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Proteomics for Biocompatibility Testing of Implant Materials

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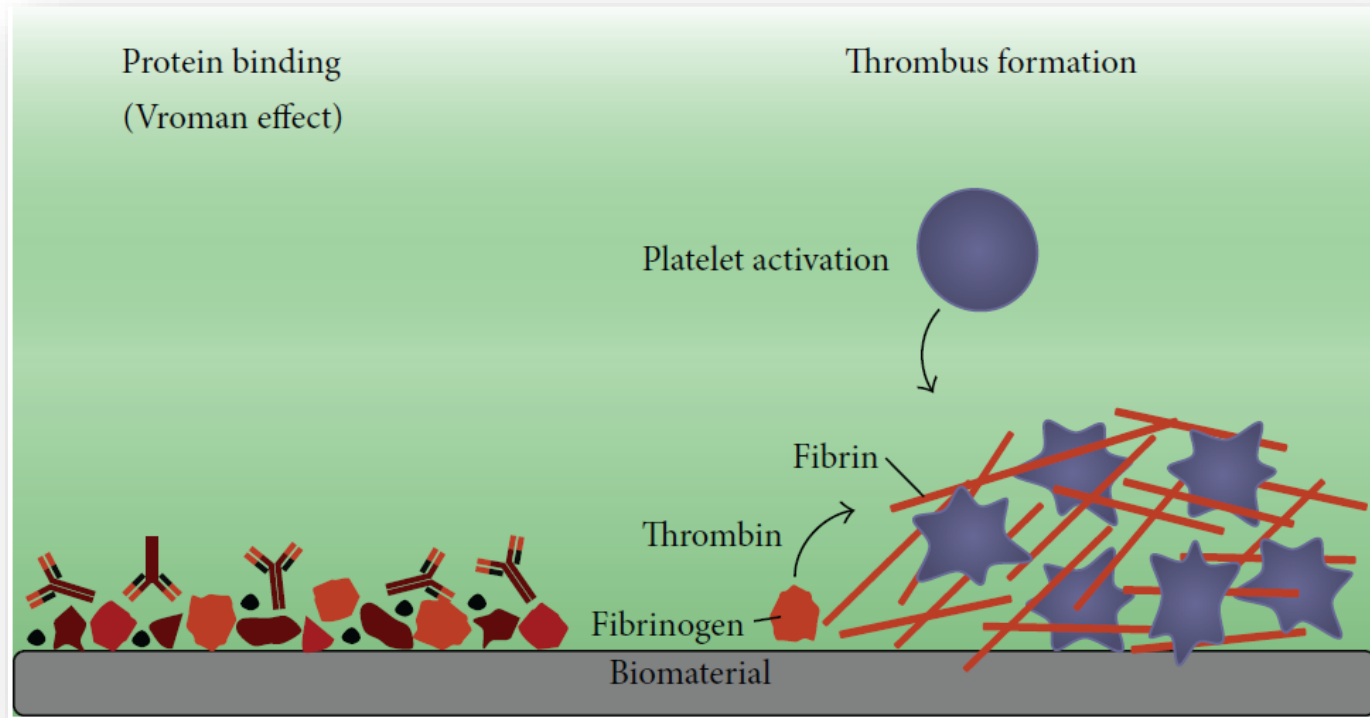


- **Implants**
 - Organism-implant interplay
- **Proteomics and its use in implantology**
 - What is proteomics
 - How can we detect proteins
 - Protein quantification
 - Application of proteomics for implantology

Implants and Organism – Implant Interplay

Foreign Body Reaction (FBR) Phase 1

Protein adsorption / deadsorption

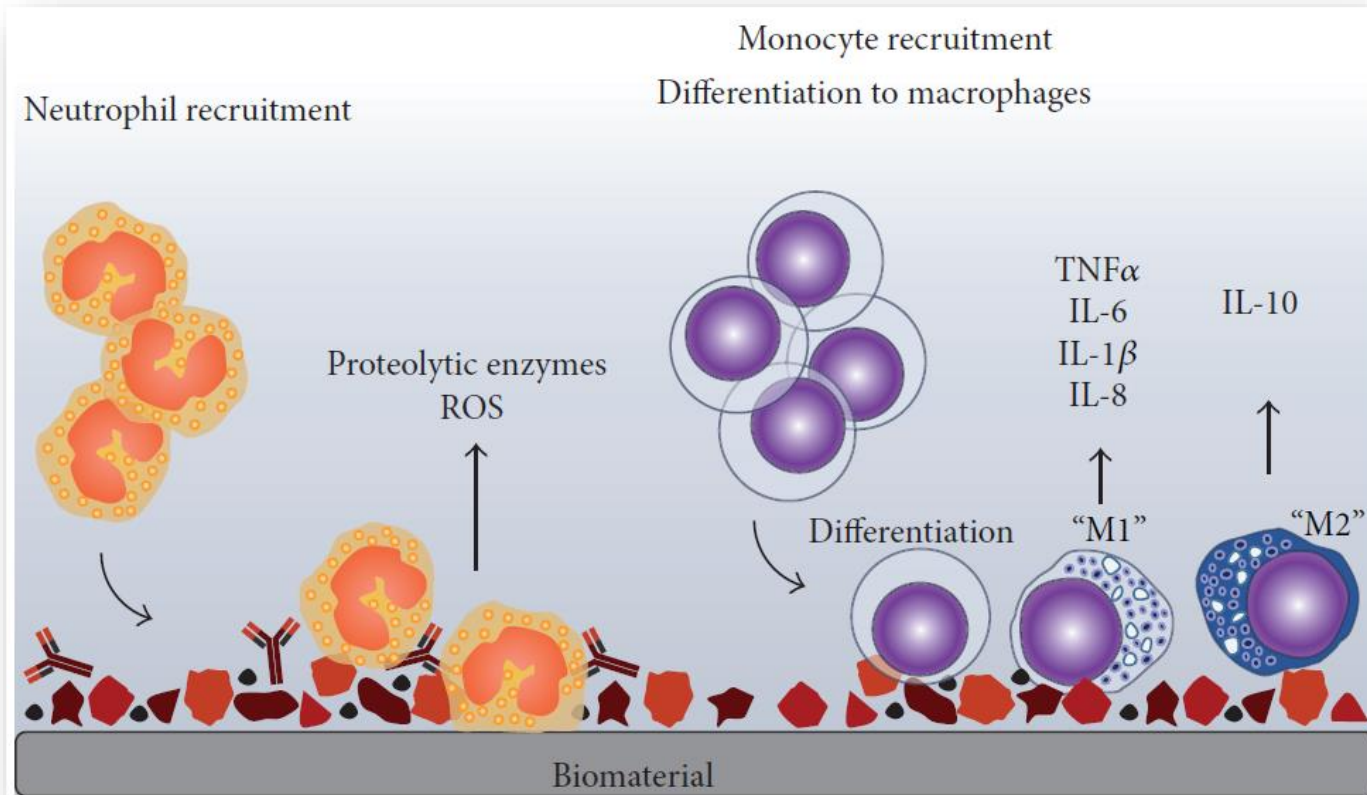


albumin, fibrinogen, fibronectin, vitronectin, gamma globulin, complement, immunomodulatory proteins

Christo et al., BioMed Research International
Volume 2015, <http://dx.doi.org/10.1155/2015/342304>

Foreign Body Reaction (FBR) Phase 2

Acute inflammation



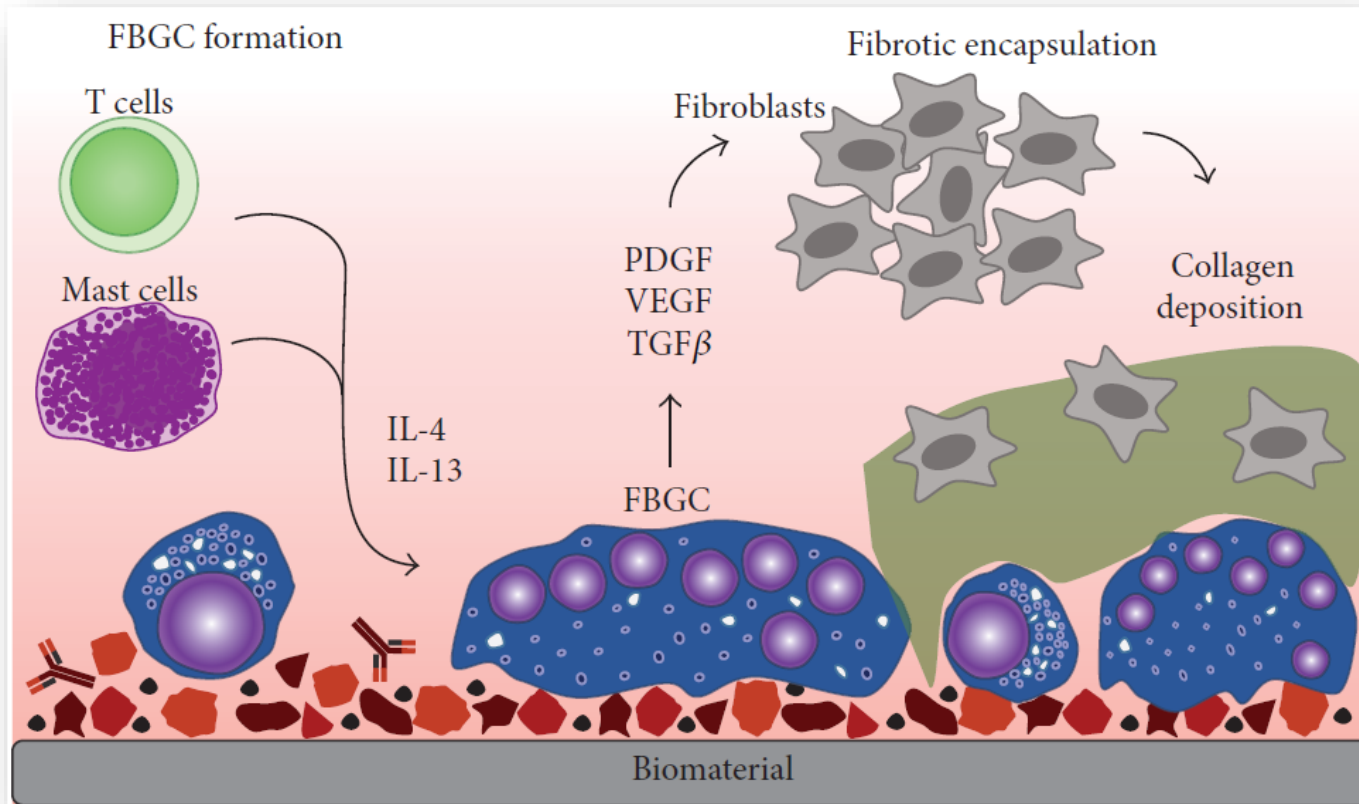
neutrophil extracellular traps
(NETs)

