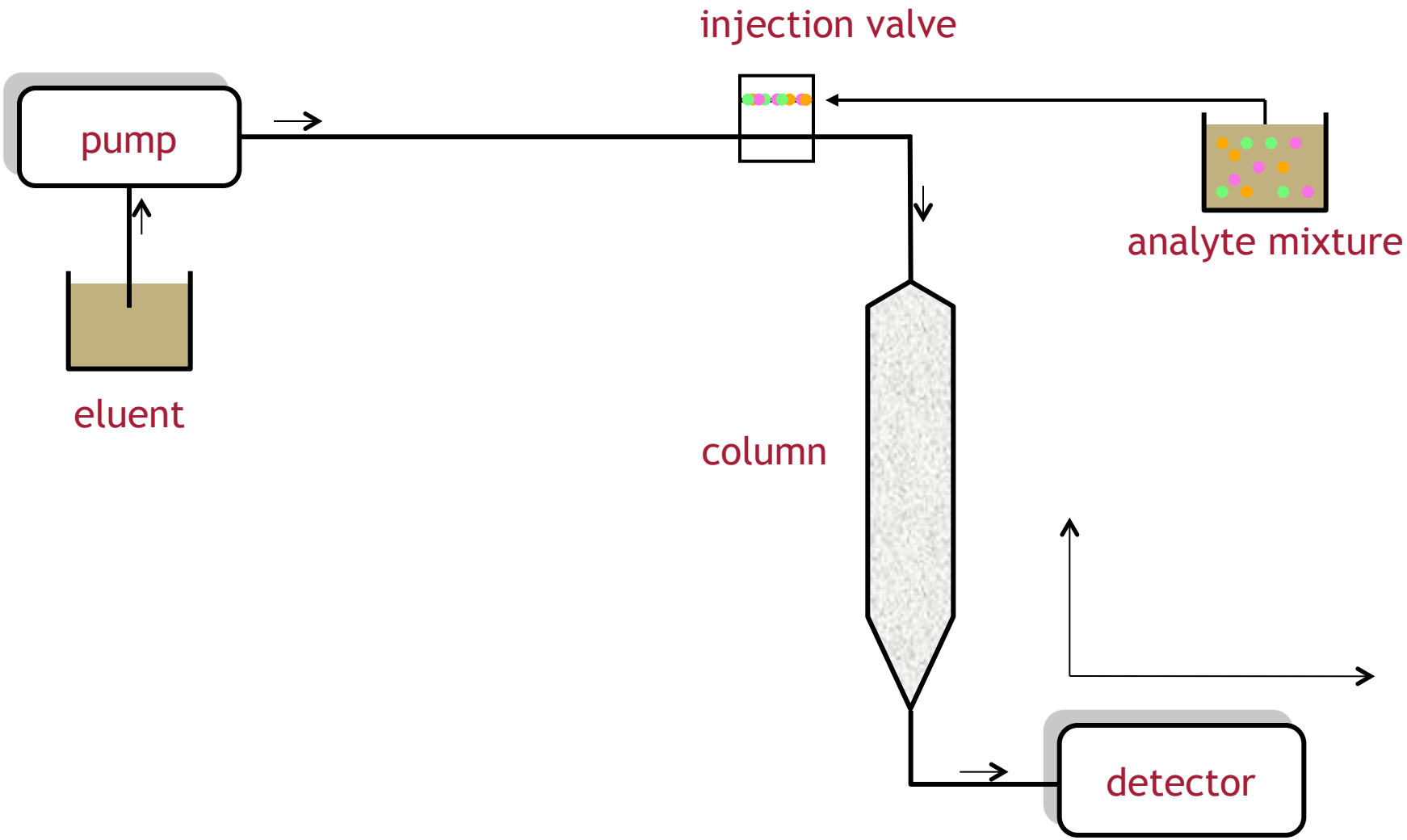

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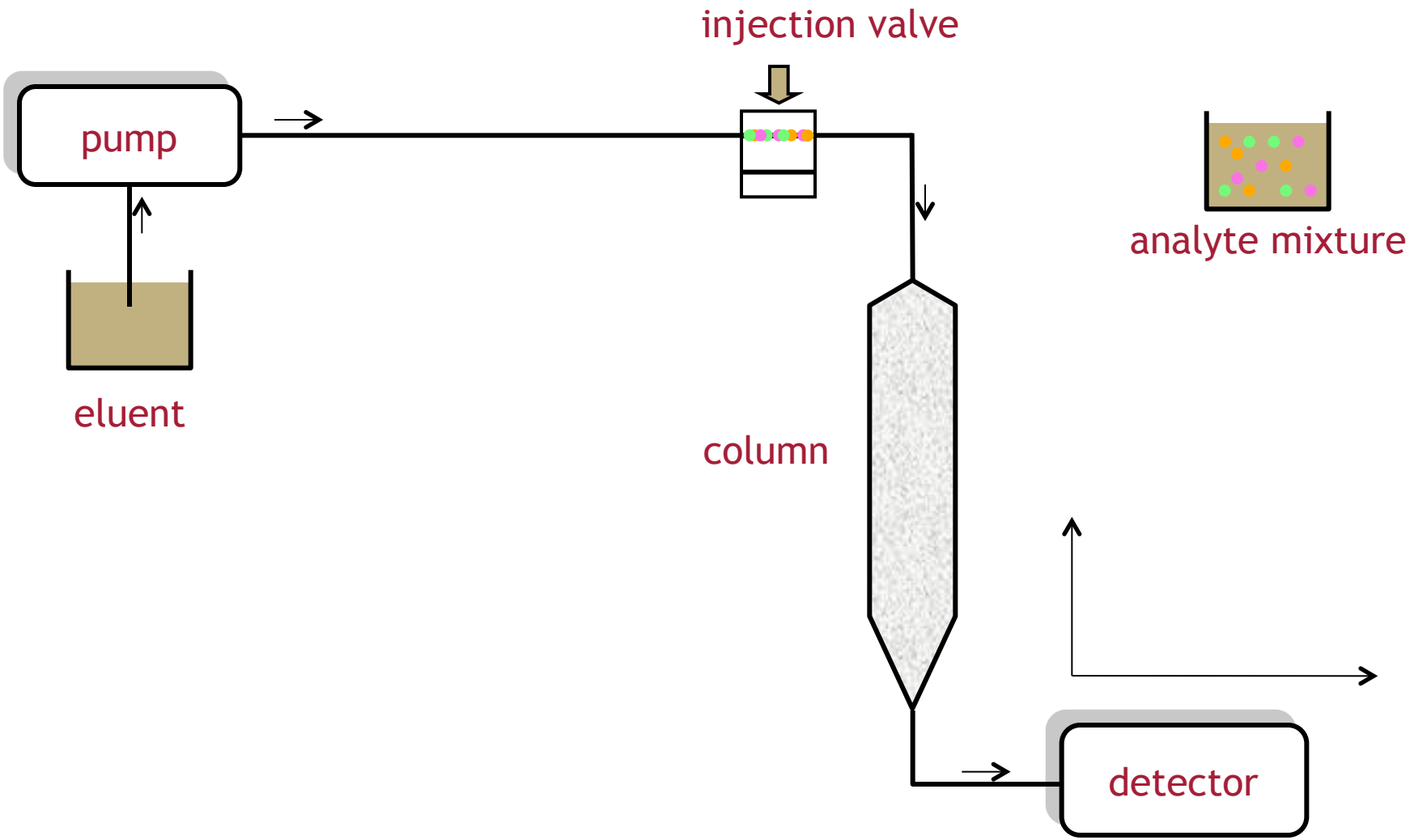

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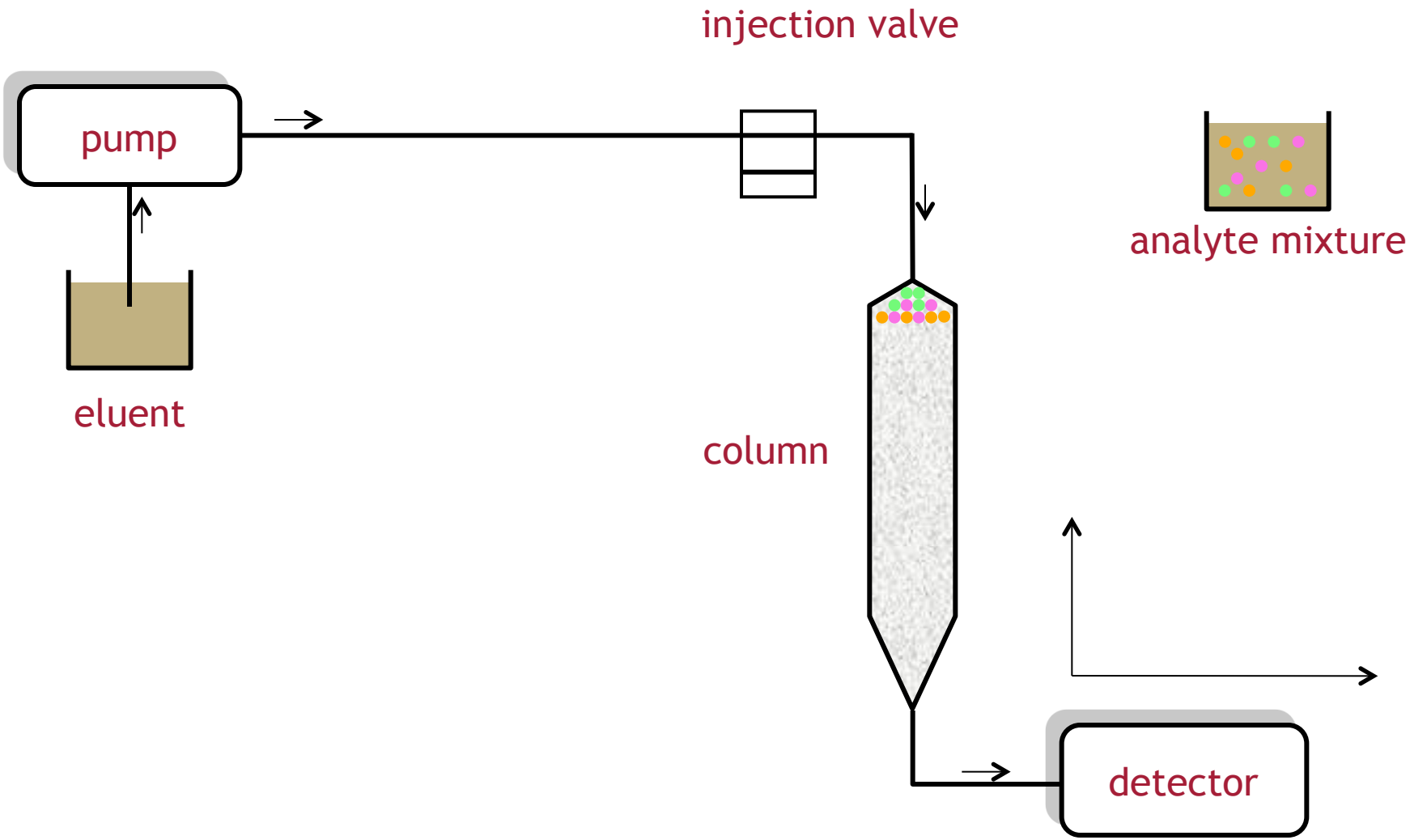

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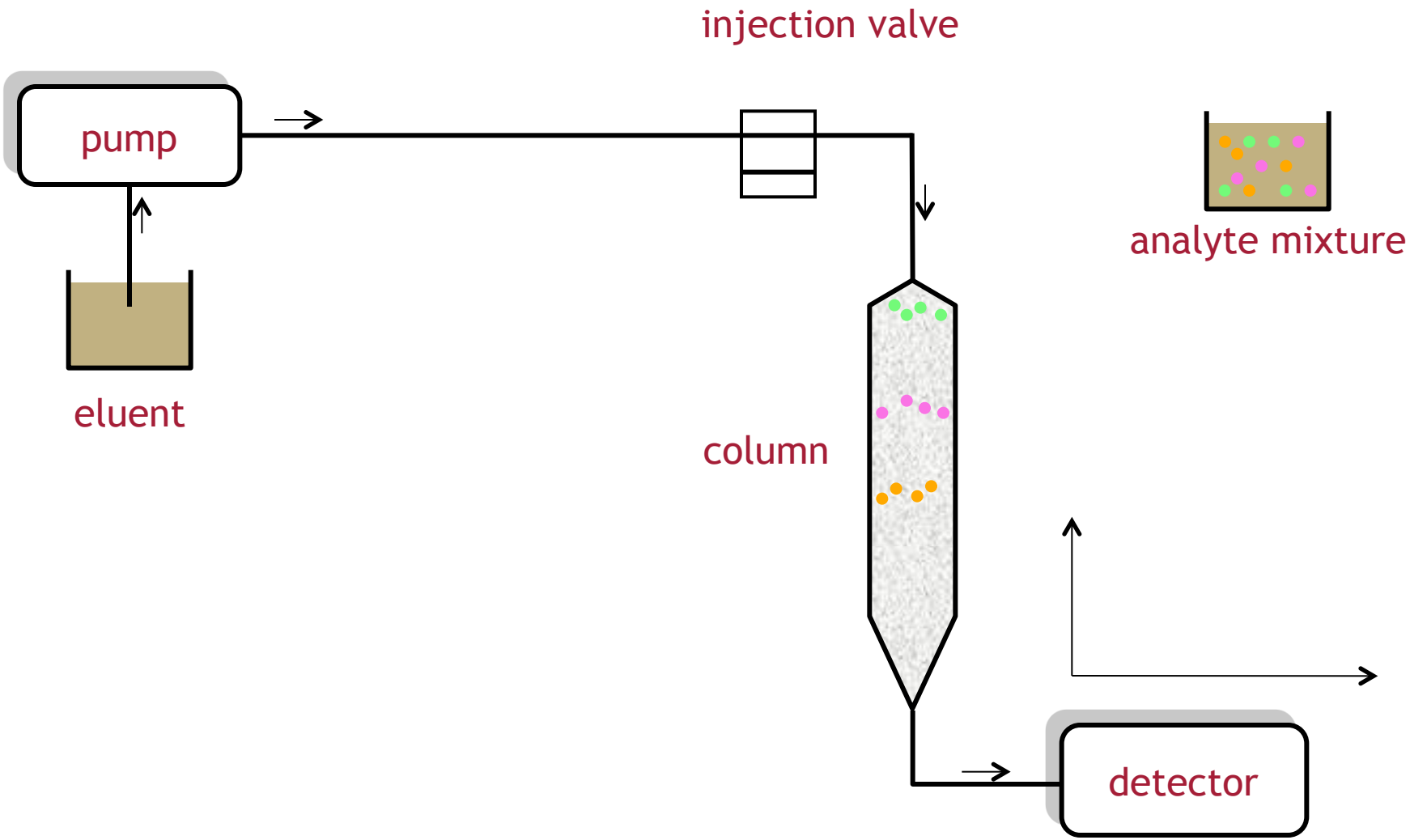

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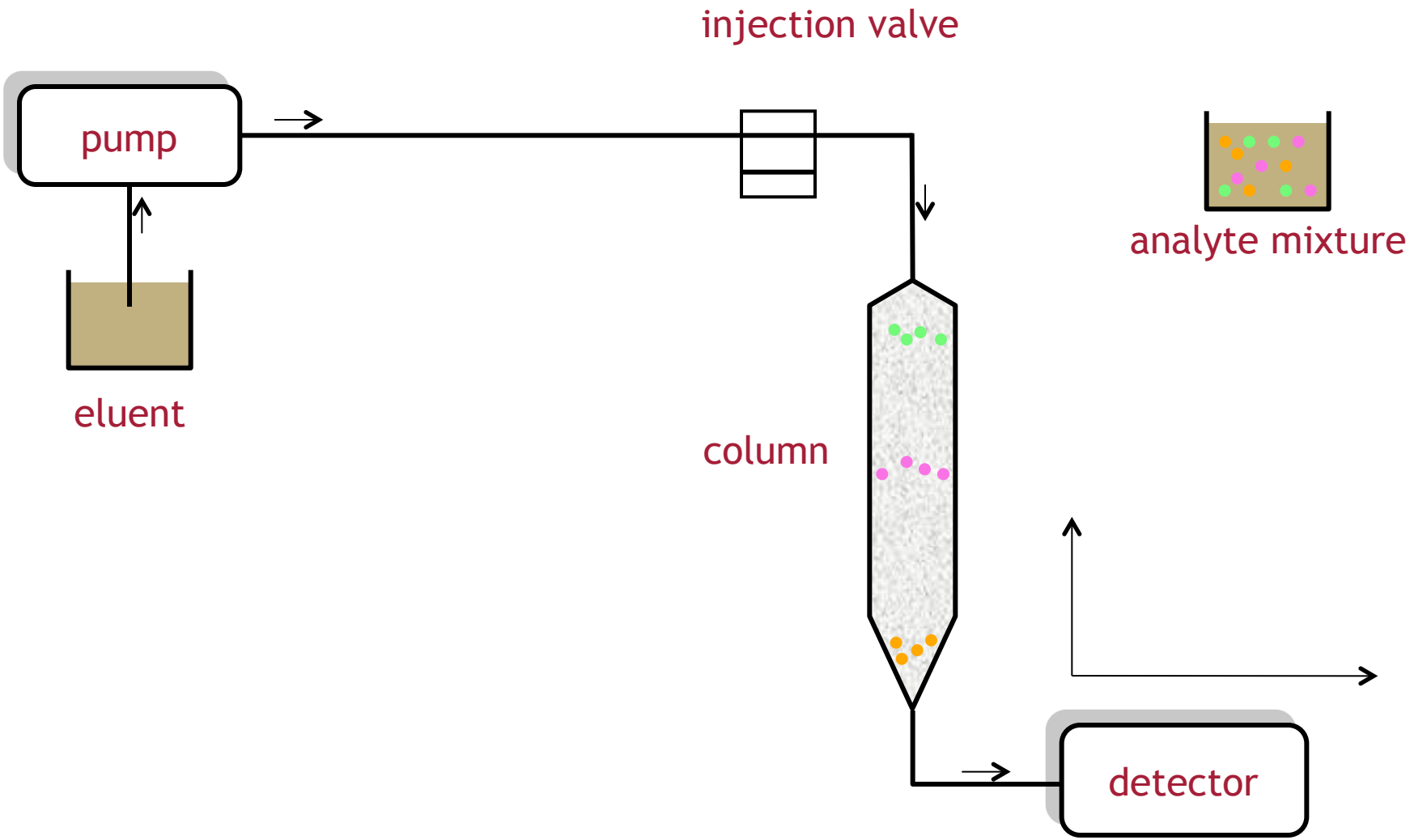

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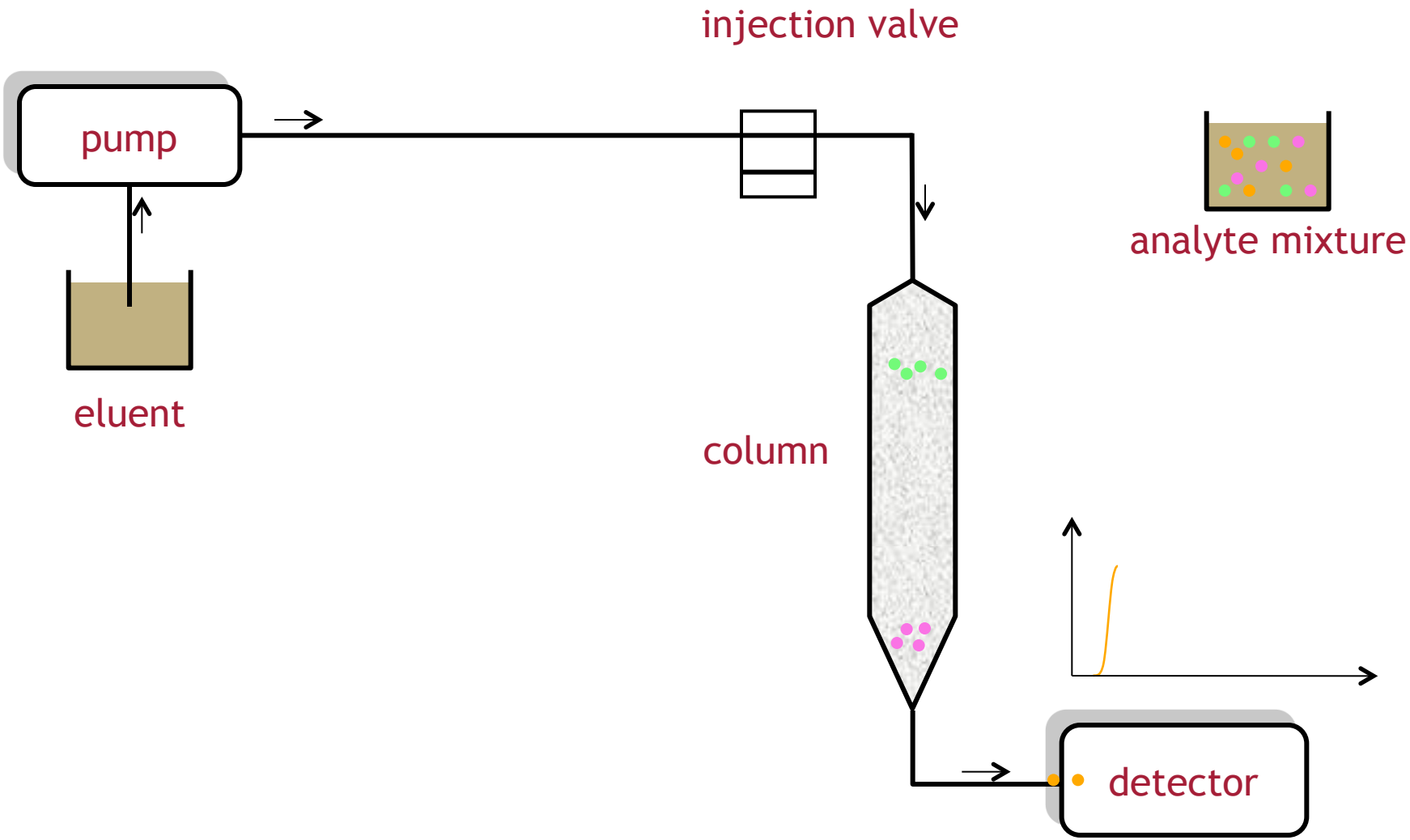

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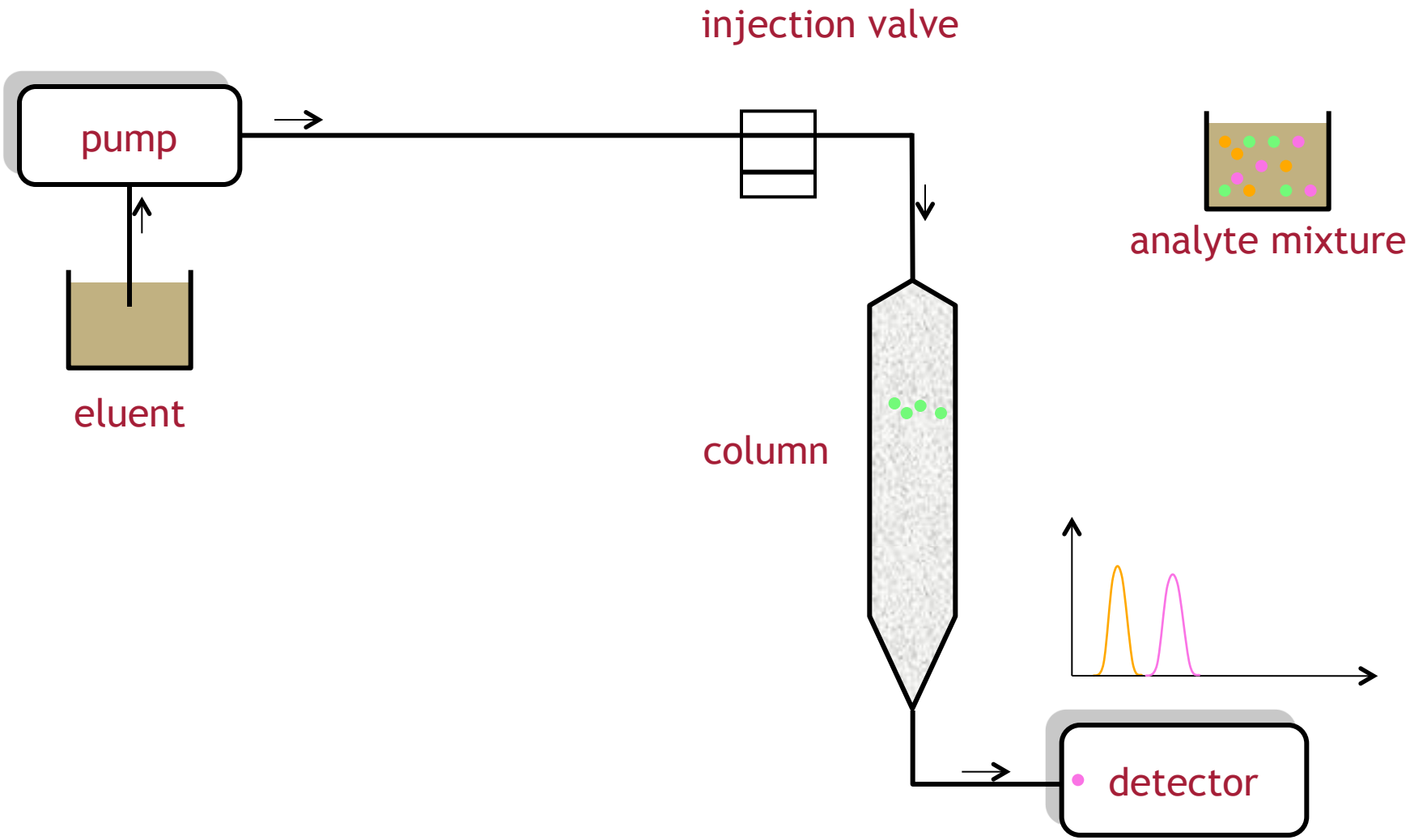

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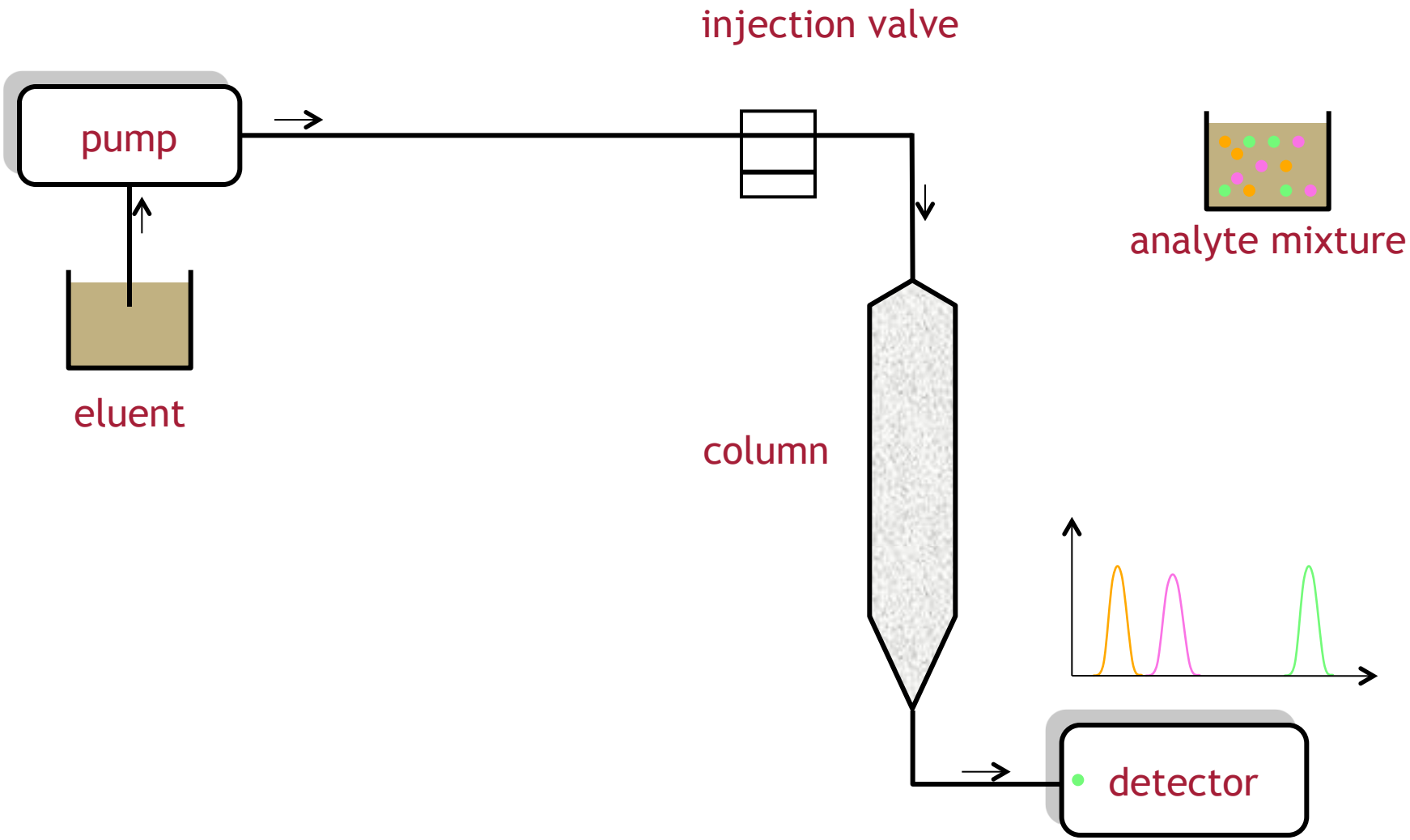
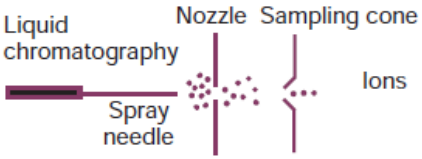


Mass spectrometry

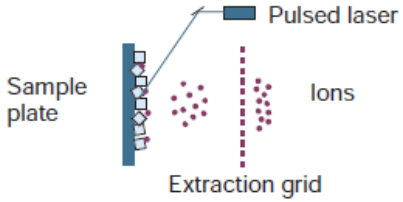
- Mass spectrometry (MS) is an analytical technique to measure the mass (or more precisely: mass-to-charge ratio, m/z) of an analyte
- MS has a long history in physics and chemistry and today the key technology in proteomics and metabolomics
- “soft ionization” methods enable its application in the bio(-analytical) sciences
- For OMICS analyses MS is usually coupled to a second separation technique (e.g. LC for proteomics and LC/GC for metabolomics)
- There are various types of mass spectrometers

Mass spectrometry

Ionization techniques



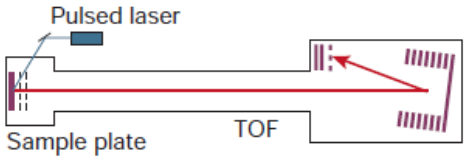
Electrospray ionization (ESI)



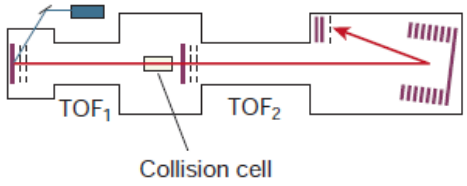
Matrix Assisted Laser Desorption/ Ionisation (MALDI)

Mass analyzers

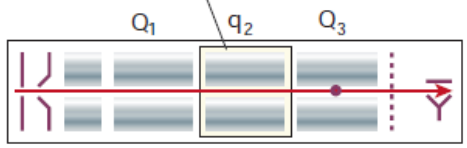
Reflector time-of-flight (TOF)



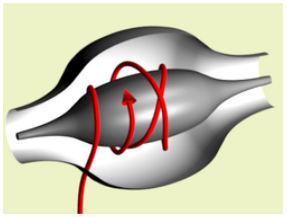
time-of-flight time-of-flight (TOF-TOF)