"frustrated" phagocytosis

Christo et al., BioMed Research International
Volume 2015, <http://dx.doi.org/10.1155/2015/342304>

Foreign Body Reaction (FBR) Phase 3

Chronic inflammation



foreign body giant cell

Christo et al., BioMed Research International
Volume 2015, <http://dx.doi.org/10.1155/2015/342304>

The Proteome and Challenges in Proteome Analysis

Defintion

The Proteome (Keith Williams and Marc Wilkins, 1994)

"The protein equivalent to the genome"

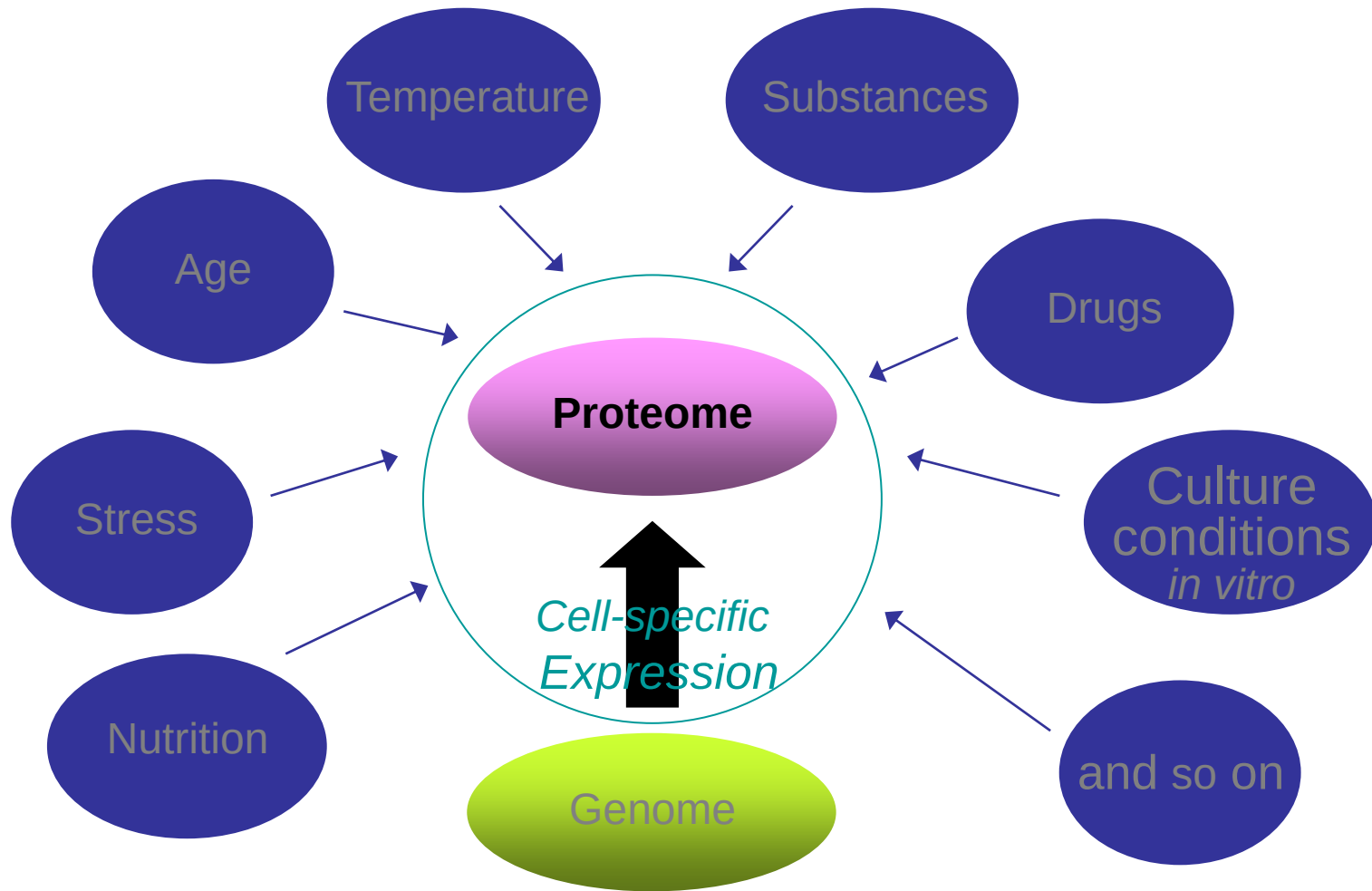
The proteome describes the **entirety of proteins** of an organism at a **certain point in time and in a certain state**.

*In contrast to the genome, the proteome is not static but **highly dynamic***

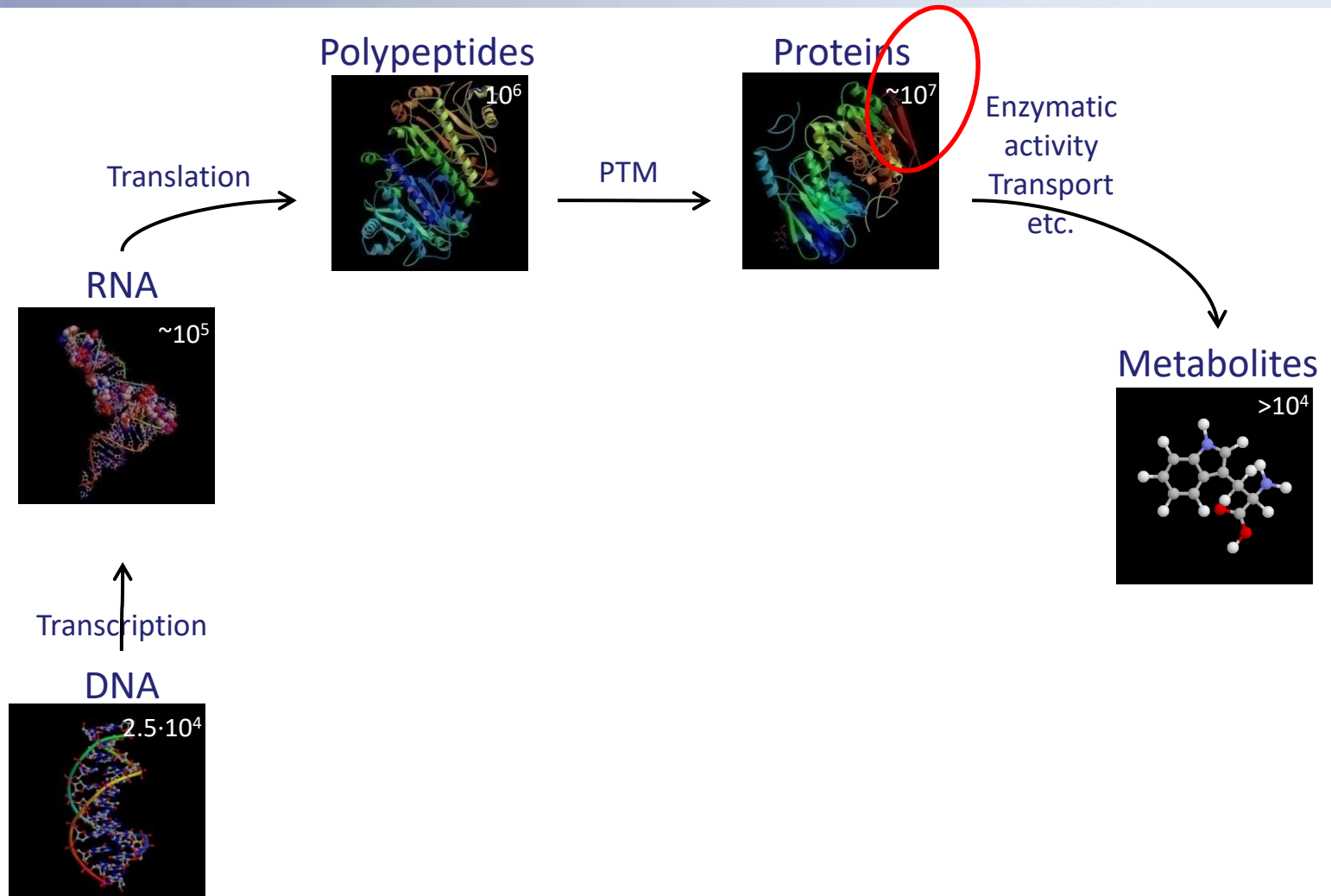
***One species,
the same genome,
different proteome(s)***