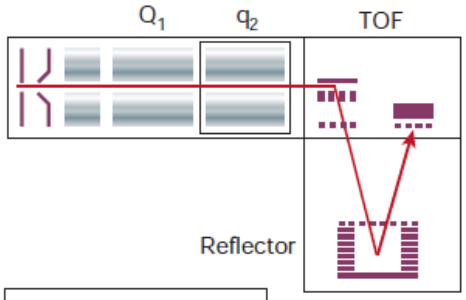
Triple Quadrupole



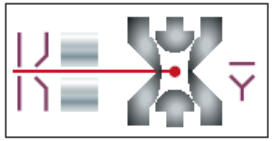
Orbitrap



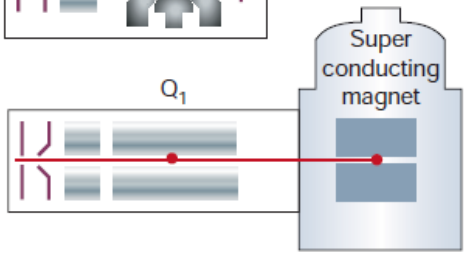
Quadrupole - time-of-flight



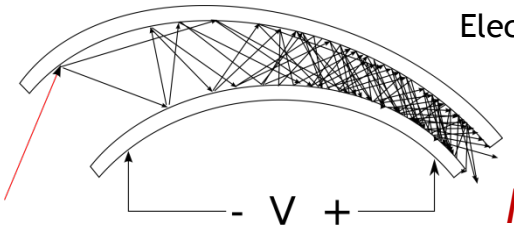
Ion trap



Fourier transform - Ioncyclotron resonance

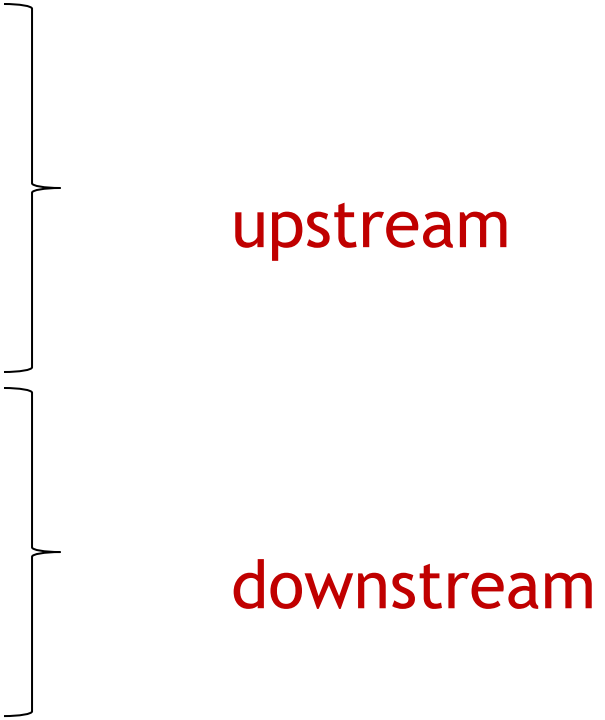


Mass detector

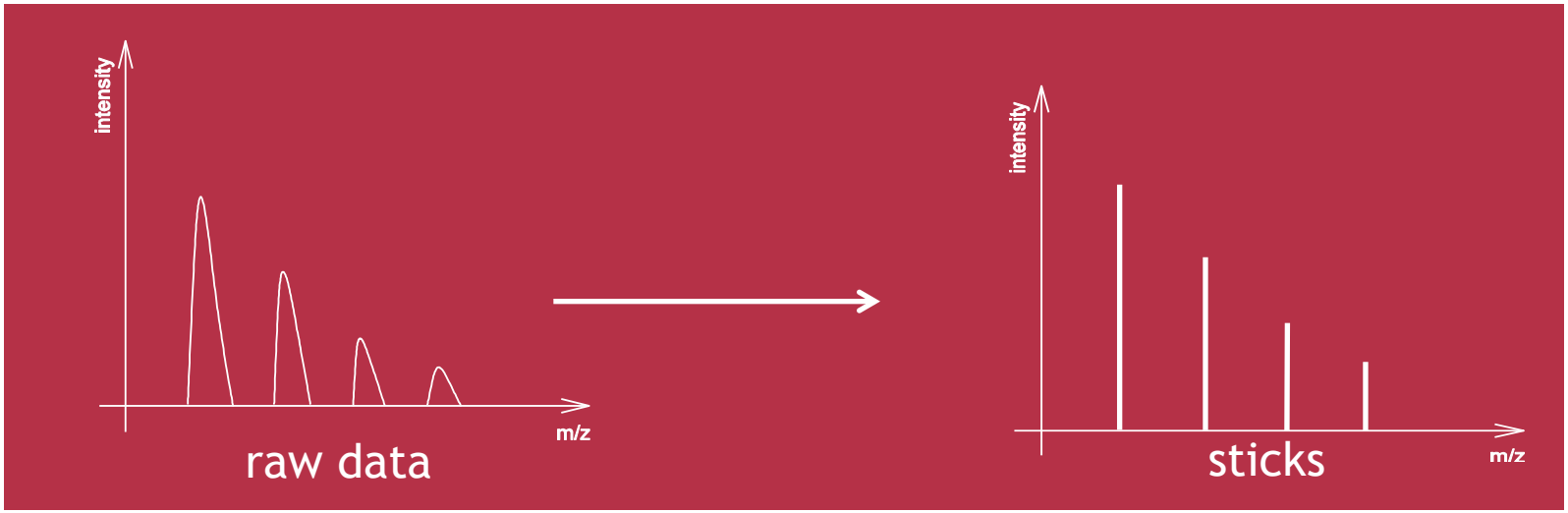


Challenges in computational MS

- Huge data sets (up to TBs per experiment)
- Ambiguity in protein identification
- Uncertainty in proteome size
- Ambiguity of masses for small molecules

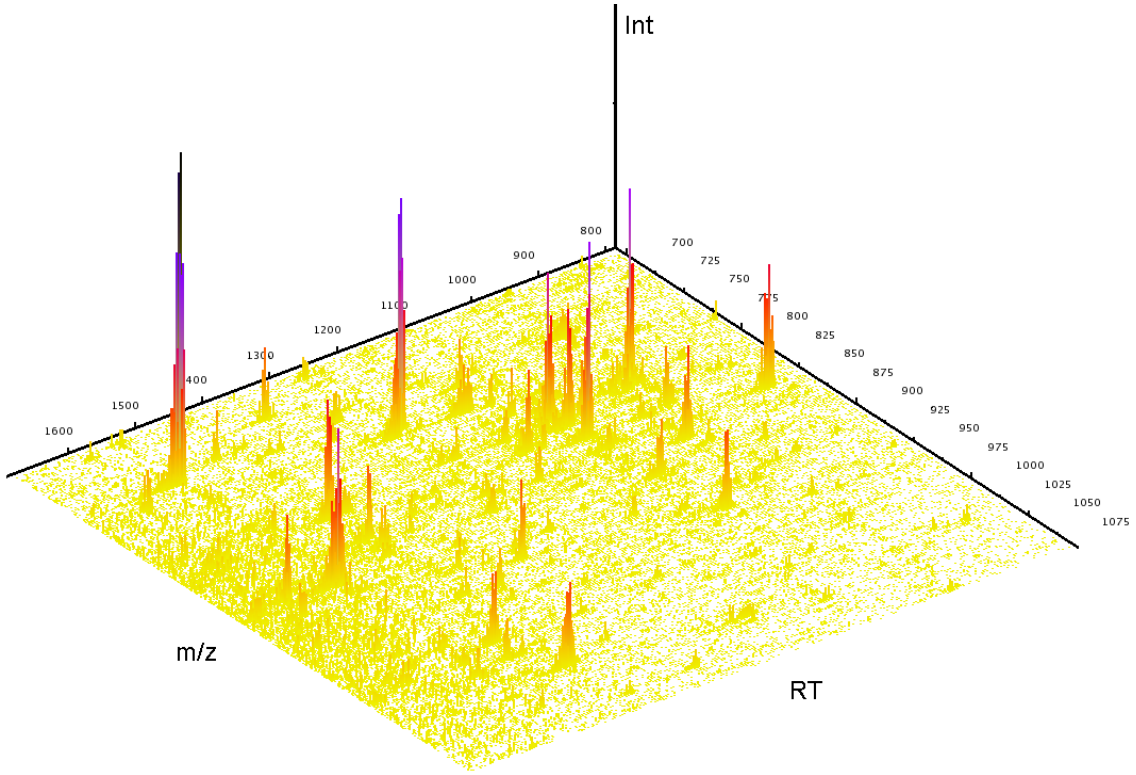


Peak Picking



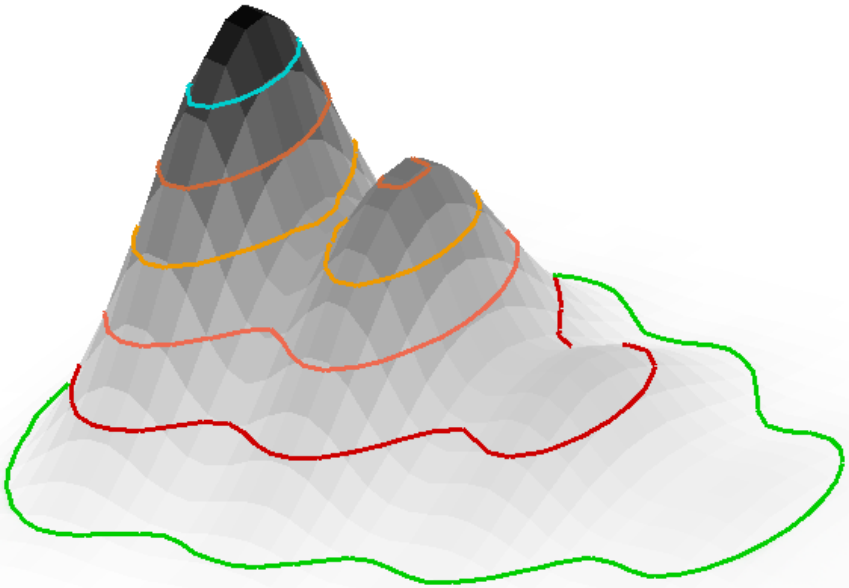
- Identify peaks
- Integrate peaks to sticks

Quantification

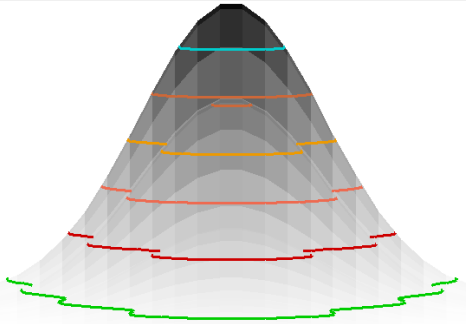


- Determine volume of each feature in a map

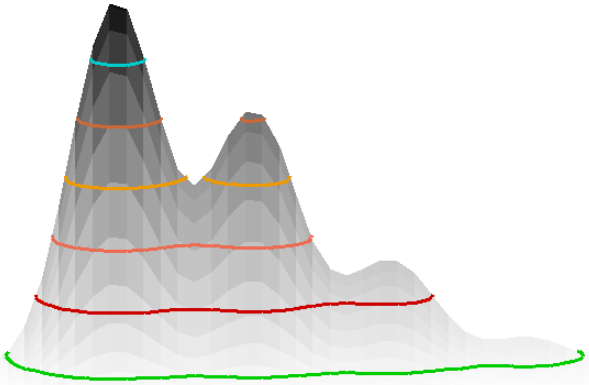
Quantification



feature model



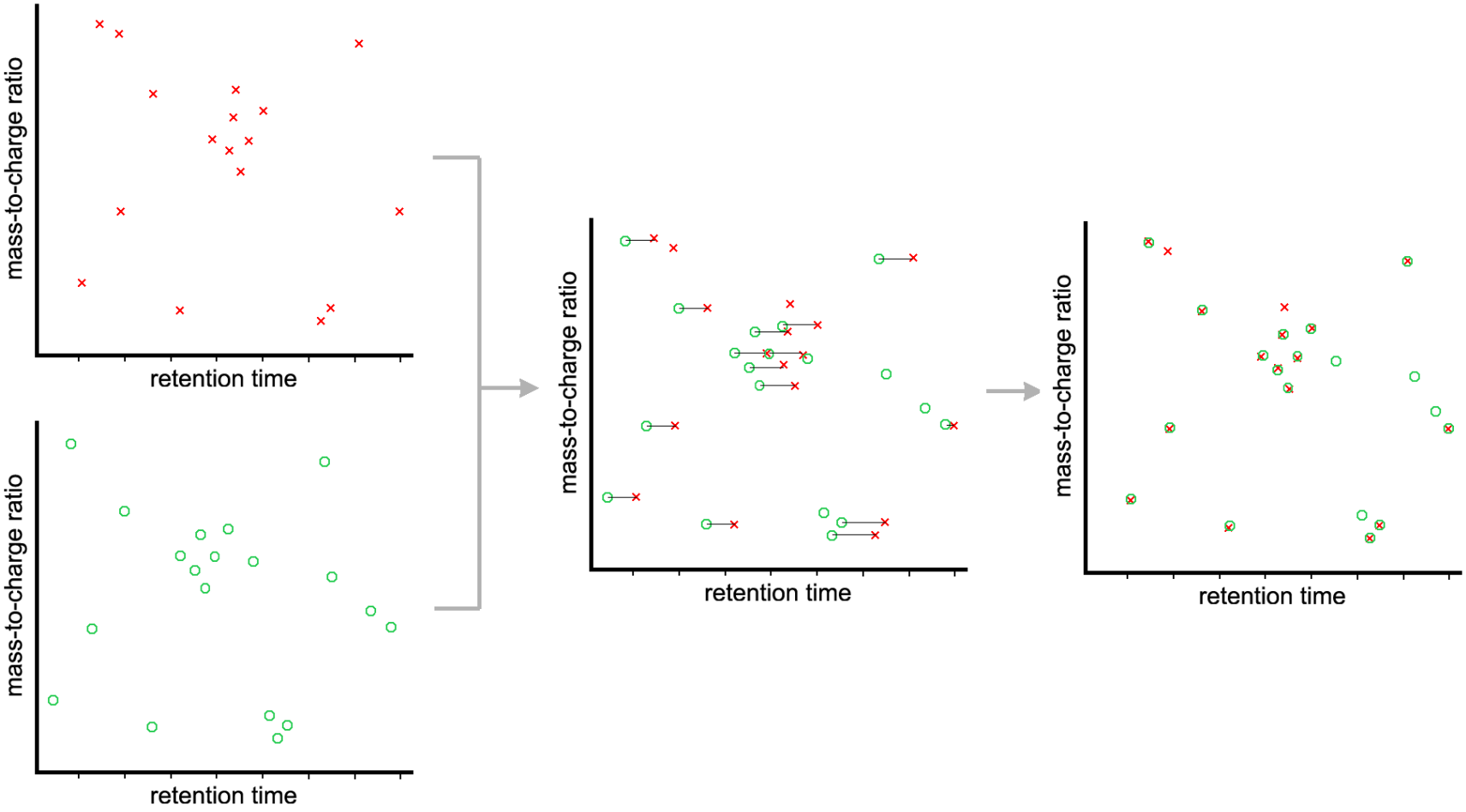
RT: elution profile



m/z: Isotopic pattern

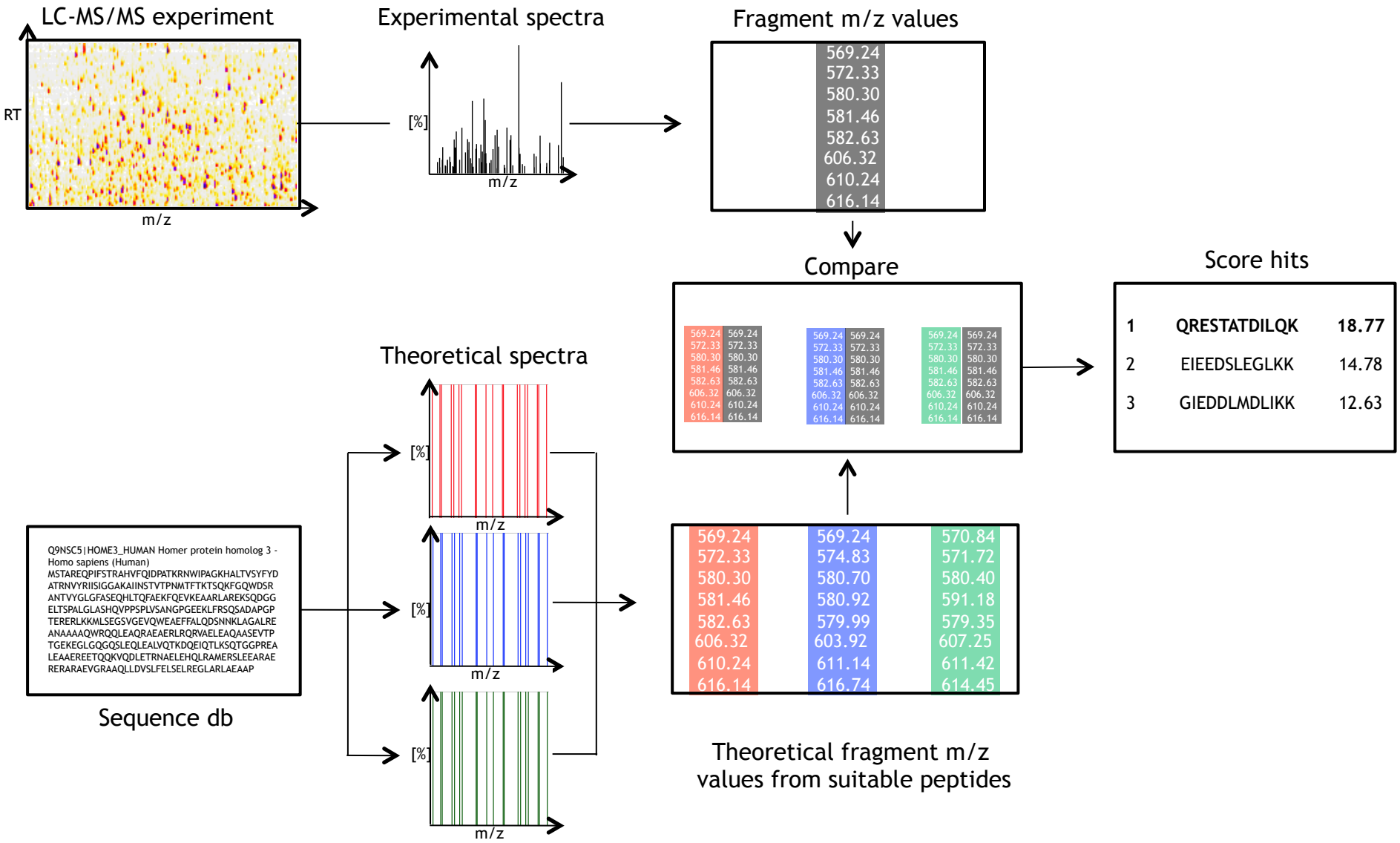
- Quantification as a 3D signal detection problem