The proteome influenced by the environment

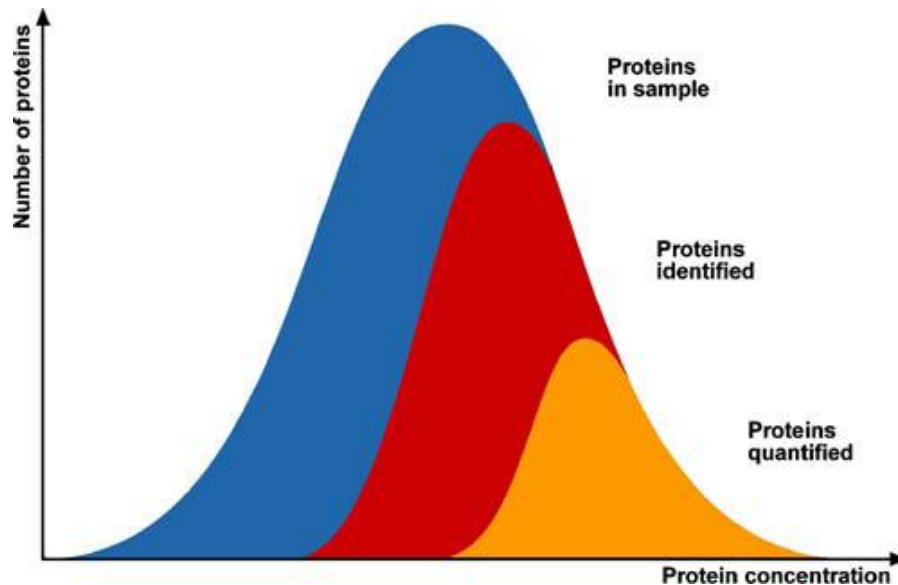


The Analytical Challenge

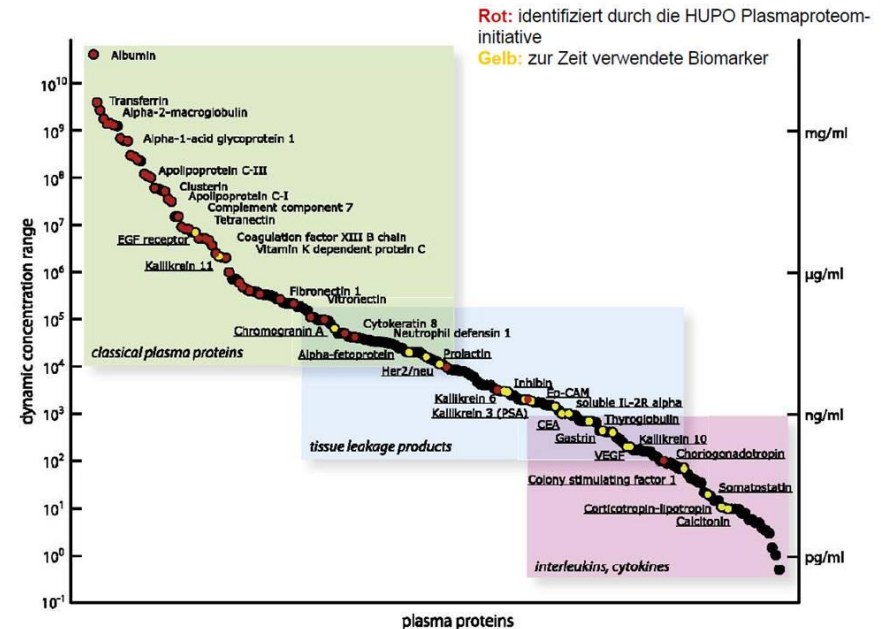


More analytical challenges

➤ Huge dynamic range (10 orders of magnitude)

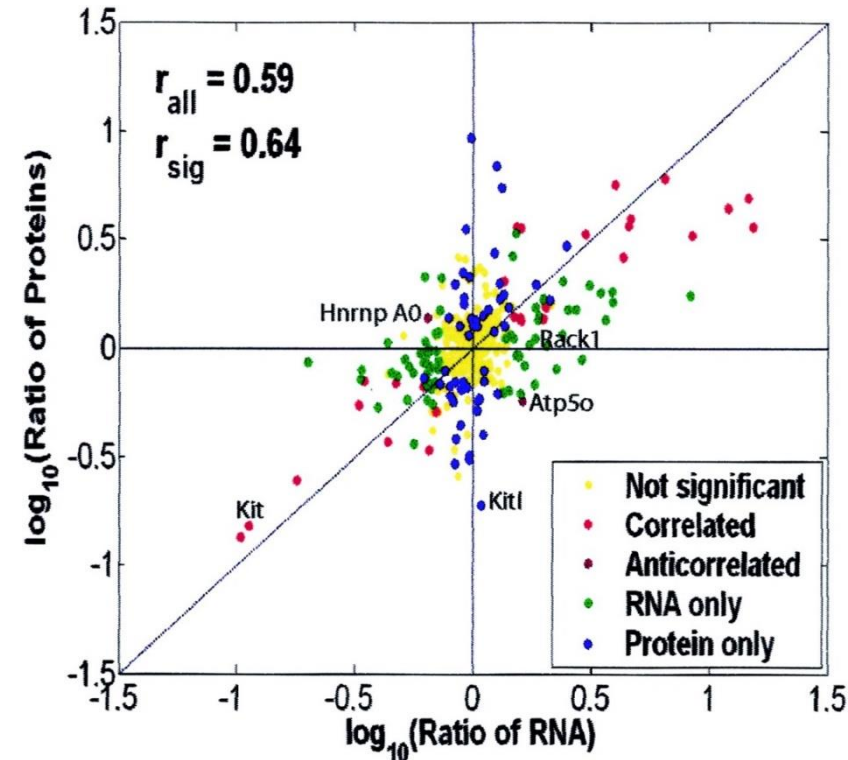


Banschkeff et al., Anal. Bioanal. Chem., 2007



RNA versus protein analysis

mRNA versus protein expression



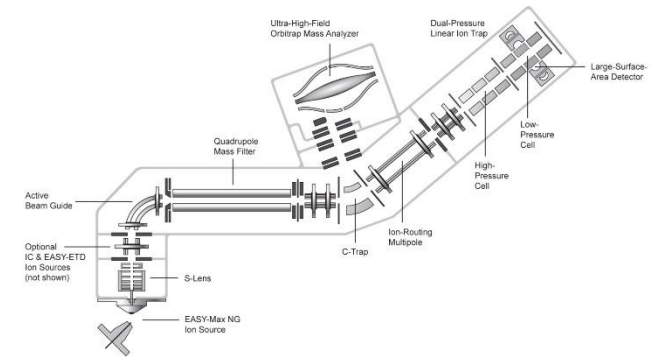
Tian Q et al. Mol Cell Proteomics
2004;3:960-969

Sub-proteome analysis:

- Proteomes of organelles (mitochondria, cilia, outer segments of photoreceptors,...)
- Body fluids
- Protein complexes
- ...

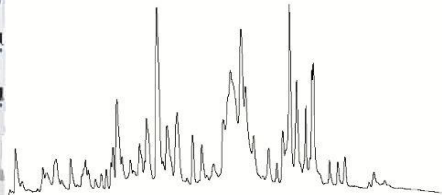
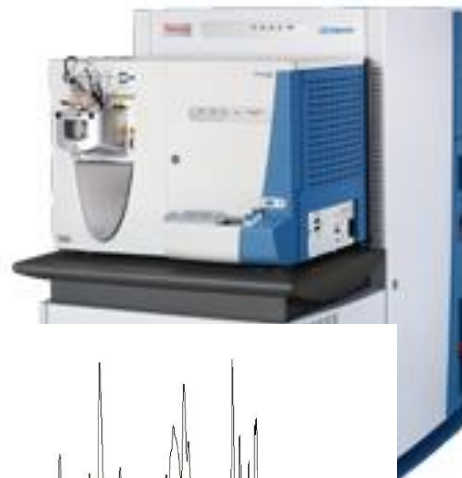
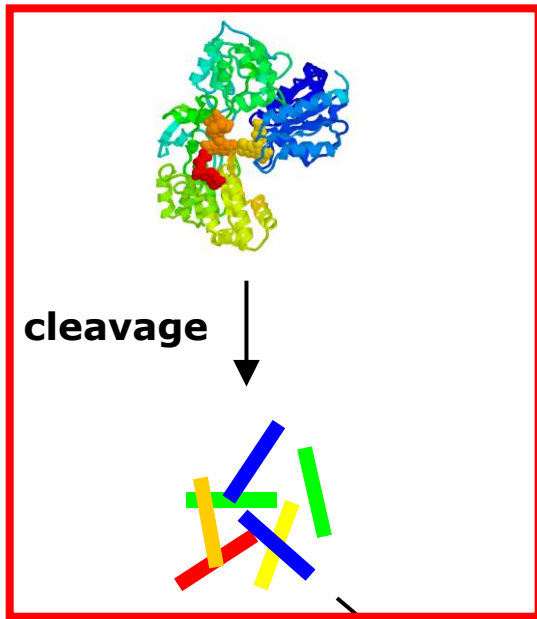
Post-translational modifications:

- Phosphorylation
- Acetylation
- Sumoylation
- ...

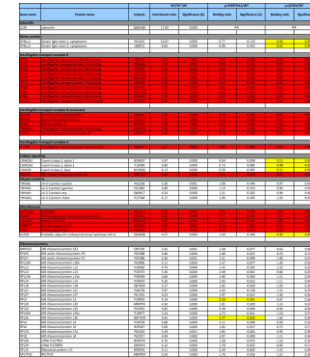


Mass spectrometry based proteomics

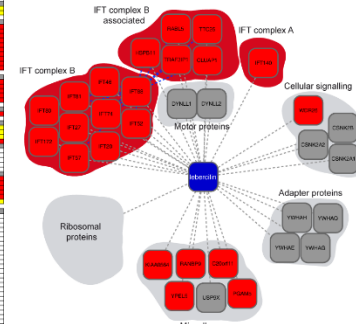
Mass spectrometry based proteomics workflow



Protein identification/quantification



A screenshot of a data table used for protein identification and quantification. The table has multiple columns, including protein names and various numerical values. Several rows are highlighted in red, indicating specific protein identifications or significant quantification results.



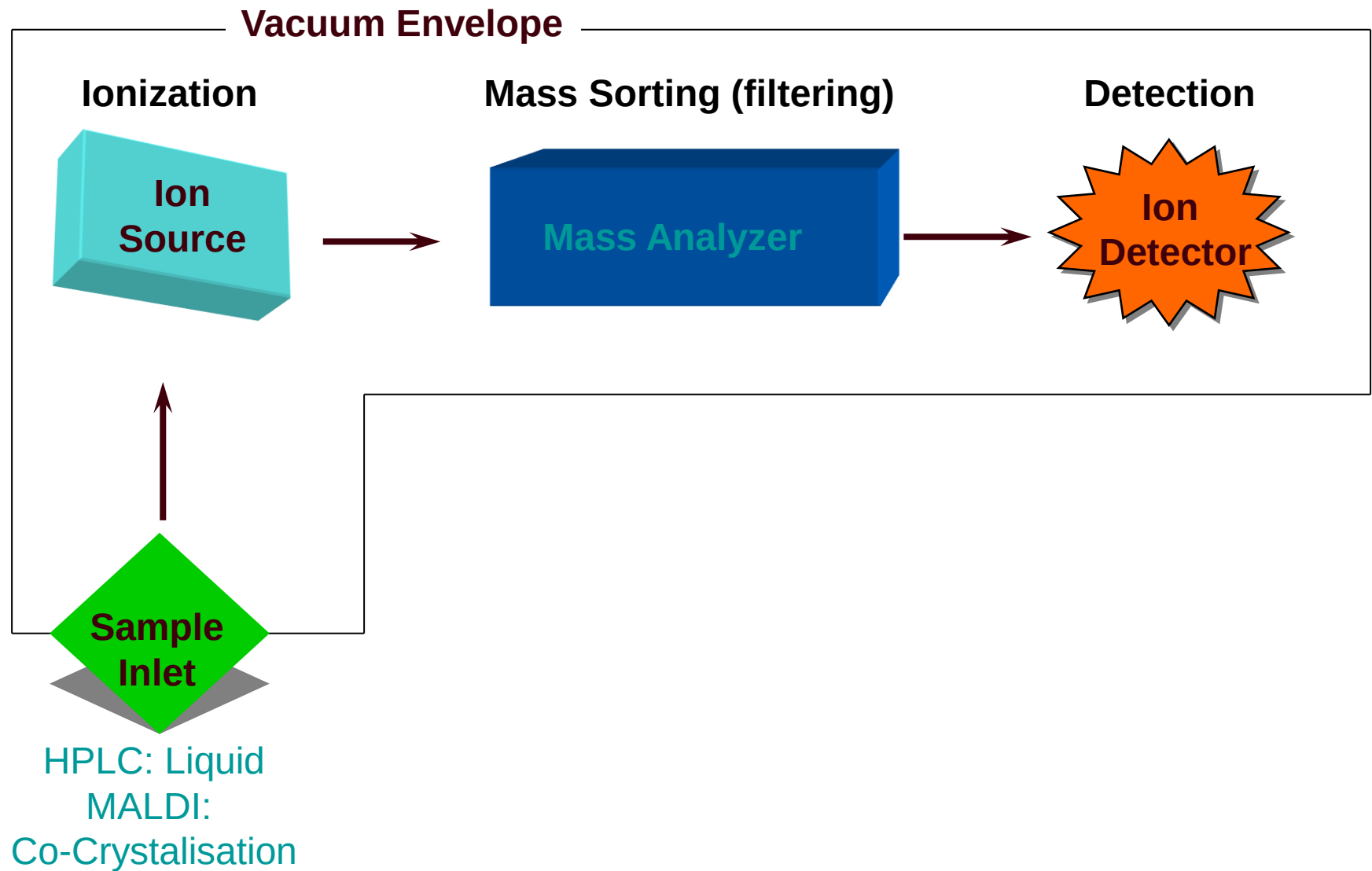
Liquid-Chromatography/ Tandem Mass spectrometry



Why do we need proteolytic cleavage

- Molecular weight of proteins varies greatly (10-500 kilo Dalton)
- MS machines are mainly suited for the analysis of molecules of up to some Dalton
- Proteolytic cleavage produces peptide fragments making the high throughput analysis and identification by MS possible
- Major disadvantage: Even higher complexity of the sample

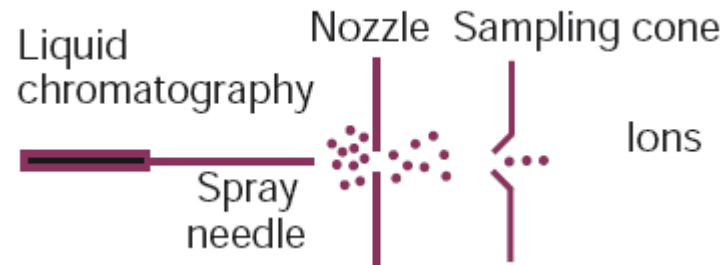
Essential components of a mass spectrometer



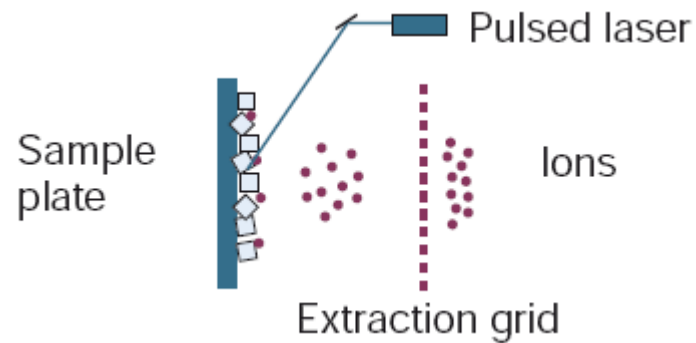
Ion sources



Electro-Spray Ionization
ESI



Matrix-Assisted laser
desorption/ionization
(MALDI)

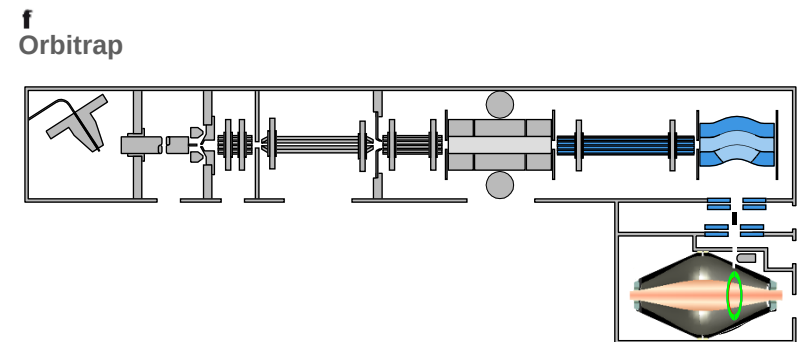
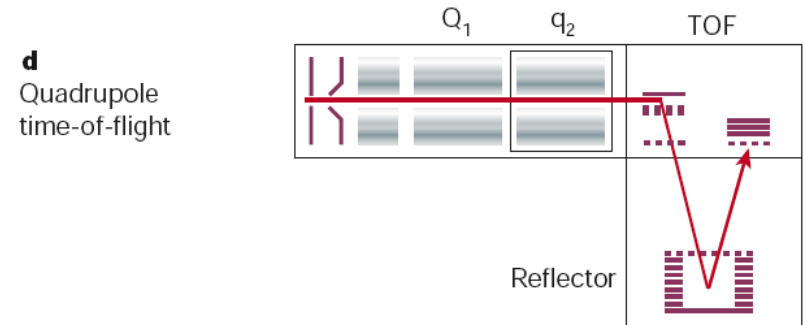
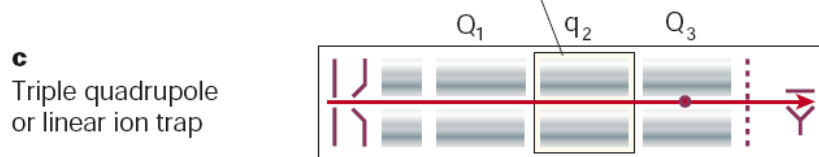
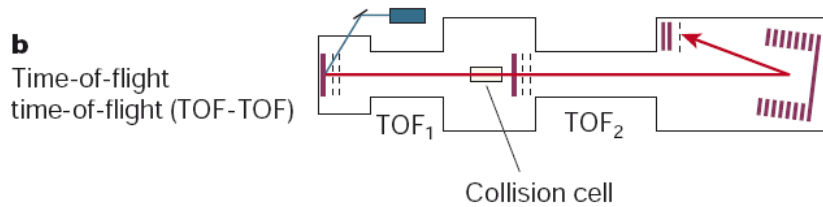
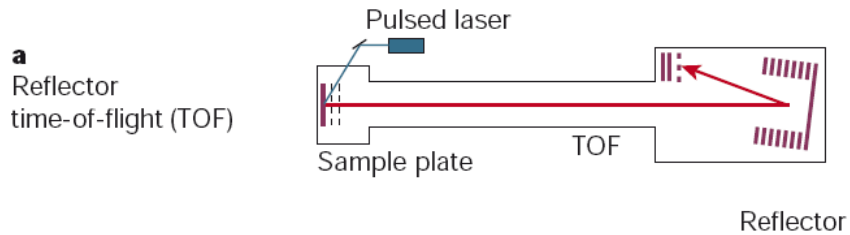