Map alignment

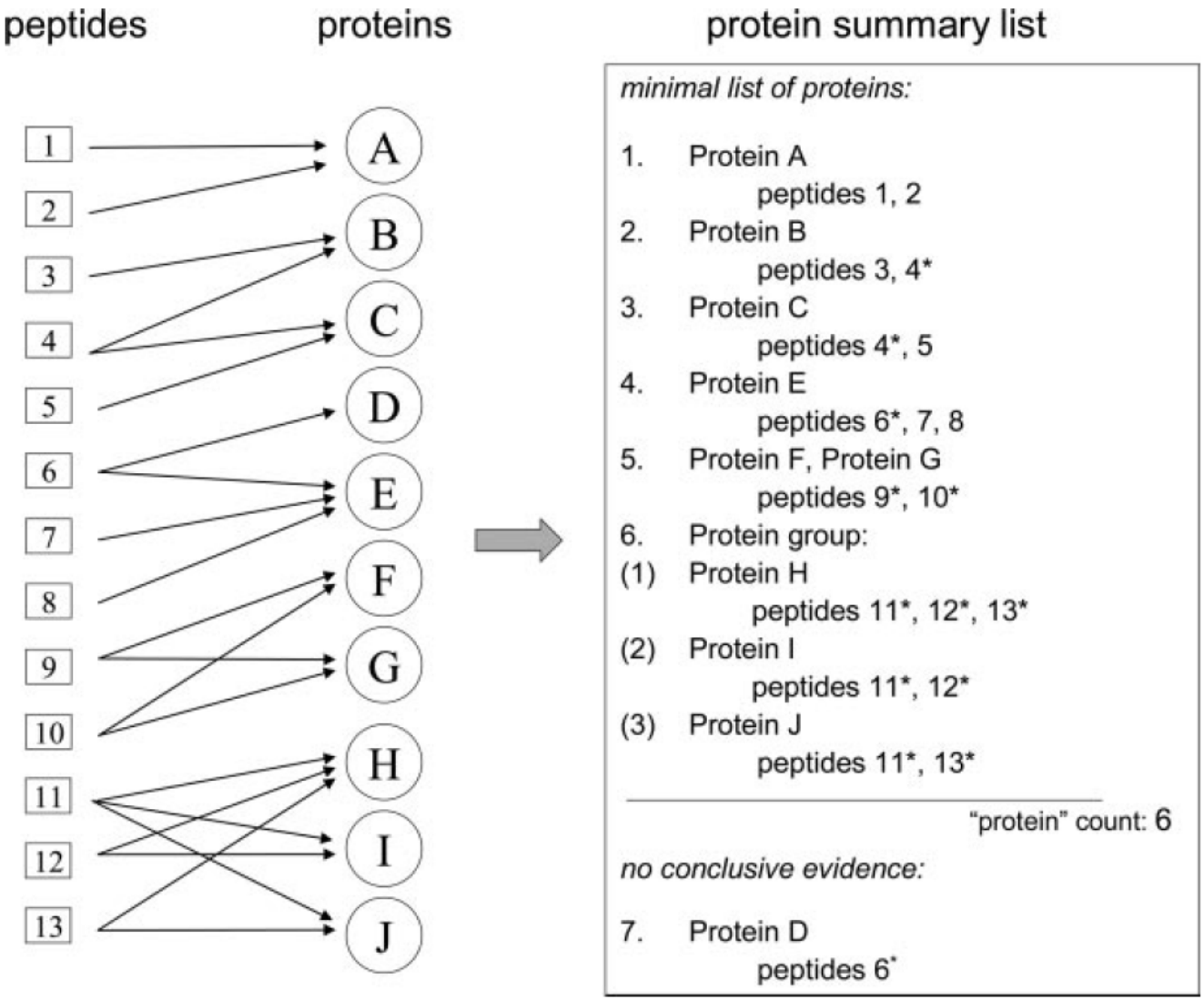


- Correct for retention time offset and distortions in label-free experiments

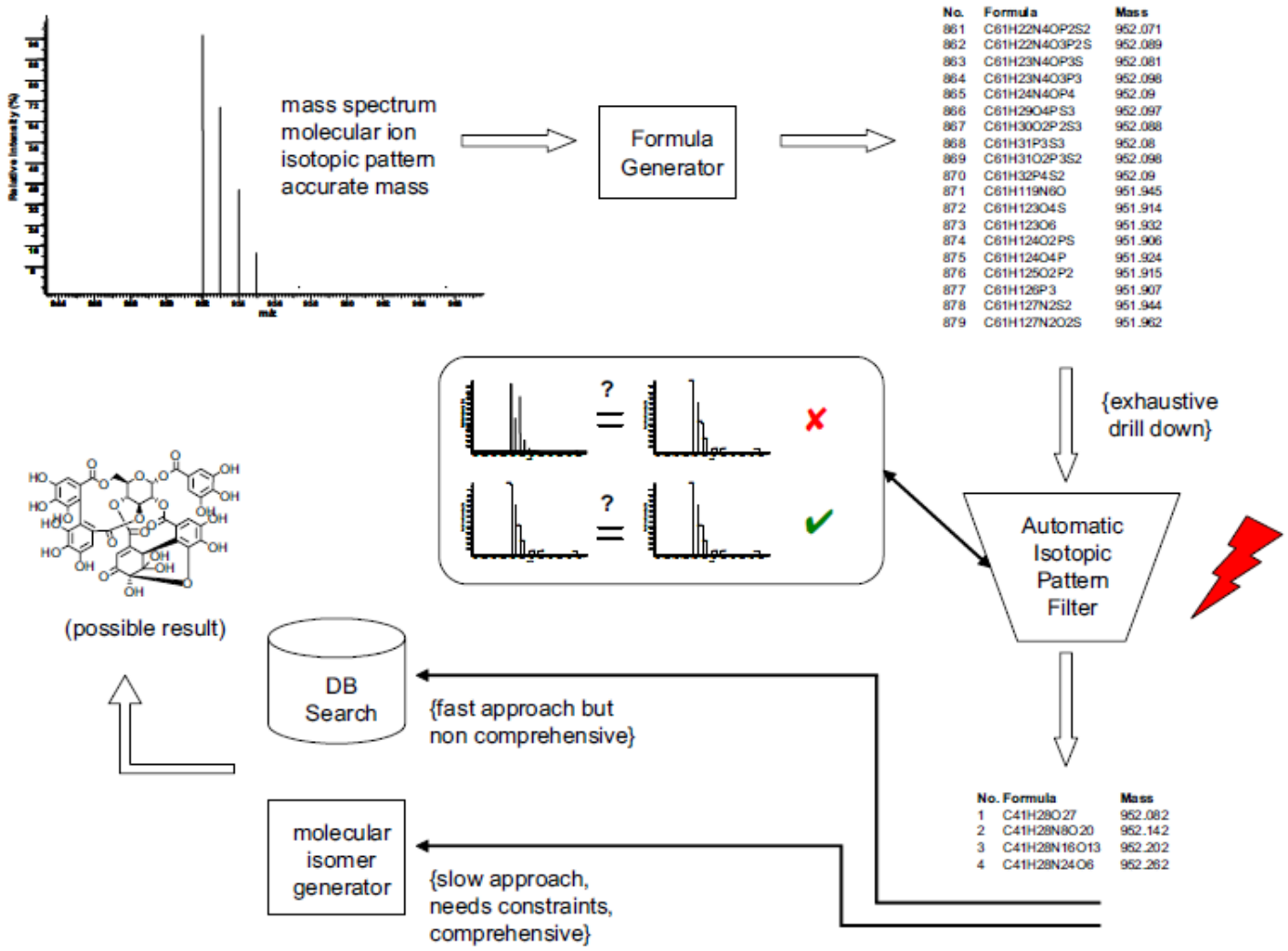
Peptide Identification



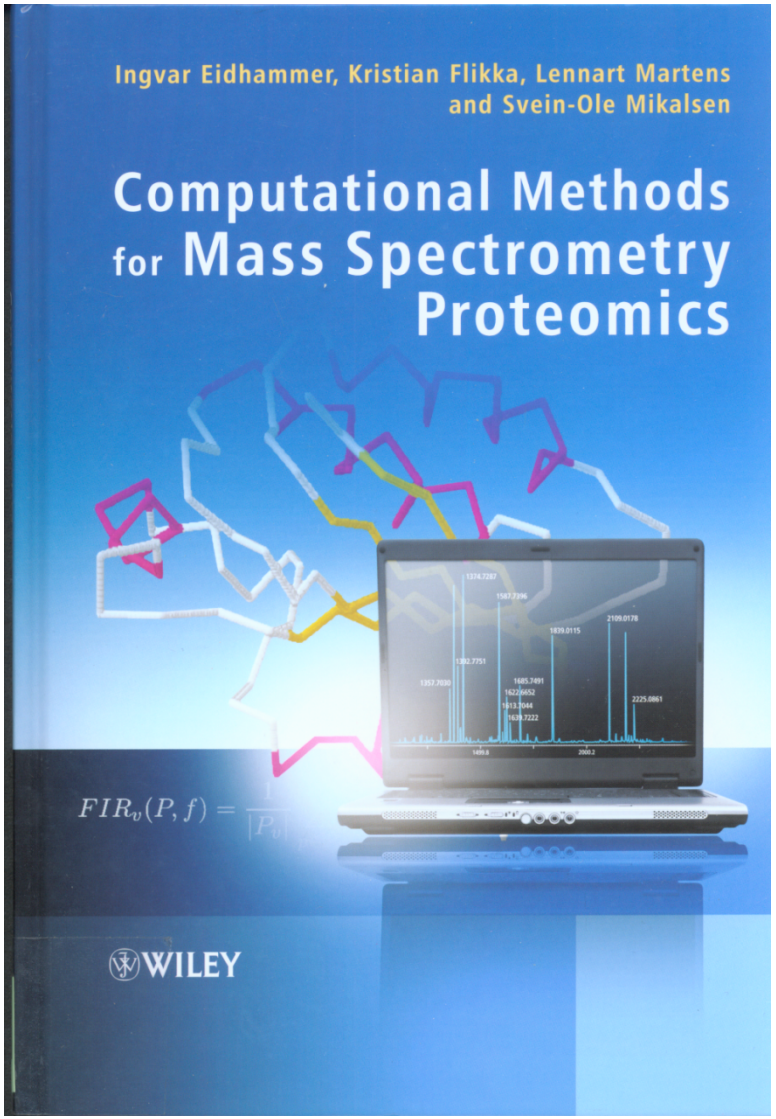
Protein inference



Metabolite identification



Textbooks



Eidhammer, Flikka, Martens, Mikalsen: Computational methods for mass spectrometry proteomics, Wiley, 2007

Good introduction:

- Biochemical basics
- Mass spectrometry
- Algorithms for protein identification/ quantification

Important papers

THE ABC'S (AND XYZ'S) OF PEPTIDE SEQUENCING

Hanno Steen and Matthias Mann‡*

Abstract | Proteomics is an increasingly powerful and indispensable technology in molecular cell biology. It can be used to identify the components of small protein complexes and large organelles, to determine post-translational modifications and in sophisticated functional screens. The key — but little understood — technology in mass-spectrometry-based proteomics is peptide sequencing, which we describe and review here in an easily accessible format.

Important papers

Mass spectrometry-based proteomics

Ruedi Aebersold* **& Matthias Mann†**

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Recent successes illustrate the role of mass spectrometry-based proteomics as an indispensable tool for molecular and cellular biology and for the emerging field of systems biology. These include the study of protein–protein interactions via affinity-based isolations on a small and proteome-wide scale, the mapping of numerous organelles, the concurrent description of the malaria parasite genome and proteome, and the generation of quantitative protein profiles from diverse species. The ability of mass spectrometry to identify and, increasingly, to precisely quantify thousands of proteins from complex samples can be expected to impact broadly on biology and medicine.

Important papers

Analysis and validation of proteomic data generated by tandem mass spectrometry

Alexey I Nesvizhskii[1], Olga Vitek[2] & Ruedi Aebersold[3,4]

The analysis of the large amount of data generated in mass spectrometry–based proteomics experiments represents a significant challenge and is currently a bottleneck in many proteomics projects. In this review we discuss critical issues related to data processing and analysis in proteomics and describe available methods and tools. We place special emphasis on the elaboration of results that are supported by sound statistical arguments.