Mass analysers

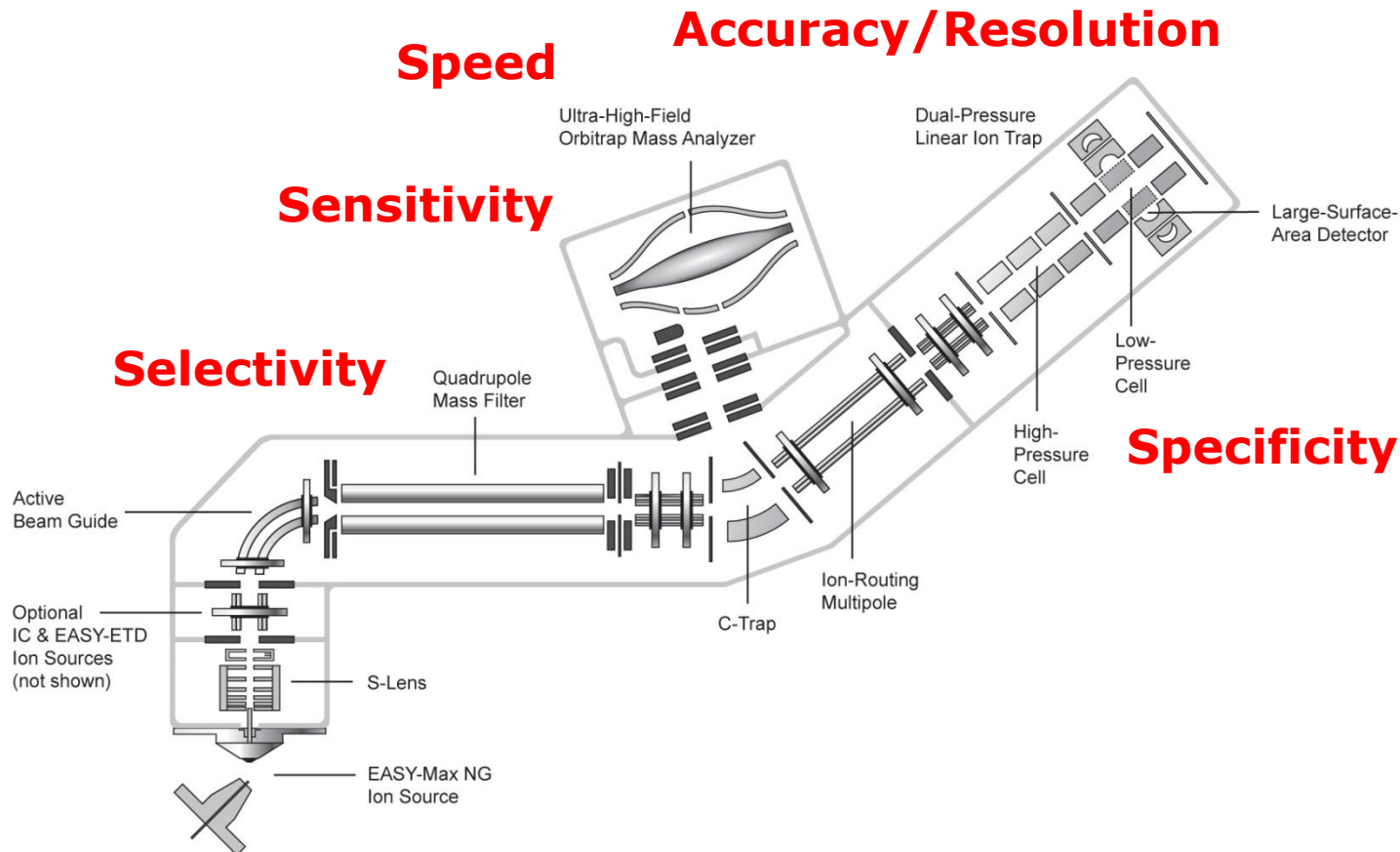
Ion source

Mass analyser

Detector



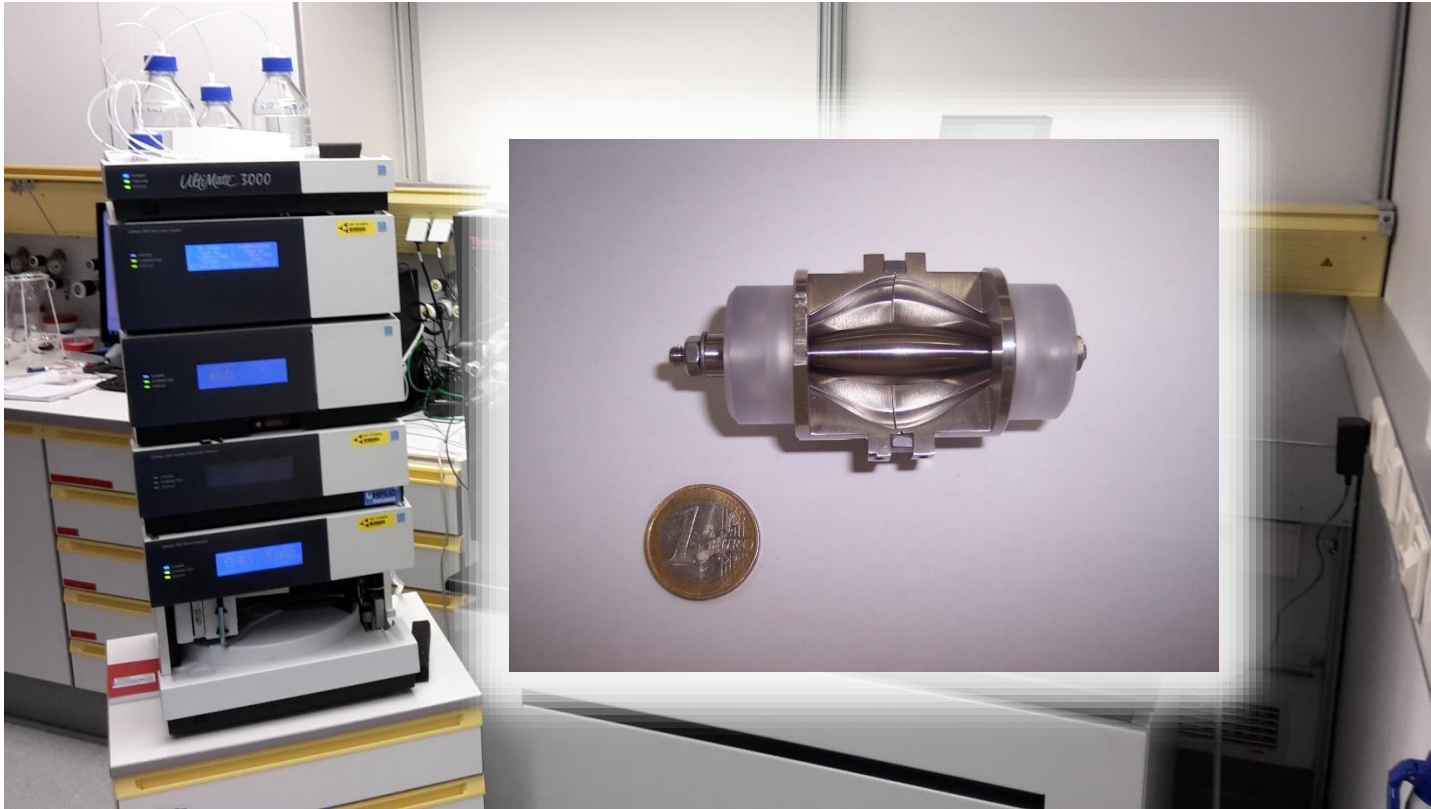
High end MS: Orbitrap Fusion



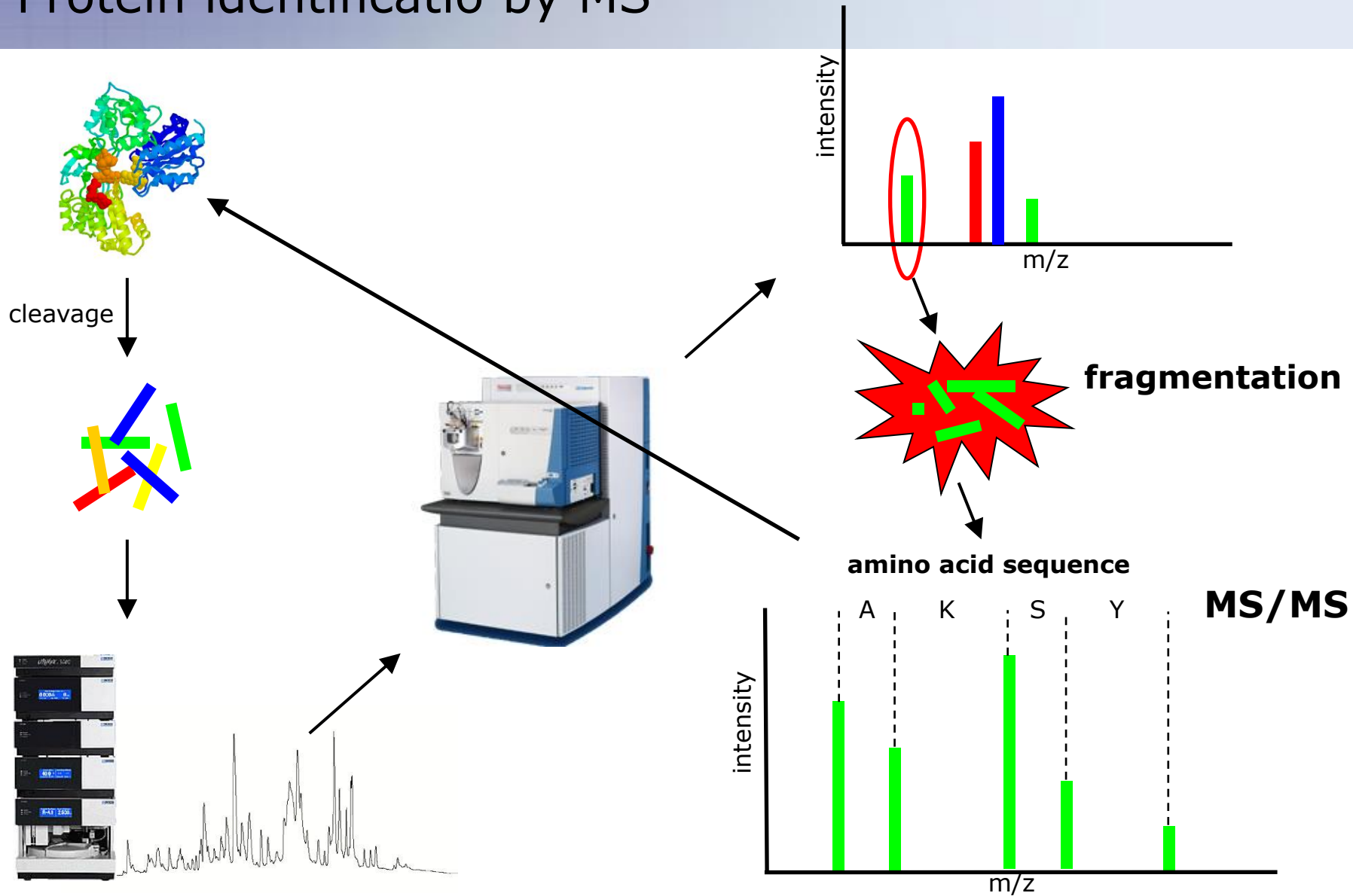
Source: Thermo Scientific



High end MS: Orbitrap Fusion

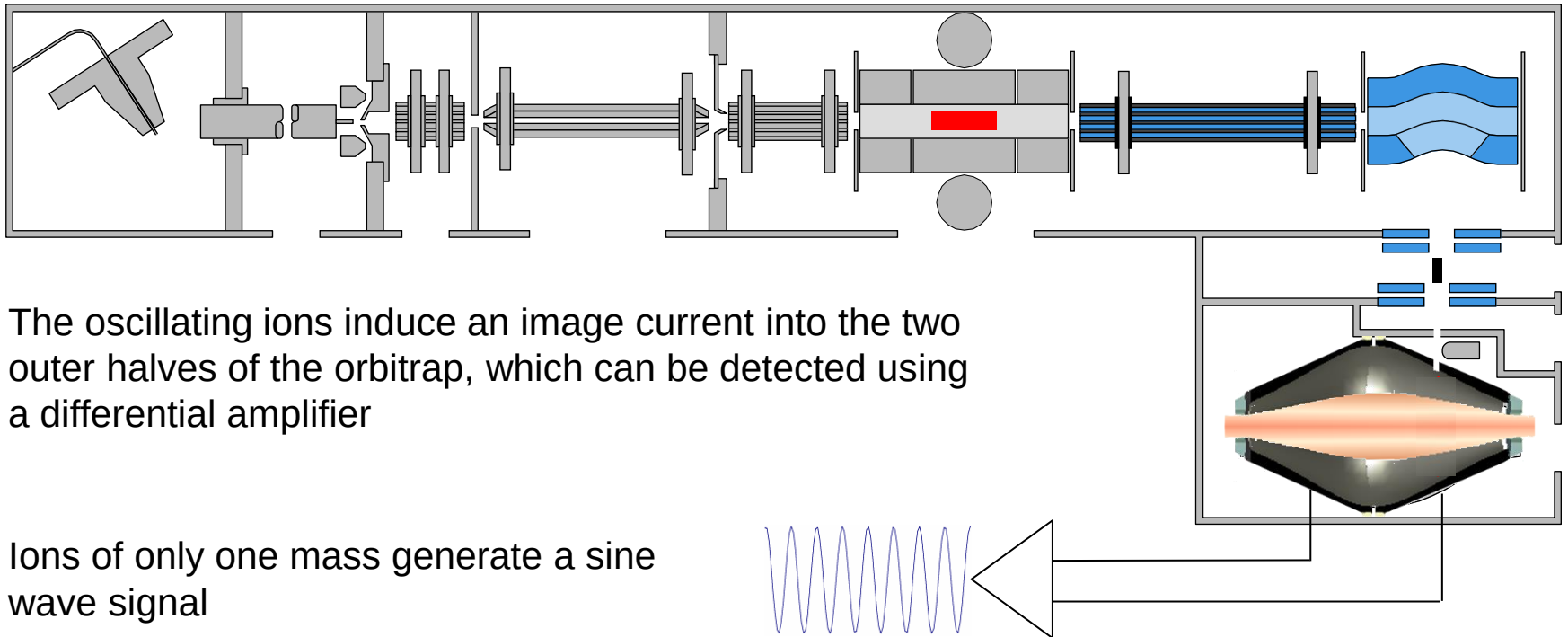


Protein identification by MS

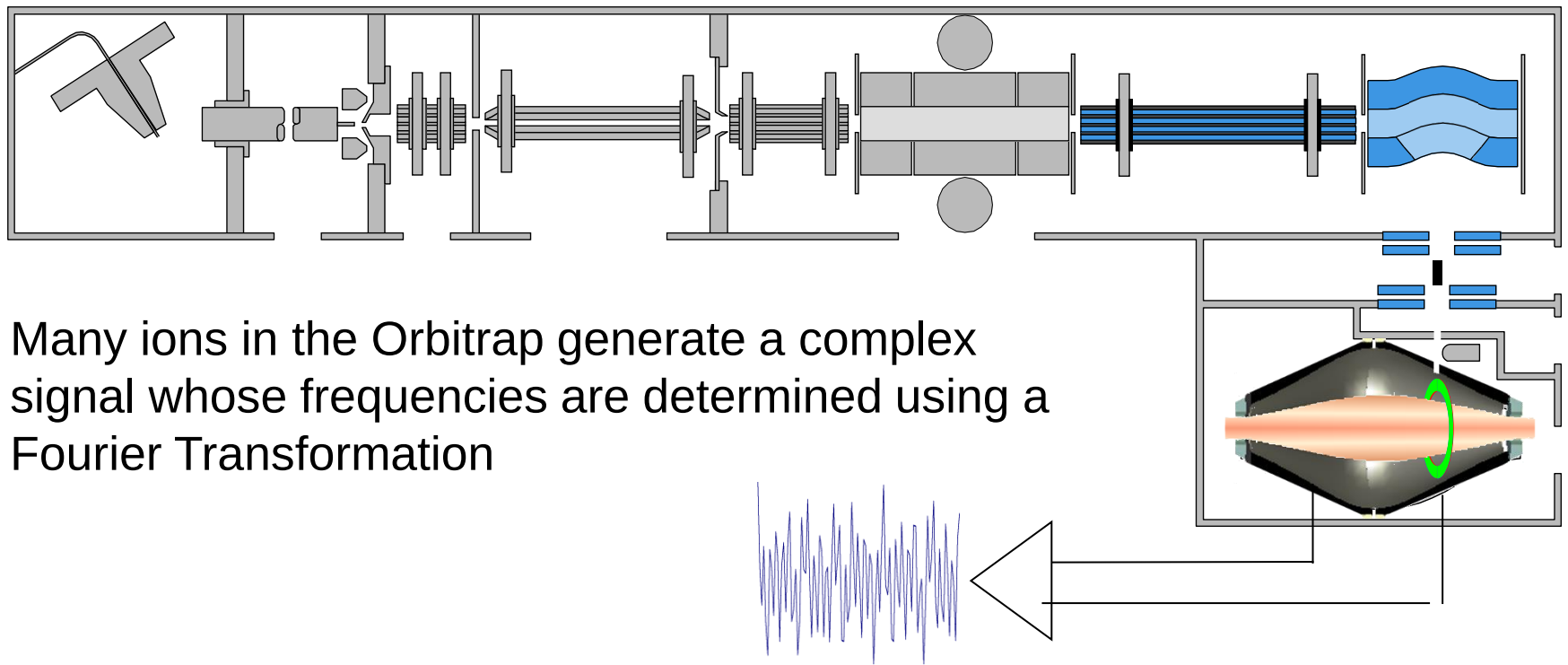


LC-MSMS on an Orbitrap

1. Ions are stored in the Linear Trap
2. are axially ejected
3. and trapped in the C-trap
4. they are squeezed into a small cloud and injected into the Orbitrap
5. where they are electrostatically trapped, while rotating around the central electrode and performing axial oscillation