Tutorial

Interpretation of Shotgun Proteomic Data

THE PROTEIN INFERENCE PROBLEM*

Alexey I. Nesvizhskii‡§ and Ruedi Aebersold‡¶

Important papers

Anal Bioanal Chem (2010) 398:2779–2788
DOI 10.1007/s00216-010-4142-5

REVIEW

Computational mass spectrometry for metabolomics: Identification of metabolites and small molecules

Steffen Neumann · Sebastian Böcker

Important papers

BRIEFINGS IN BIOINFORMATICS. VOL 8. NO 5. 279–293
Advance Access publication July 11, 2007

doi:10.1093/bib/bbm030

Current Progress in computational metabolomics

David S. Wishart

Submitted: 2nd April 2007; Received (in revised form): 15th June 2007

Important papers

BMC Bioinformatics

BioMed Central

Research article

Open Access

Metabolomic database annotations *via* query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm

Tobias Kind* and Oliver Fiehn

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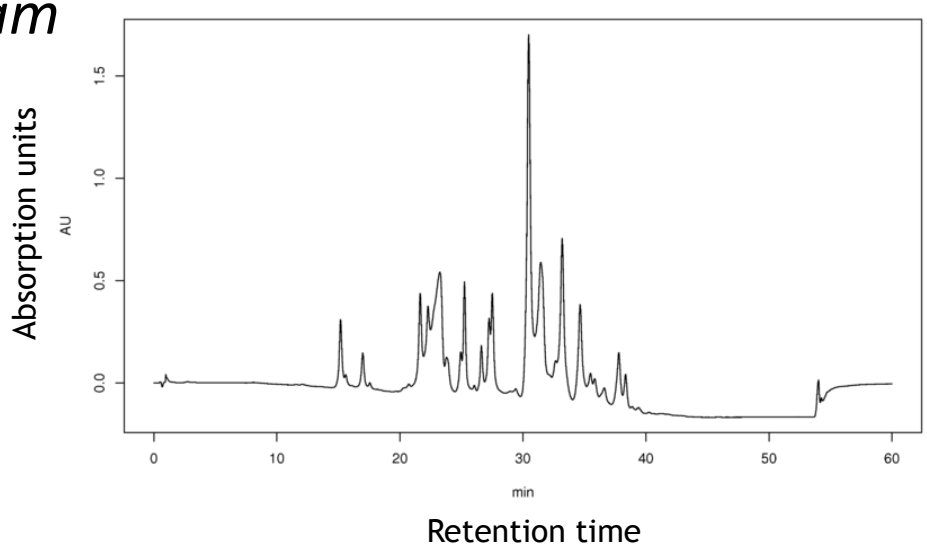
Chromatography

- Family of techniques used to separate a mixture into individual components
- Separation by passing the mixture through immobilized porous substance
- Individual components interact to different degrees
- **Retention time** :≔ time an individual component takes to pass through the system
- Chromatography has a long tradition and is a scientific field in itself
- In Proteomics/ Metabolomics: used for separation

Detectors used for HPLC

- The detectors registers the components as they pass
- Detection of light absorption
  - Components absorb light at certain wavelength (e.g., 280 nm for aromatic amino acids)
- The detector can also be a mass spectrometer
- It registers a diagram of showing the intensity of eluting components as a function of retention time. This diagram is called *chromatogram*



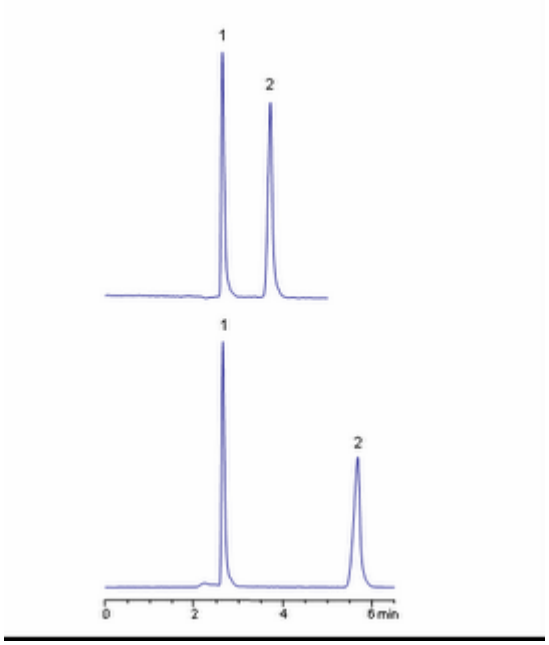
Good HPLC performance

- Find an optimal balance between the components' affinity for the stationary phase and the solubility of the components in the mobile phase
  - Different components should migrate at different rates
  - Narrow elution peak of different components
  - Ideally elution peaks of different components should not overlap
- Challenge:
  - Achieve different rates of migration for the different components
  - Narrow elution peaks
- Difficulties:
  - Noise. *Signal-to-noise* ratios can be used to quantify how well a real signal can be differentiated from background signal
  - Baseline drift. The baseline is recorded when only the mobile phase elutes. This can vary over time.

Good HPLC performance

The good

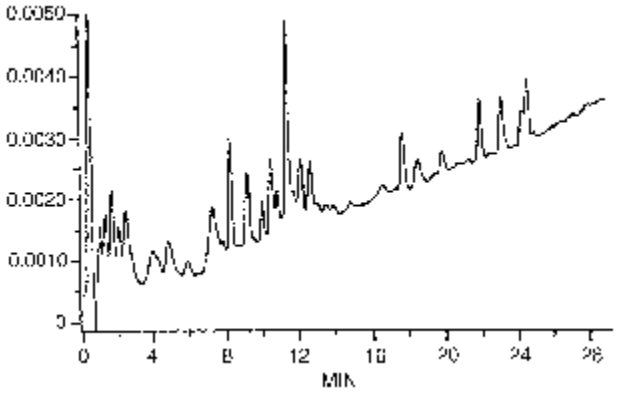
- Peaks are well separated
- Peaks are sharp
- Peaks come down to baseline

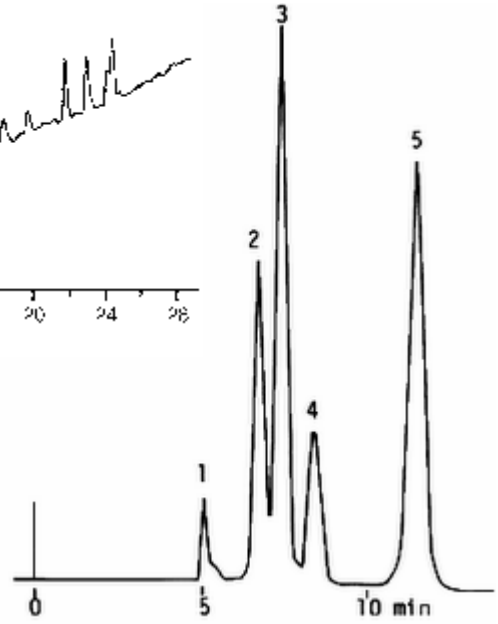


Faculty of Pharmacy; Cairo University

The bad

- Peaks are not well resolved
- Baseline is drifting upwards





HPLC methods

- Essential components are
  - Stationary phase
    - Interaction of stationary phase with components
  - Mobile phase
    - Solubility of components in mobile phase
  - Components (analytes)
- Most common HPLC methods
  - Reversed-phase (RP) chromatography
  - Strong cation/anion exchange (SCX/SAX) chromatography
  - Affinity chromatography
  - Size exclusion chromatography

Reversed-phase chromatography

- Easy to couple to MS (via ESI)
- Stationary phase: surface-modified silica (most commonly alkyl chains: (C_4, C_8 or C_{18}); comparable to fatty acid chains)
- Mixture of two solutions used as a mobile phase
  - Solution A is used to inject sample. It is usually water with a small amount of organic acid (e.g., 0.01% formic acid (FA))
  - Solution B is mainly organic solvent (e.g., 90% [ACN], 0.01% FA)

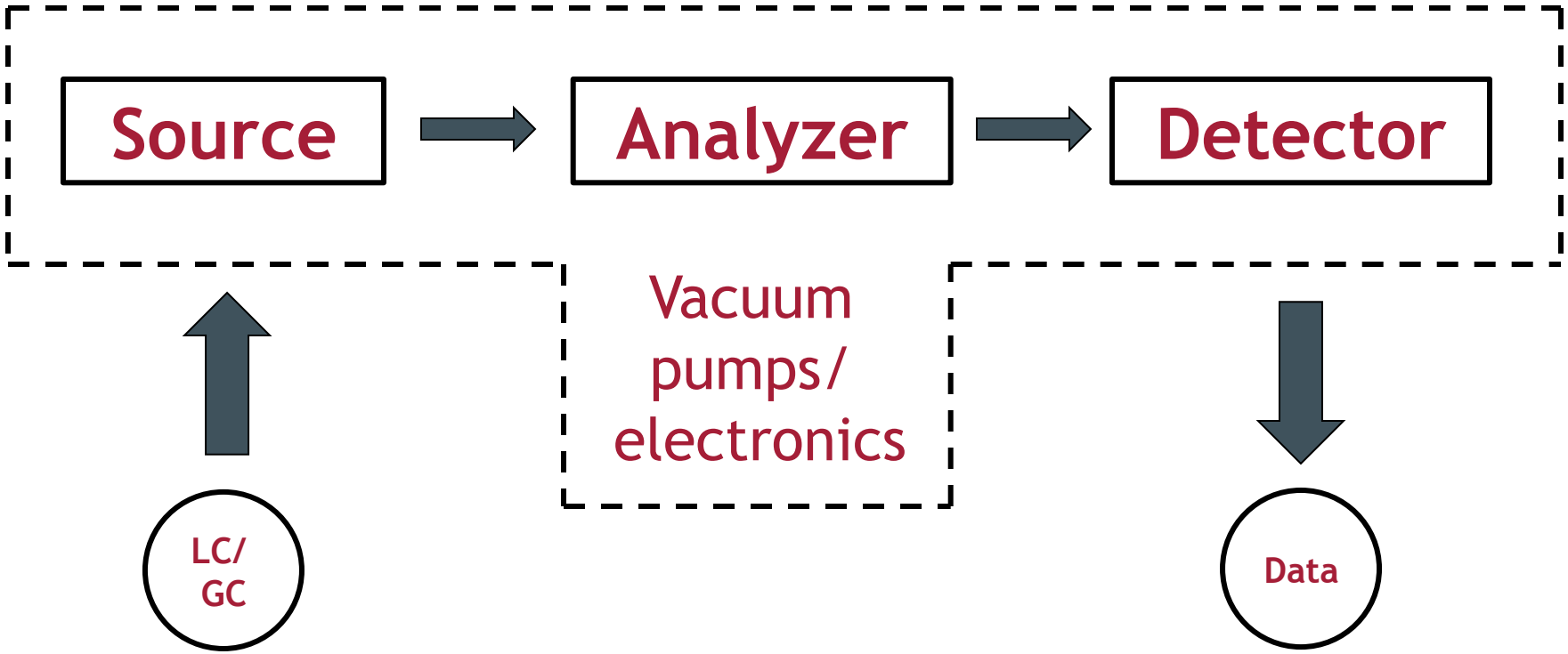
SCX (mainly for proteomics)

- Opposite charges attract each other
- Net charge of a peptide depends on pH
- Stationary phase SCX:
  - Surface modified by sulfonic acid groups (neg. charged at pH > 2-3)
- Peptides injected at low pH (~3), thus positively charged (cations)
- The more positive the charges – the stronger the interaction
- For elution, increase ionic strength within solution B (using salt)

Calculating retention time

- Exact calculation is difficult due to high number of parameter that influence retention time
- Prediction of retention using machine learning works well
  - Pfeifer et al., BMC Bioinformatics. 2007 Nov 30;8:468.
    -Support vector machines-
  - Oh et al., Bioinformatics. 2007 Jan 1;23(1):114-8. Epub 2006 Nov 8.
    -Artificial neural networks-

Schema Mass spectrometer



Ionization methods

- ElectroSpray Ionization (ESI) *-soft ionization-*
- Matrix Assisted Laser Desorption/ Ionization (MALDI)
  -soft ionization-
- Electron Impact (EI) *-hard ionization- (not suited for proteomics)*
- Other methods:
  - Particle bombardment; Field Desorption; Field Ionization;

The following (ionization) is partly based on education material from the Genetics department, Wisconsin University, USA, http://skop.genetics.wisc.edu/AhnaMassSpecMethodsTheory.ppt

Soft ionization methods

- Soft ionization techniques keep the molecule of interest fully intact
- Electro-spray ionization first conceived in 1960's by Malcolm Dole but put into practice in 1980's by John Fenn (Yale)
- MALDI first introduced in 1985 by Franz Hillenkamp and Michael Karas (Frankfurt)
- Made it possible to analyze large molecules via inexpensive mass analyzers such as quadrupole, ion trap and TOF

The Nobel Prize in Chemistry 2002

"for the development of methods for identification and structure analyses of biological macromolecules"

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"

John B. Fenn

1/4 of the prize

USA

Virginia Commonwealth

Koichi Tanaka

1/4 of the prize

Japan

Shimadzu Corp.
Kyoto, Japan

Kurt Wüthrich

1/2 of the prize

Switzerland

Eidgenössische Technische

The Nobel Prize in Chemistry 2002
Press Release
Advanced Information
Information for the Public
Presentation Speech
Illustrated Presentation

John B. Fenn
Nobel Lecture
Banquet Speech
Nobel Diploma
Prize Award Photo
Other Resources

Koichi Tanaka
Nobel Lecture
Interview
Nobel Diploma
Prize Award Photo
Other Resources

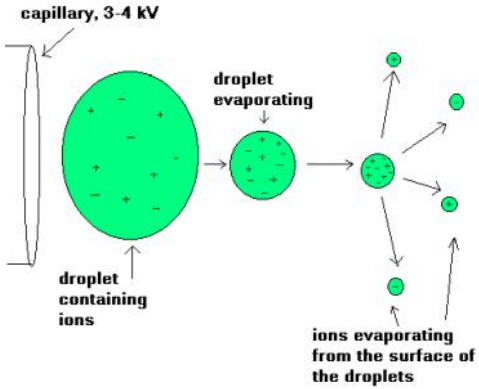
Kurt Wüthrich
Nobel Lecture
Interview
Nobel Diploma
Prize Award Photo
Educational
Other Resources

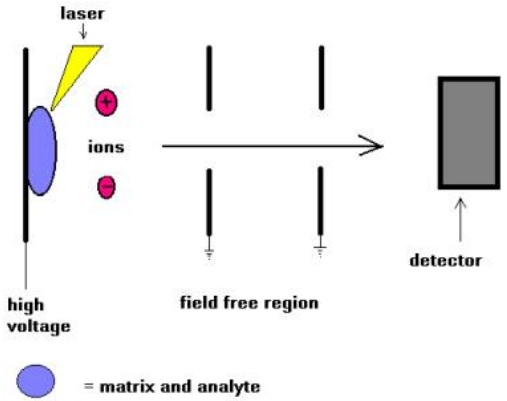
2001

The 2002 Prize in:
Physics
Chemistry
Physiology or Medicine

Soft ionization methods

- **Electrospray mass spectrometry (ESI-MS)**
  - Liquid containing analyte is forced through a steel capillary at high voltage to electrostatically disperse analyte. This induces charged droplets. Ions are formed by extensive evaporation





- **Matrix-assisted laser desorption ionization (MALDI)**
  - Analyte (protein) is mixed with large excess of matrix (small organic molecule)
  - Irradiated with short pulse of laser light. Wavelength of laser is the same as absorbance maximum of matrix.

Electrospray ionization

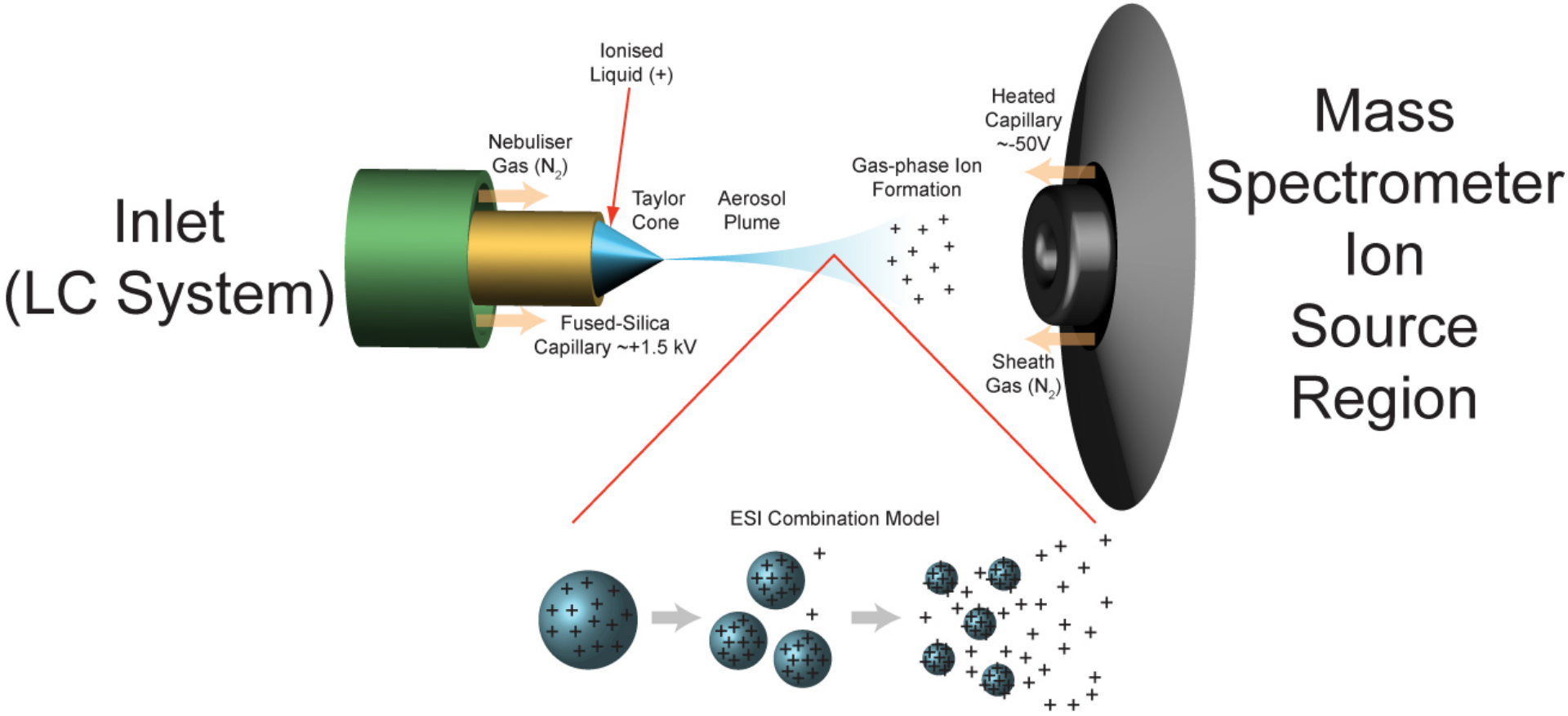
- Sample dissolved in polar, volatile buffer (no salts) and pumped through a stainless steel capillary (70 - 150 μm) at a rate of 10-100 μL/min (for nano spray this can also be at 200 nl/min)

- High voltage (3-4 kV) applied at tip along with flow of nebulizing gas causes the sample to "nebulize" or aerosolize

- Aerosol is directed through regions of higher vacuum until droplets evaporate to near atomic size (still carrying charges)

ESI

Electrospray Ionisation (ESI) and Ion Source Overview



ESI

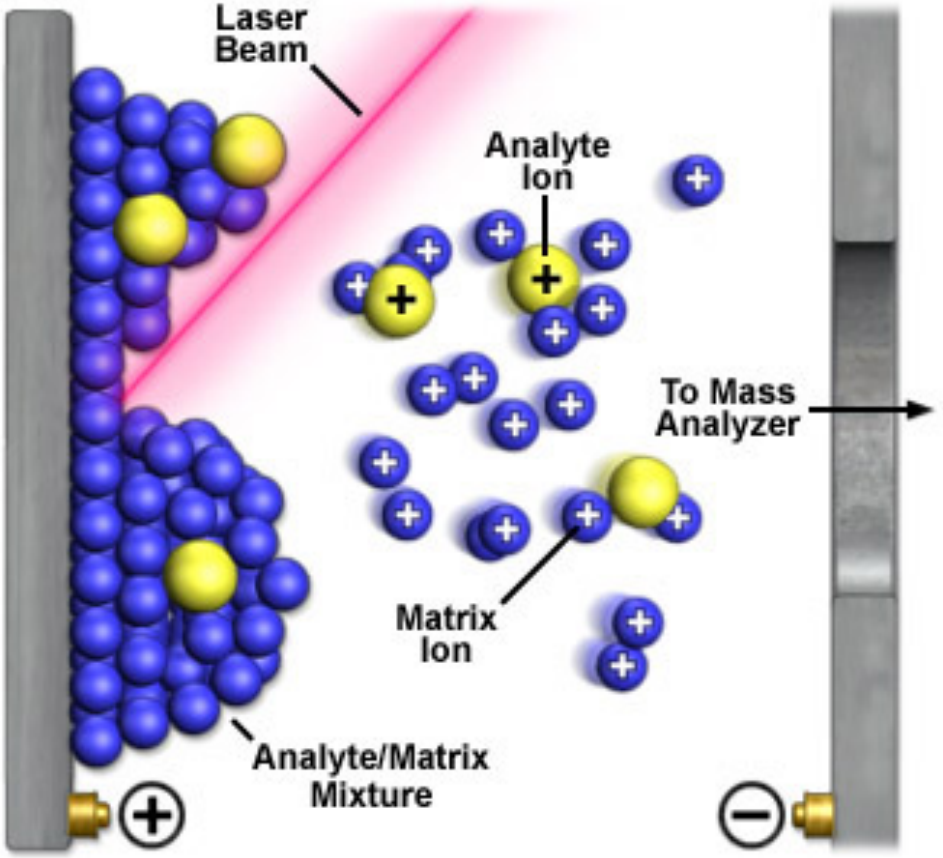
- Very sensitive technique, requires less than a picomole of material
- Strongly affected by salts and detergents
- Positive ion mode measures $(M + H)^+$ (add formic acid to solvent)
- Negative ion mode measures $(M - H)^-$ (add ammonia to solvent)

Positive or negative mode

- Functional groups that readily accept H^+ (such as amide and amino groups found in peptides and proteins) can be ionized using positive mode ESI.

- Functional groups that readily lose a proton (such as carboxylic acids and hydroxyls as found in nucleic acids and sugars) should be ionized using negative mode ESI

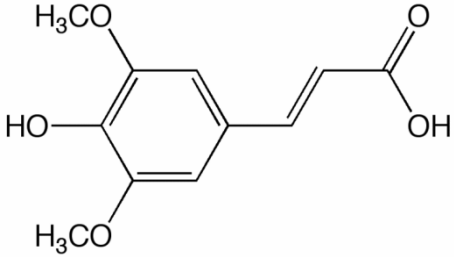
MALDI


Laser
Beam
Analyte
Ion
To Mass
Analyzer
Matrix
Ion
Analyte/Matrix
Mixture

MALDI

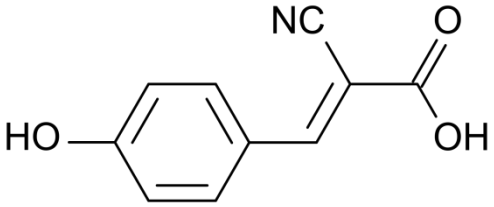
- Sample is ionized by bombarding sample with laser light
- Sample is mixed with a UV absorbant matrix (sinapinic acid for proteins, 4-hydroxycinnamic acid for peptides)
- Light wavelength matches that of absorbance maximum of matrix so that the matrix transfers some of its energy to the analyte (leads to ion sputtering)

Sinapinic acid



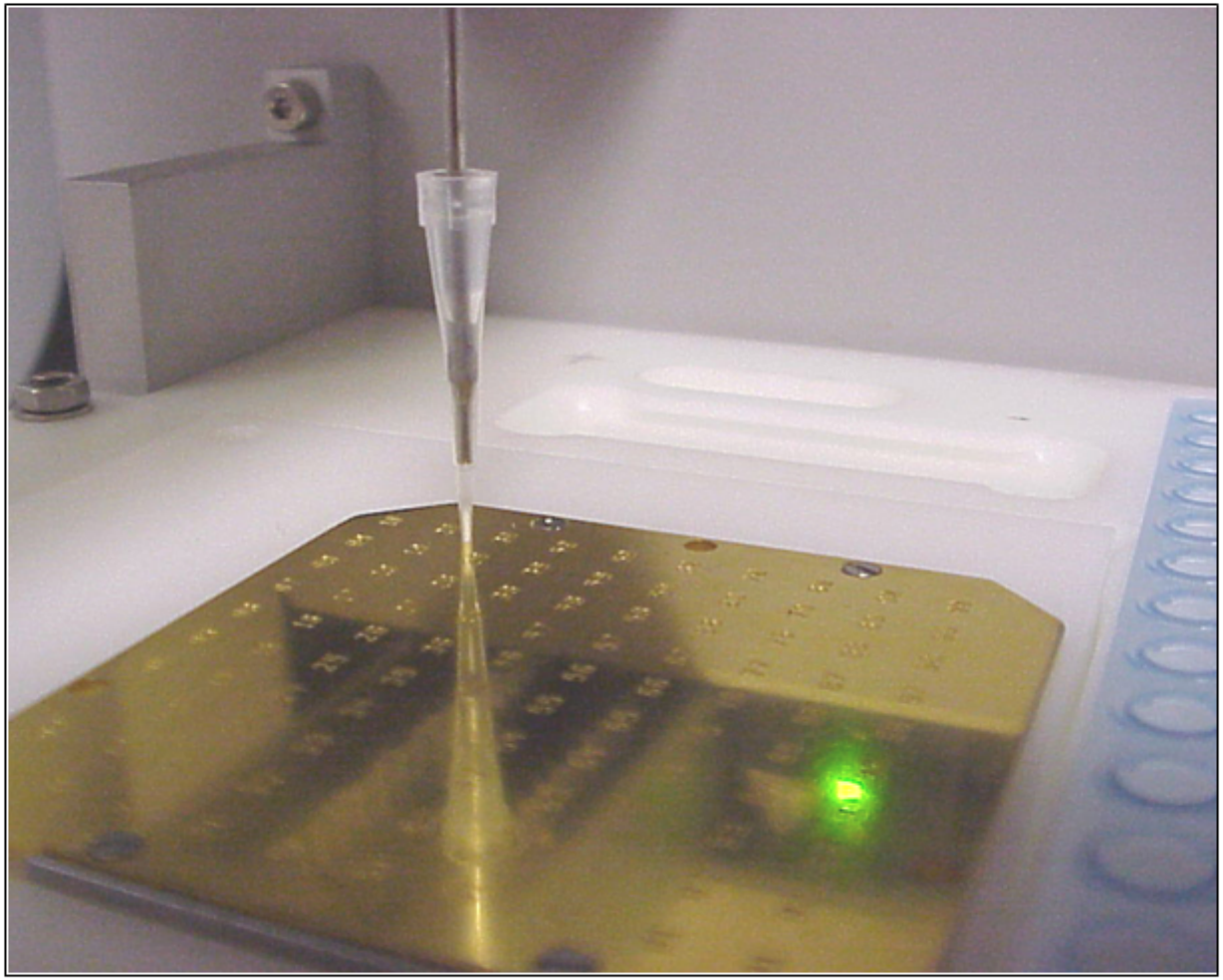
http://upload.wikimedia.org/wikipedia/commons/6/6f/Sinapinic_acid.gif

4-hydroxycinnamic acid

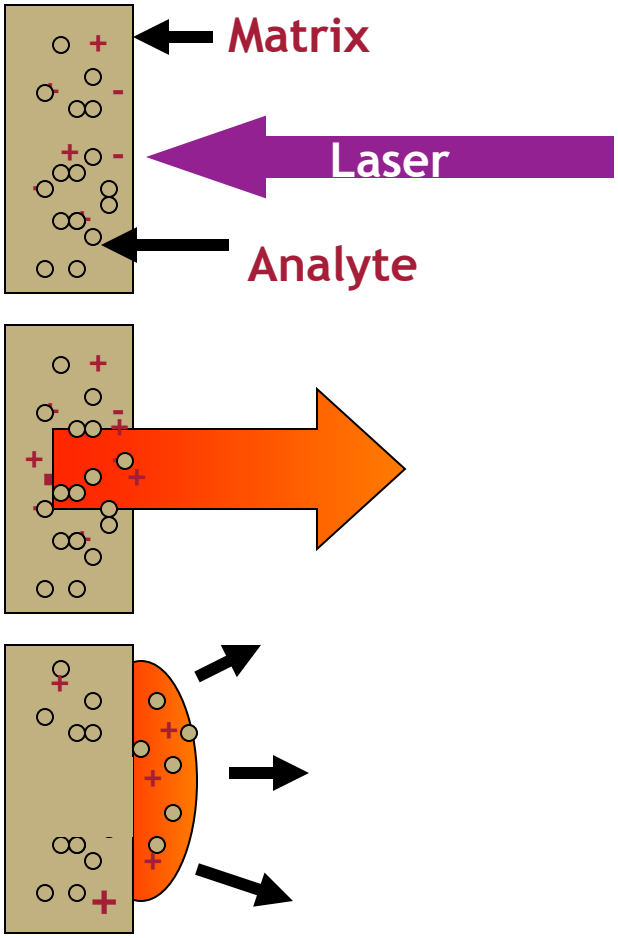


https://commons.wikimedia.org/wiki/File:%CE%91-cyano-4-hydroxycinnamic_acid.svg

Spotting on a MALDI plate



MALDI ionization



- Absorption of UV radiation by chromophoric matrix and ionization of matrix
- Dissociation of matrix, phase change to super-compressed gas, charge transfer to analyte
- Expansion of matrix, analyte trapped in expanding matrix plume (explosion/"popping")

MALDI

- Unlike ESI, MALDI generates spectra that have just a singly charged ion
- Positive mode generates ions of $(M + H)^+$
- Negative mode generates ions of $(M – H)^-$
- Generally more robust that ESI (tolerates salts and nonvolatile components)
- Easier to use and maintain, capable of higher throughput
- Requires 10 μL of 1 pmol/μL sample

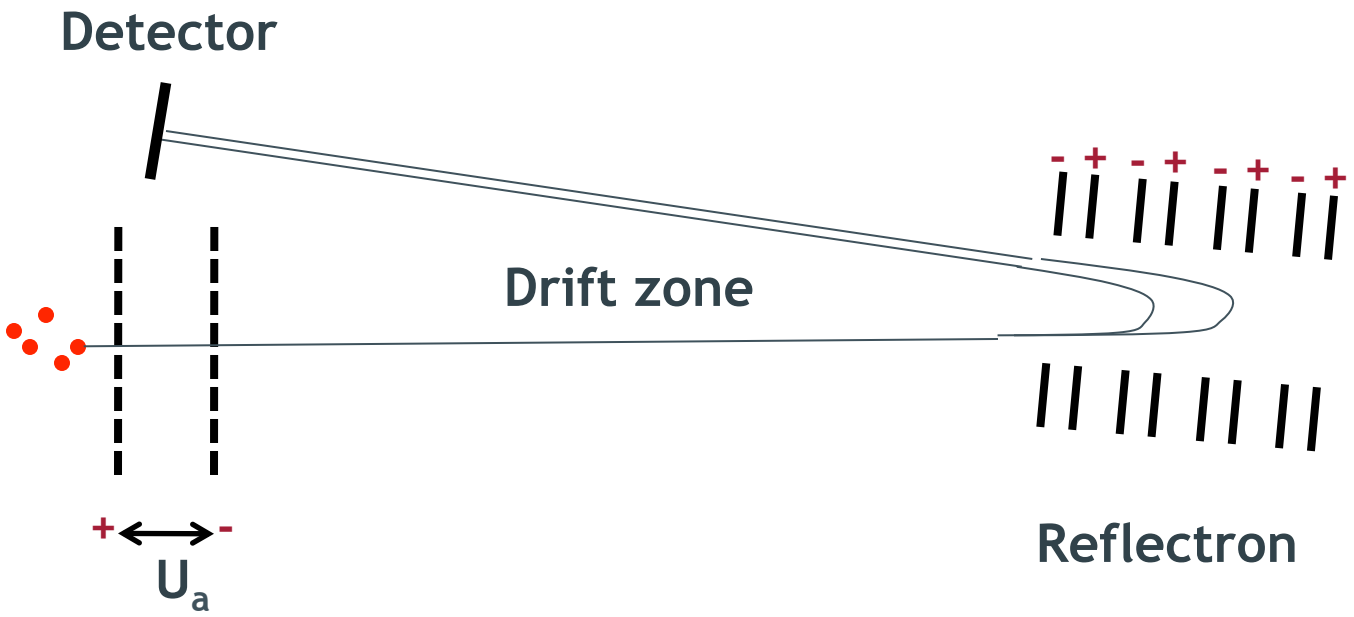
Mass analyzers

- Main operations:
  - Separate peptides
  - Selection of ions (within appropriate m/z)
  - (Fragmentation of selected precursor ions)
  - Measure the m/z of ions

Mass analyzers

- TOF
- Ion trap
- Quadrupole
- Orbitrap
- …

Mass Analyzer: Time of Flight

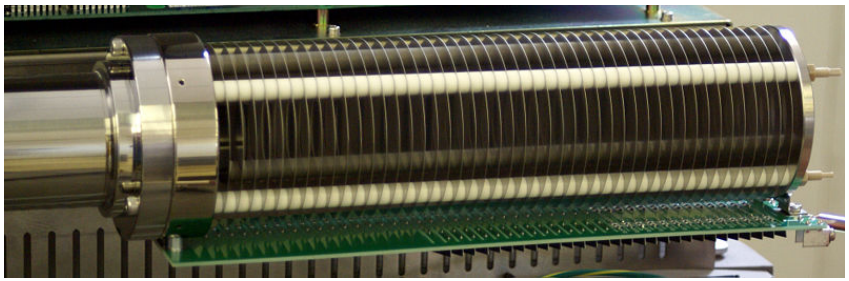


Time-of-flight mass analyzer (TOF):

- Ions are extracted from the ion source through an electrostatic field in pulses in a field-free drift zone
- An ‘electrostatic mirror’ (reflectron) reflects the ions back onto the detector
- Detector counts the particles and records the time of flight between extraction pulse and a particle hitting the detector