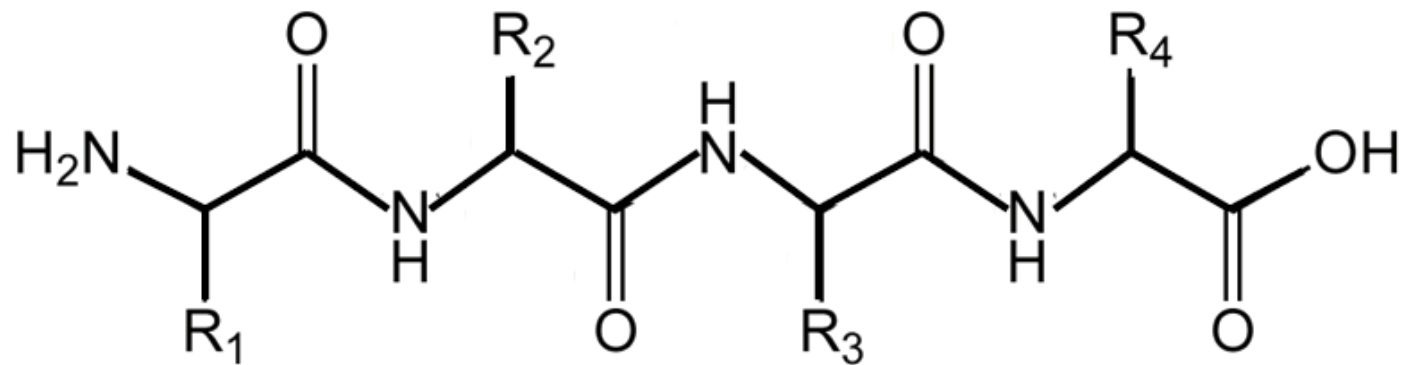


LC-MSMS on an Orbitrap

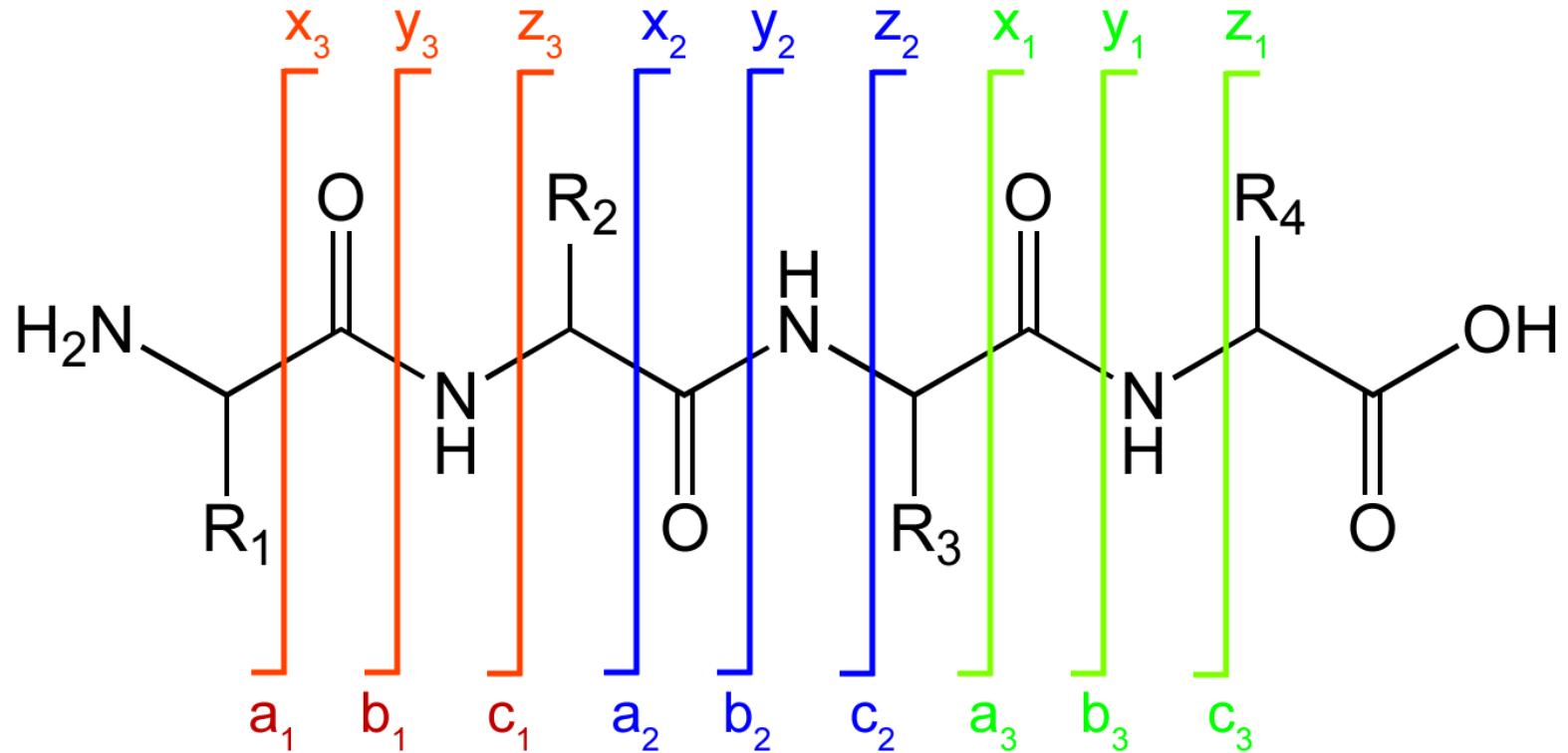


Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

Peptide Fragmentation



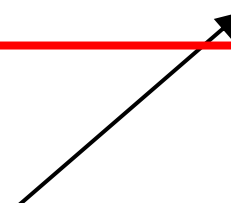
Peptide Fragmentation



Source: Wikipedia



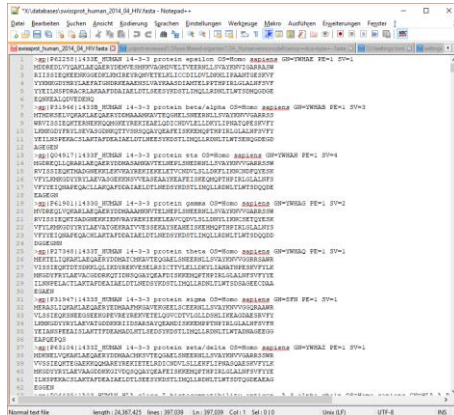
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Identification by sequence tags

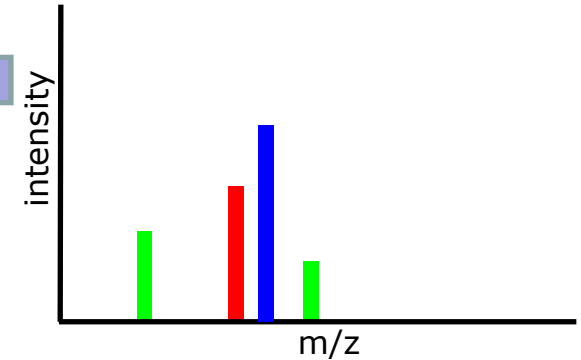
Peptide database (i.e human)
→ in-silico cleaved

Measured
molecular weight



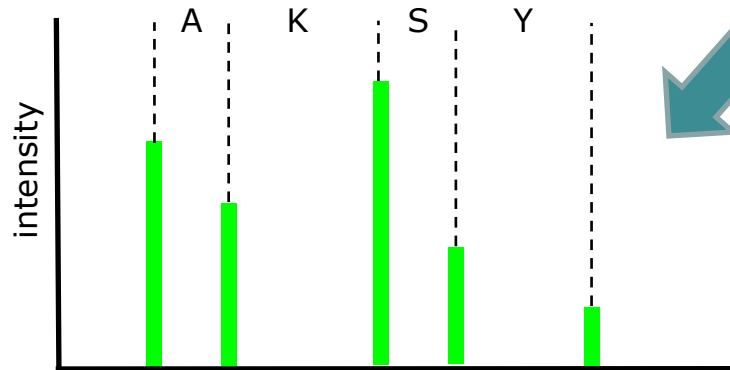
Search engines

(Mascot, MaxQuant, OMSSA, X!tandem,...)