Mass Analyzer: Time of Flight

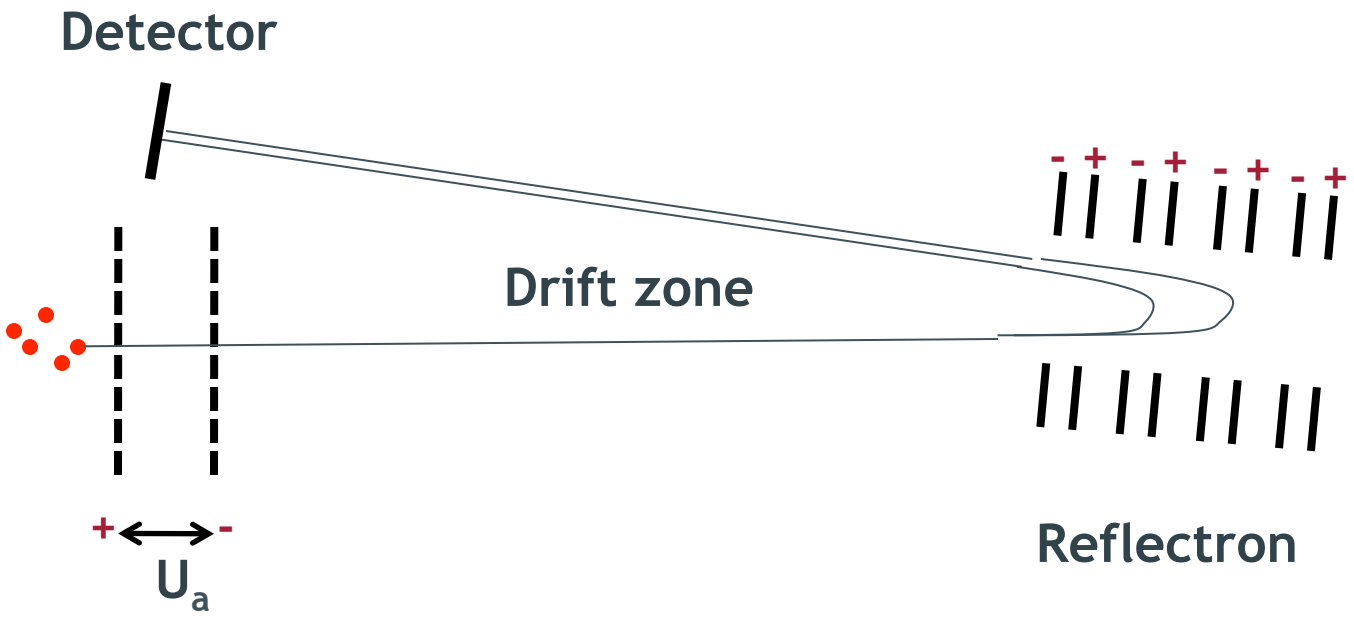
- **Drift tubes** have sizes of over a meter in real-world instruments
- A **reflectron** doubles the drift length, and thus the instrument's resolution
- It also focuses the ions onto the detector



Reflectron

http://www.biochem.mpg.de/nigg/research/koerner/instruments/absatz_pic_reflexIII.jp
http://upload.wikimedia.org/wikipedia/commons/thumb/d/d8/Reflectron.jpg/800px-Reflectron.jp

Mass Analyzer: Time of Flight



- The kinetic energy transferred to the ions depends on the acceleration voltage U_a and the particle's charge
- Lighter particles fly faster that heavier particles of the same charge
- Hence, they arrive later at the detector
- The time of flight is thus a measure of the particle's mass

Mass Analyzer: Time of Flight

- Energy transferred to an ion with charge q accelerated by an electrostatic field with acceleration voltage U_a:

$$E_{pot} = qU_a$$

- This energy is obviously converted into kinetic energy as the ion accelerates:

$$E_{kin} = \tfrac{1}{2}\, mv^2 = qU_a$$

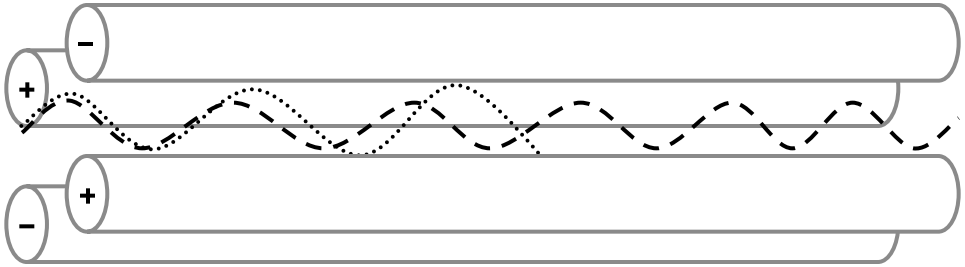
- For a given path length s from extraction to detector, the time of flight t is thus

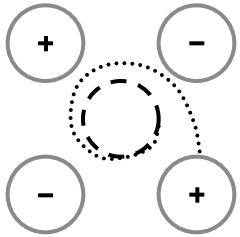
$$t = s / v$$

- Time of flight for a given path length and acceleration voltage, which are instrument parameters, depends on the ion's charge and mass only

Mass Analyzer: Quadrupole

- Oscillating electrostatic fields stabilize the flight path for a specific mass-to-charge ratio – these ions will pass through the quadrupole
- Ions with different *m/z* will be accelerated out of the quadrupole
- Changing the frequency allows the selection of a different *m/z*





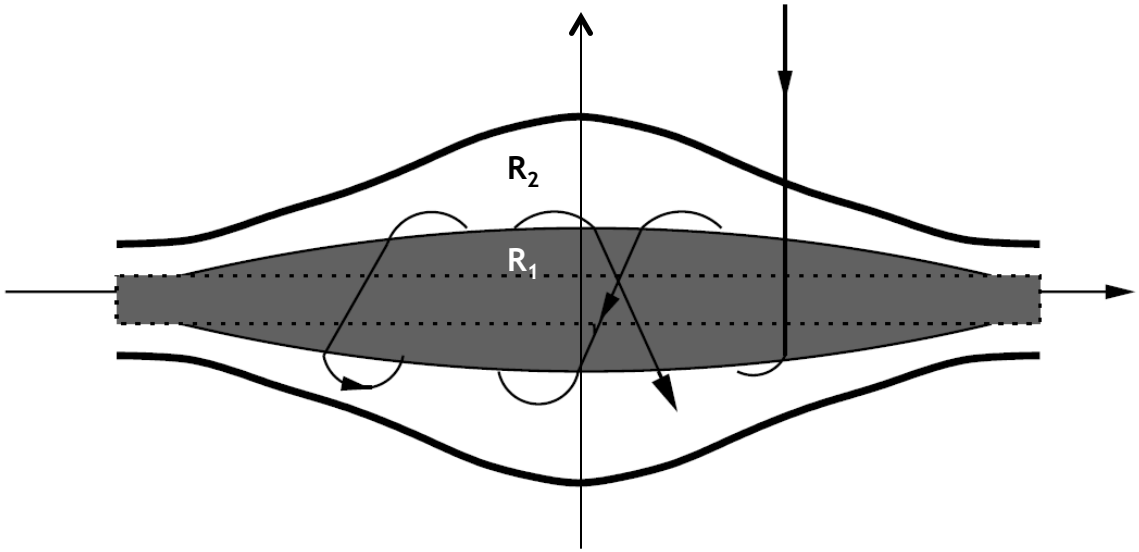
--- stable ion path

····· unstable ion path

Ion Trap

- Ions are captured in a region of a vacuum system or tube
- Trapping of ions is based on a combination of magnetic and electric fields
- There is a long history of ion trapping and a variety of different technologies have emerged over the years
  - Penning trap
  - Paul trap
  - Kingdon trap

Orbitrap (based on Kingdon trap)



- Outer and inner coaxial electrode with radii R_2 and R_1, respectively
- Electrostatic field
- Ions form harmonic oscillation along the axis of the electrostatic field
- The harmonic oscillator with frequency ω is used to determine m/z, with $\omega = \sqrt{kz/m}$, where *k* is a constant

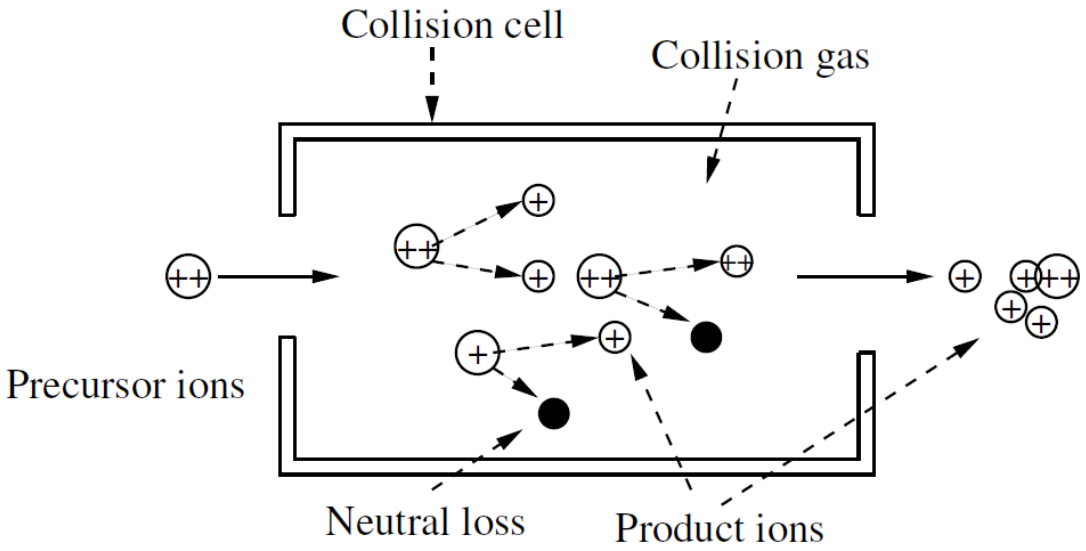
Tandem MS

- Uses two mass-to-charge measurements to analyze *precursor* and *product* ions
- Fragmentation is used to dissociate the analytes into smaller fragments
- MS/MS capable instruments
  - Same mass analyzers are used: *in-time set-up*
  - Different analyzers are used (hybrid instruments): *in-space set-up*

Tandem MS fragmentation methods

- Different fragmentation techniques
  - **Collision-Induced-Dissociation (CID)**
  - Pulsed Q Dissociation (PQD)
  - Electron transfer dissociation (ETD)
  - Electron capture dissociation (ECD)

Collision-induced dissociation



- Two colliding molecules
- Fragmentation is performed in collision cell
- Inert collision gas (e.g., Ar, He) is used for collision
- Precursor that reaches energy threshold will fragment into products and/or neutral losses
- Typical settings: high (>1000 eV) or low energy (<100 eV) CID
- Peptides are fragmented at the peptide bond !

Hybrid mass spectrometer

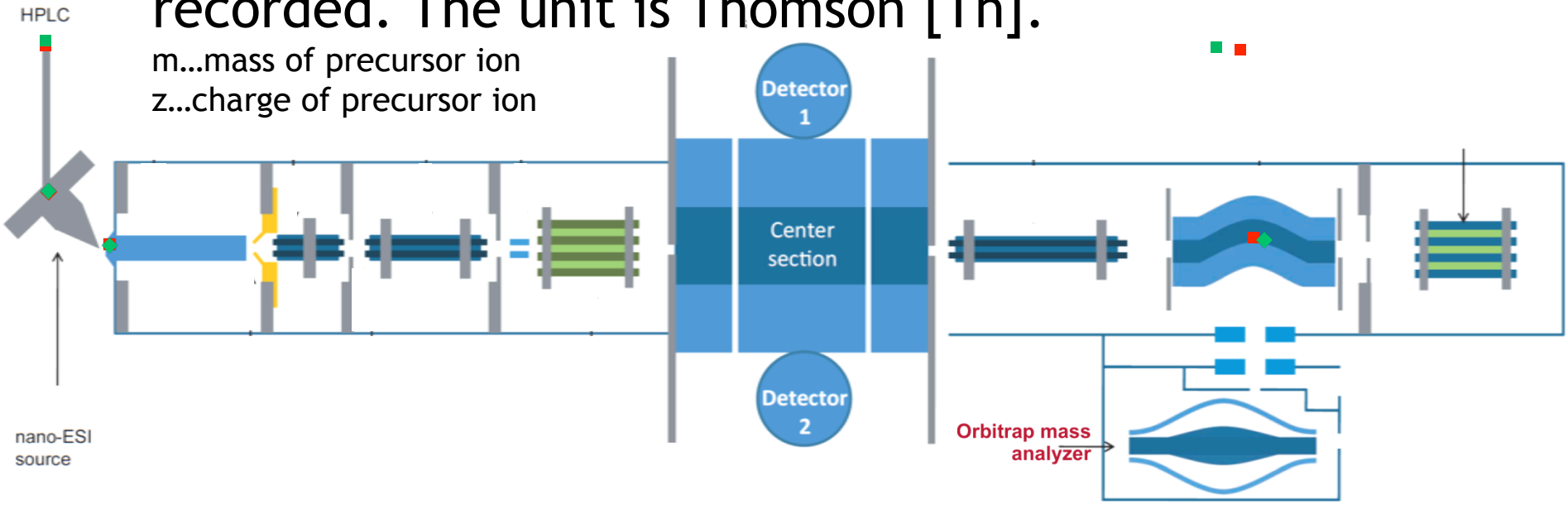
Different hybrid mass spectrometers are used for different applications. The most frequently used combinations are

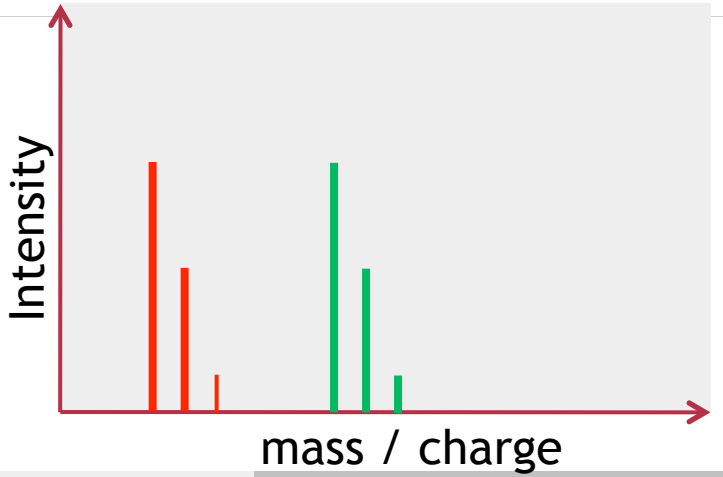
- Q-TOF
- Q-Trap
- **LTQ (linear ion trap)- Orbitrap**

LTQ-Orbitrap – MS

Mass-to-charge ratios (**m/z**) are recorded. The unit is Thomson [Th].

m…mass of precursor ion
z…charge of precursor ion

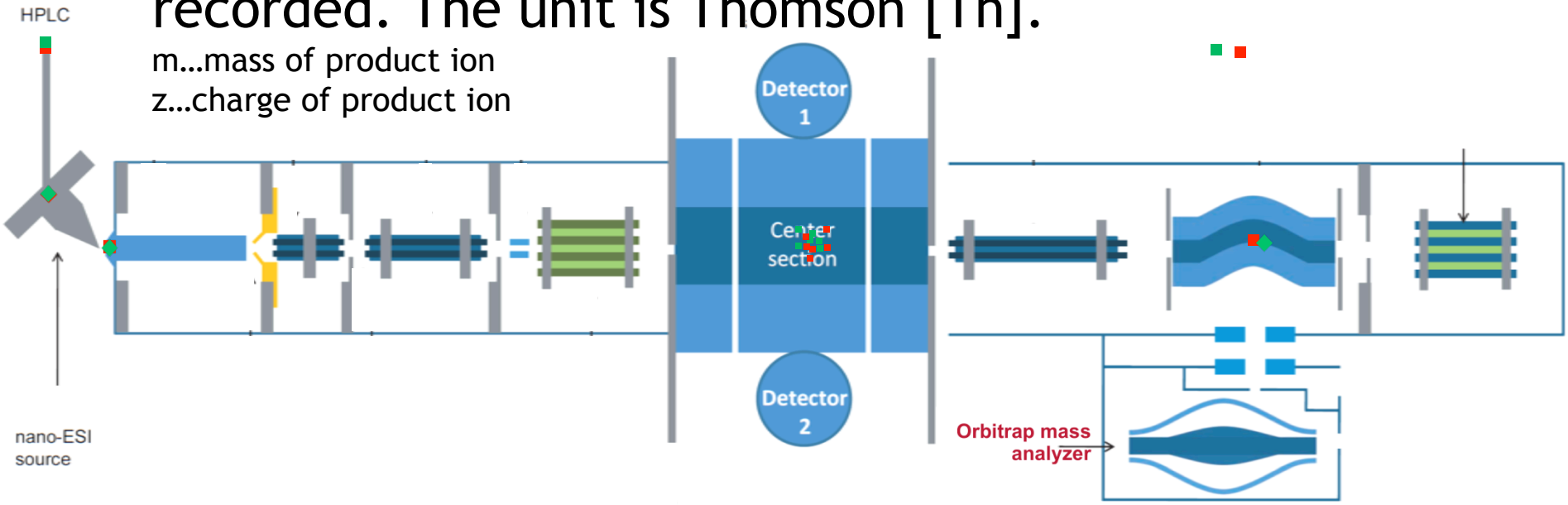


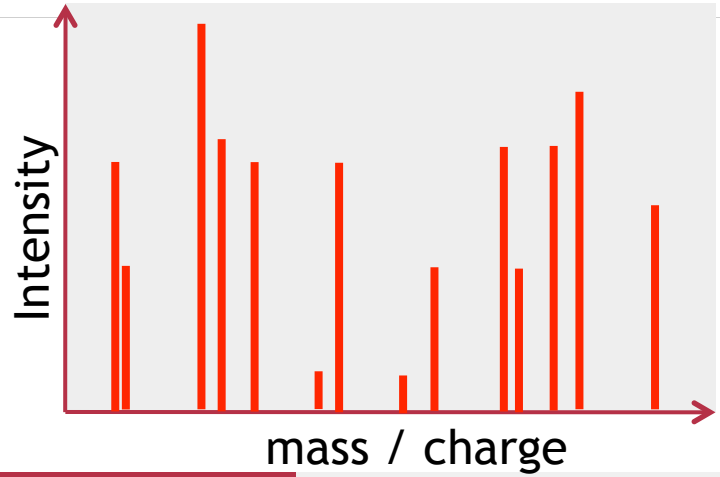


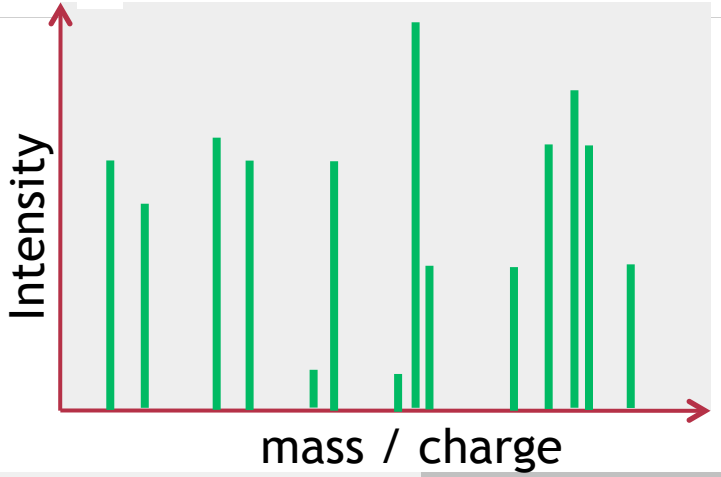
LTQ-Orbitrap – MS/MS

Mass-to-charge ratios (**m/z**) are recorded. The unit is Thomson [Th].

m…mass of product ion
z…charge of product ion

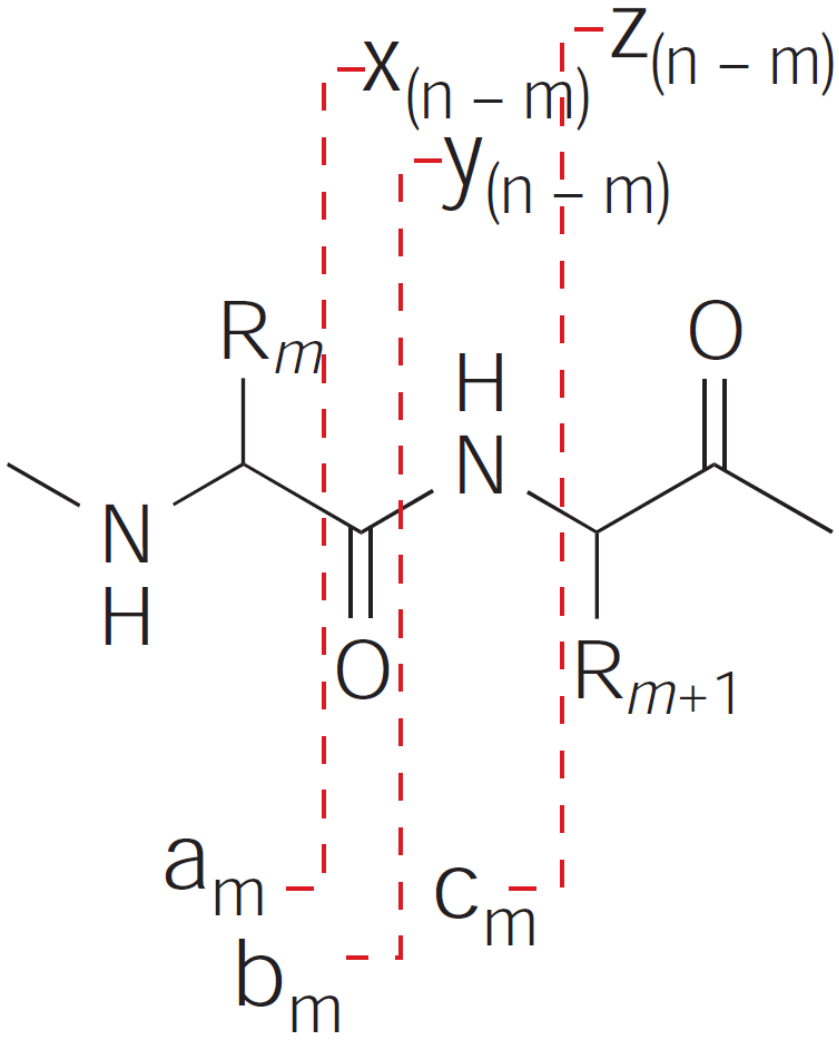






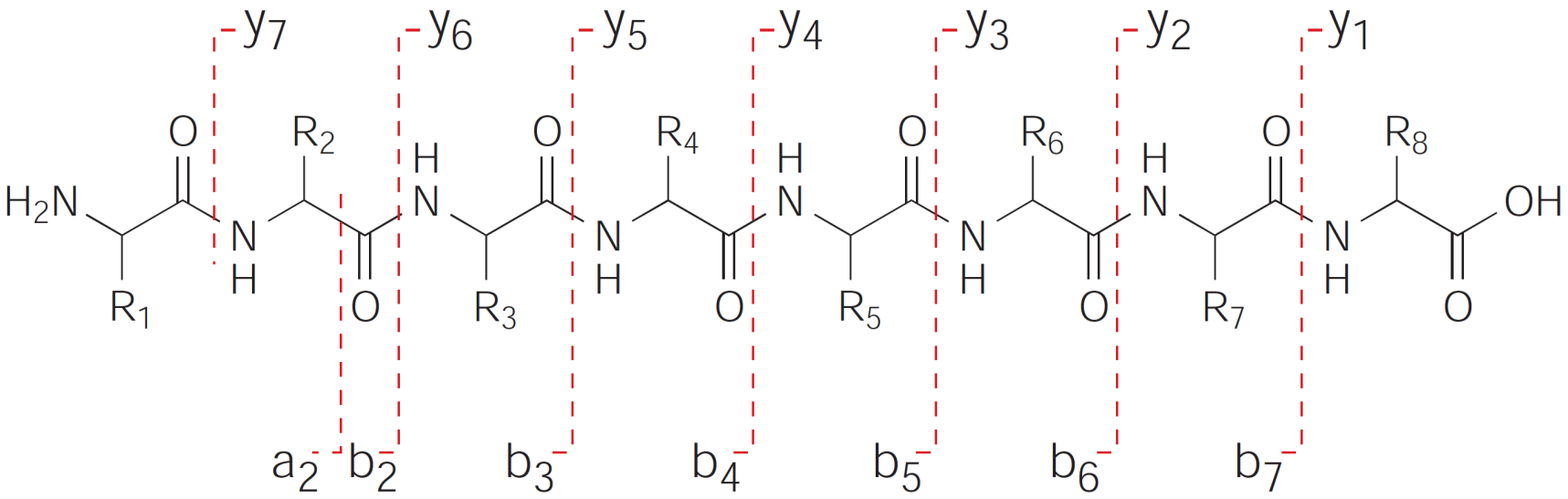
Product ion generation

A peptide of length *n* can potentially give rise to a,b,c and x,y,z ions. This example shows the fragments that can be produced between amino acids R_m and R_{m+1}



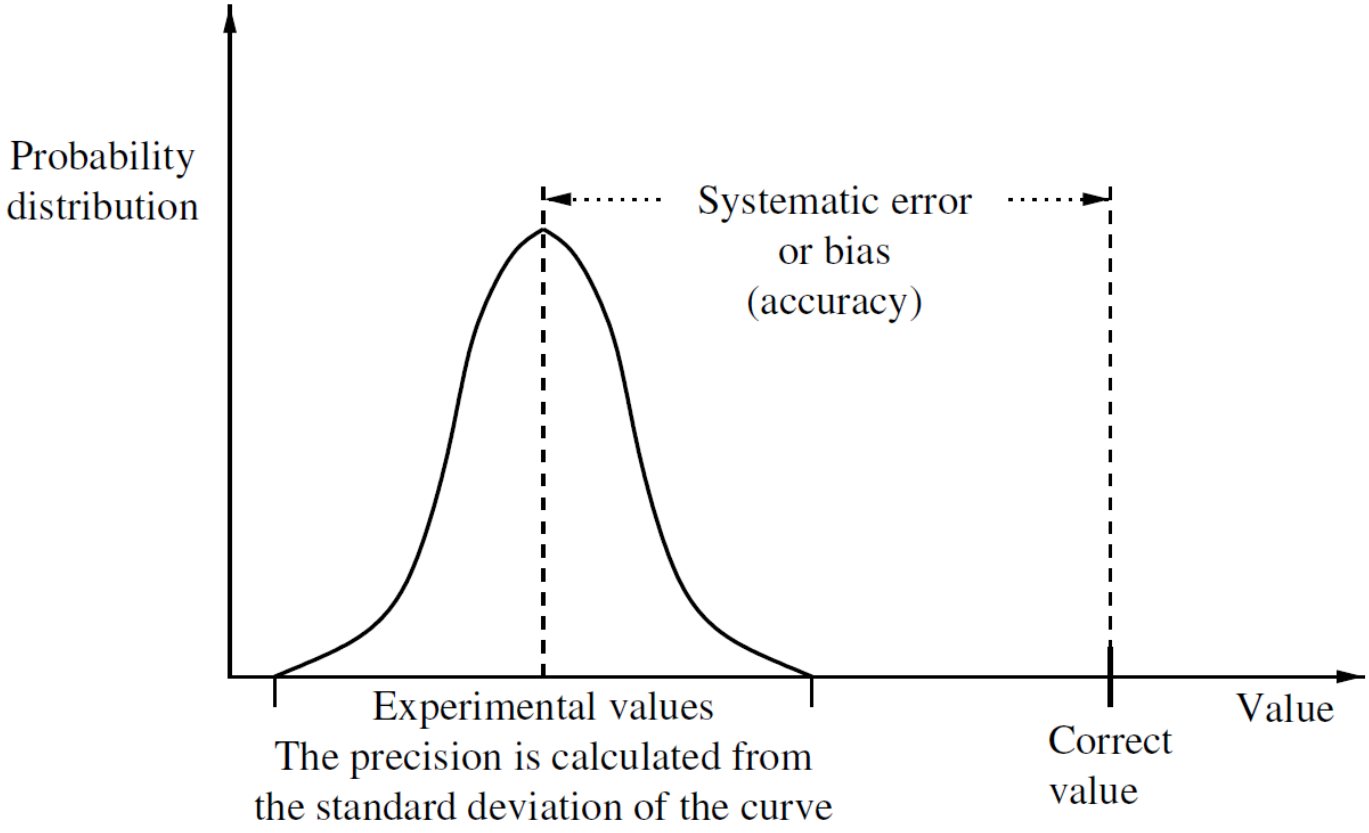
b/y ions in CID

CID fragmentation predominately produces b and y ions



Accuracy vs. precision …

… (of a mass measurement)

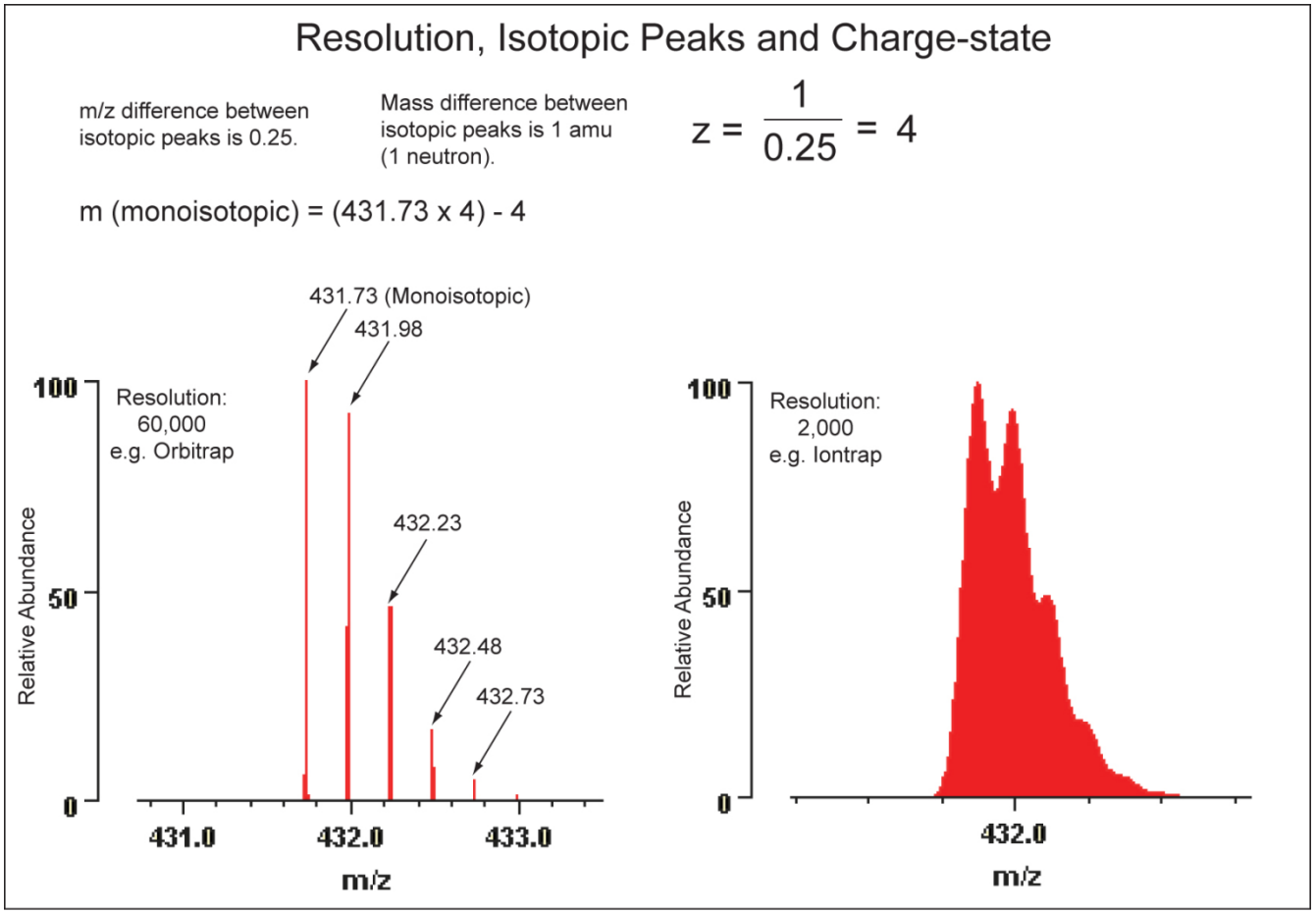


Resolution

(peak width definition)

Resolution:= For a single peak made up of singly charged ions at mass m in a mass spectrum, the resolution may be expressed as $m/\Delta m$, where Δm is the width of the peak at a height which is a specified fraction of the maximum peak height. It has been standardized to use 50% of the maximum peak height. FMWH (Full Width at Half Maximum) is commonly used. Note that resolution is dimensionless. Furthermore, in proteomics it has become common to report the resolution for ions at 400 Th.

Charge states



- Charge state determination is easy if the resolution is high enough
- For low resolution data this can become difficult

Sources

- Eidhammer et al., Computational Methods for Mass Spectrometry Proteomics. Wiley. 2007.