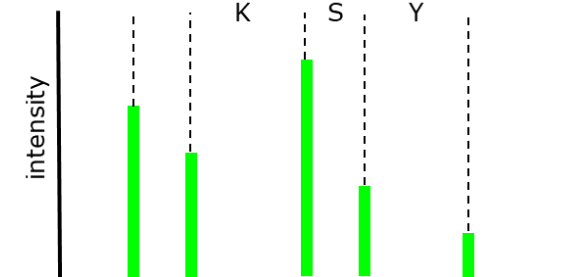


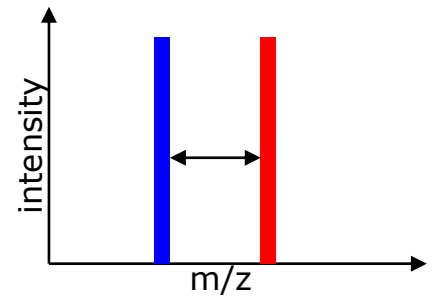
List of potential peptides
P1, P2,

Peptide ID and annotation



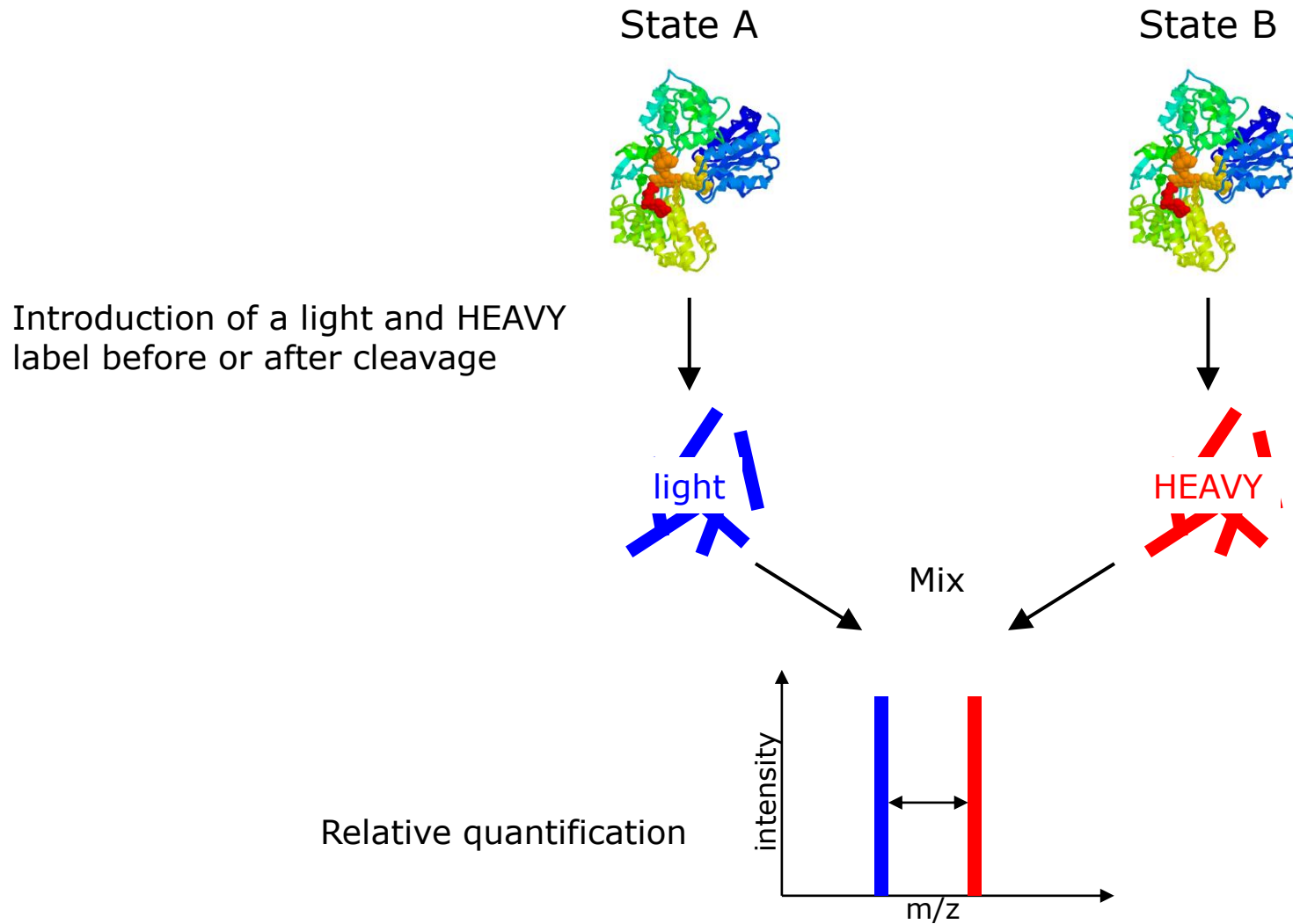
Sequence tag



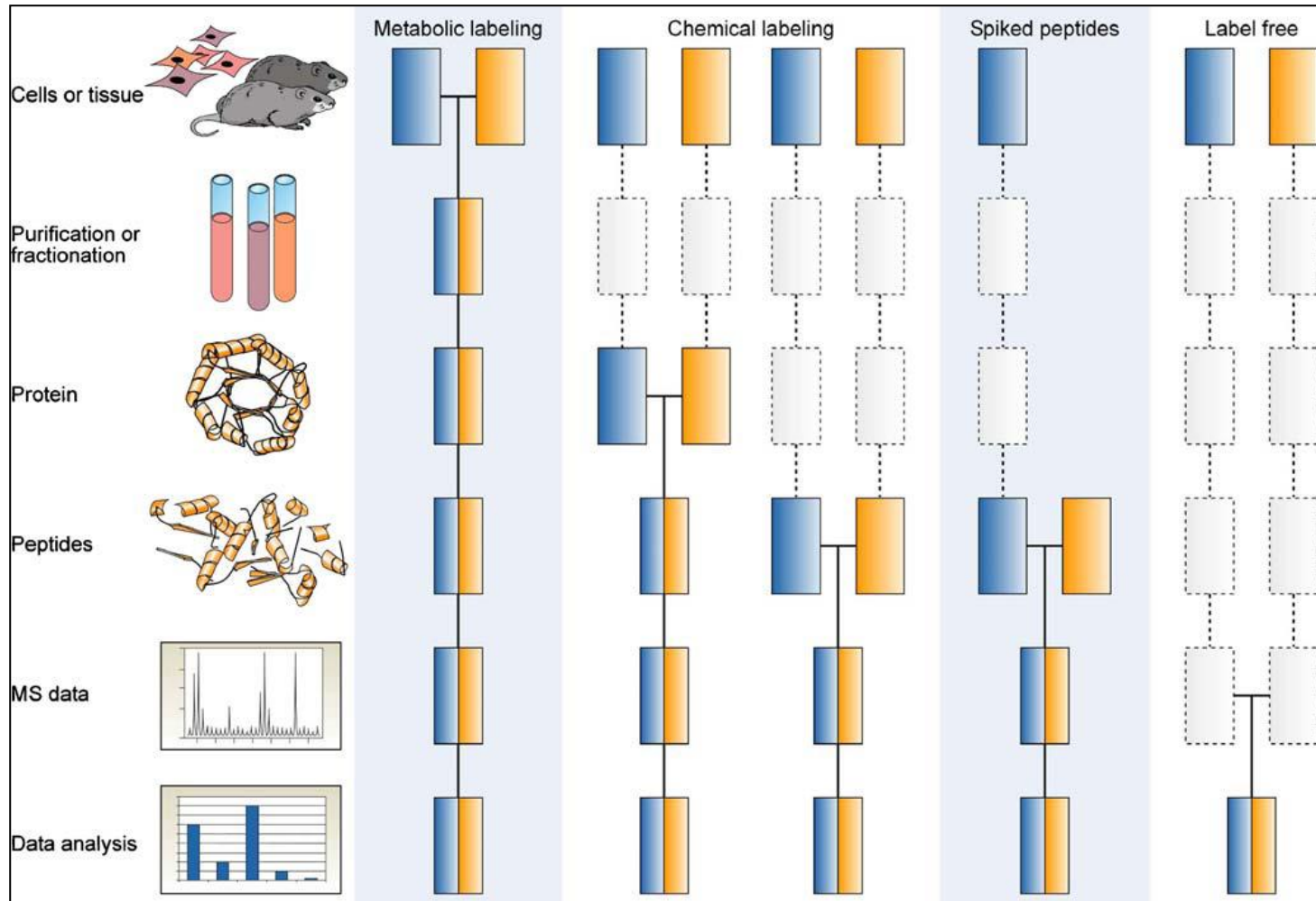


Protein Quantification

MS-based quantification: Principle



MS-based quantification: An overview

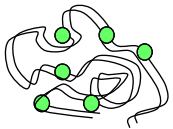


Banscheff et al., Anal. Bioanal. Chem., 2007

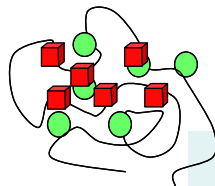
MS-based quantification: Absolute quantification

Quantification is achieved using stable isotope dilution (SID)

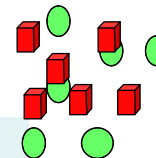
Sample with analytes



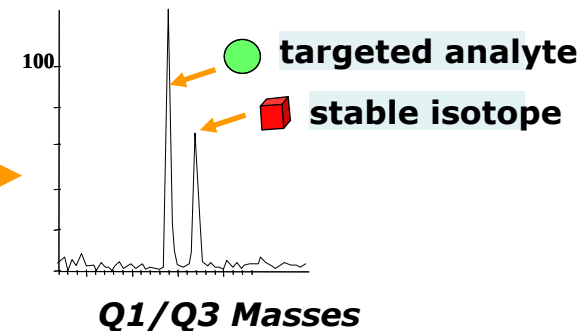
Combined during sample processing



Specificity and identification via SRM / MRM pairs



Extraction etc.



Quantification by MS/MS

Mixture of Internal Standards

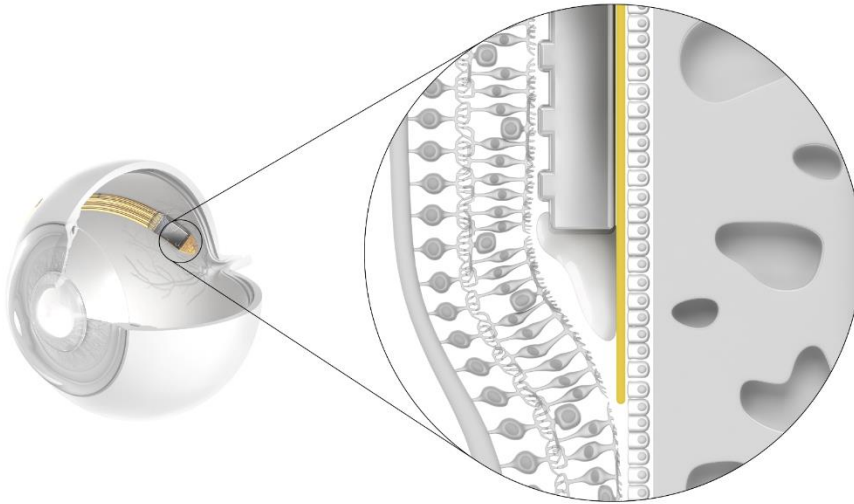


Application to Implantology

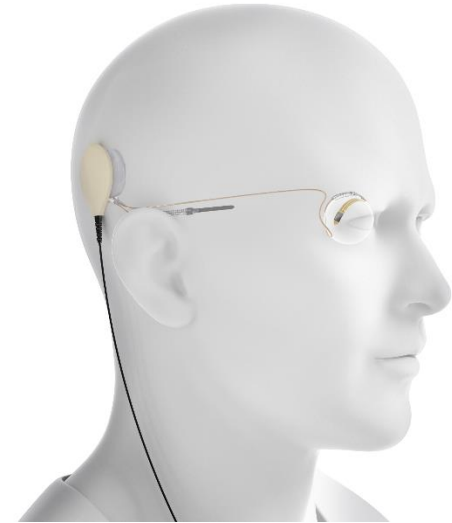


The Retina Implant

Retina implant



Transmitter

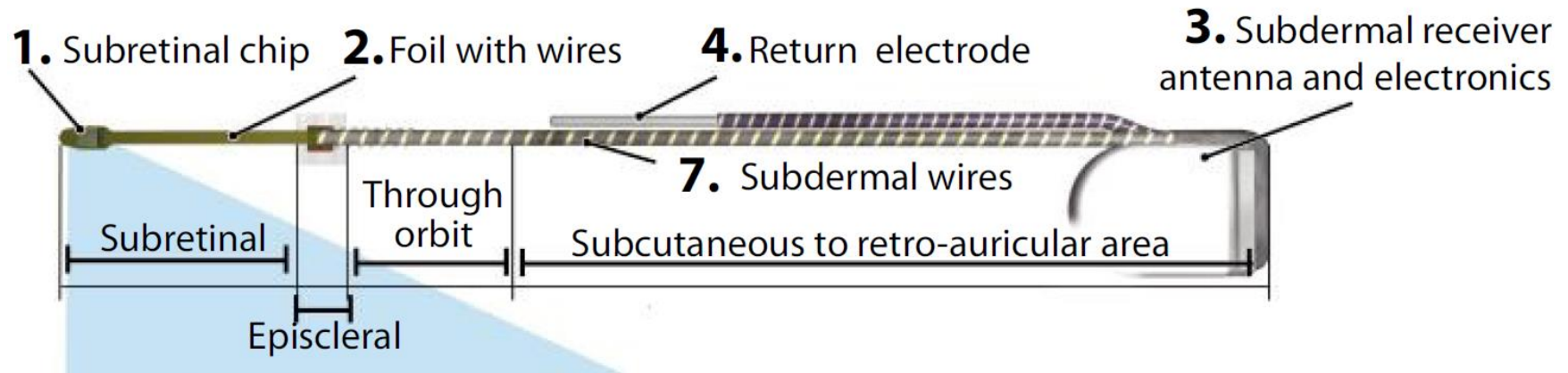


Control unit



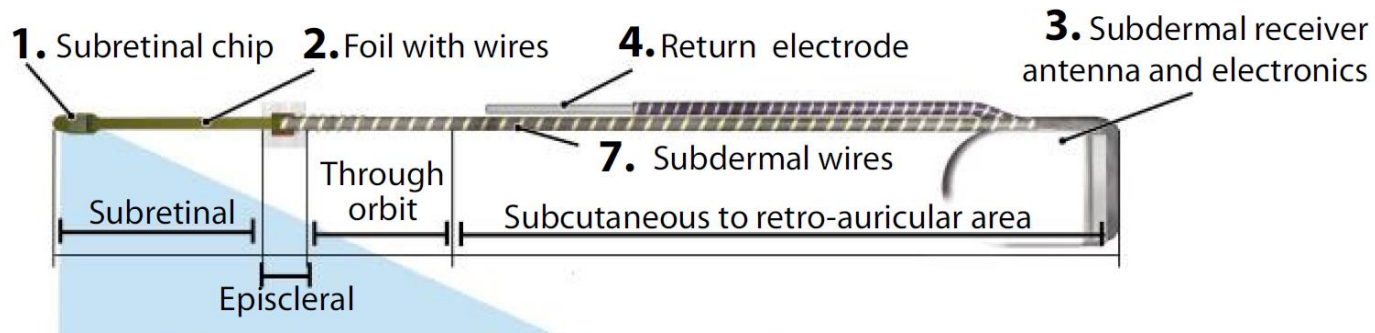
Zrenner E., Sci Transl Med. 2013 Nov 6;5(210):210ps16
<https://www.retina-implant.de>

The Retina Implant



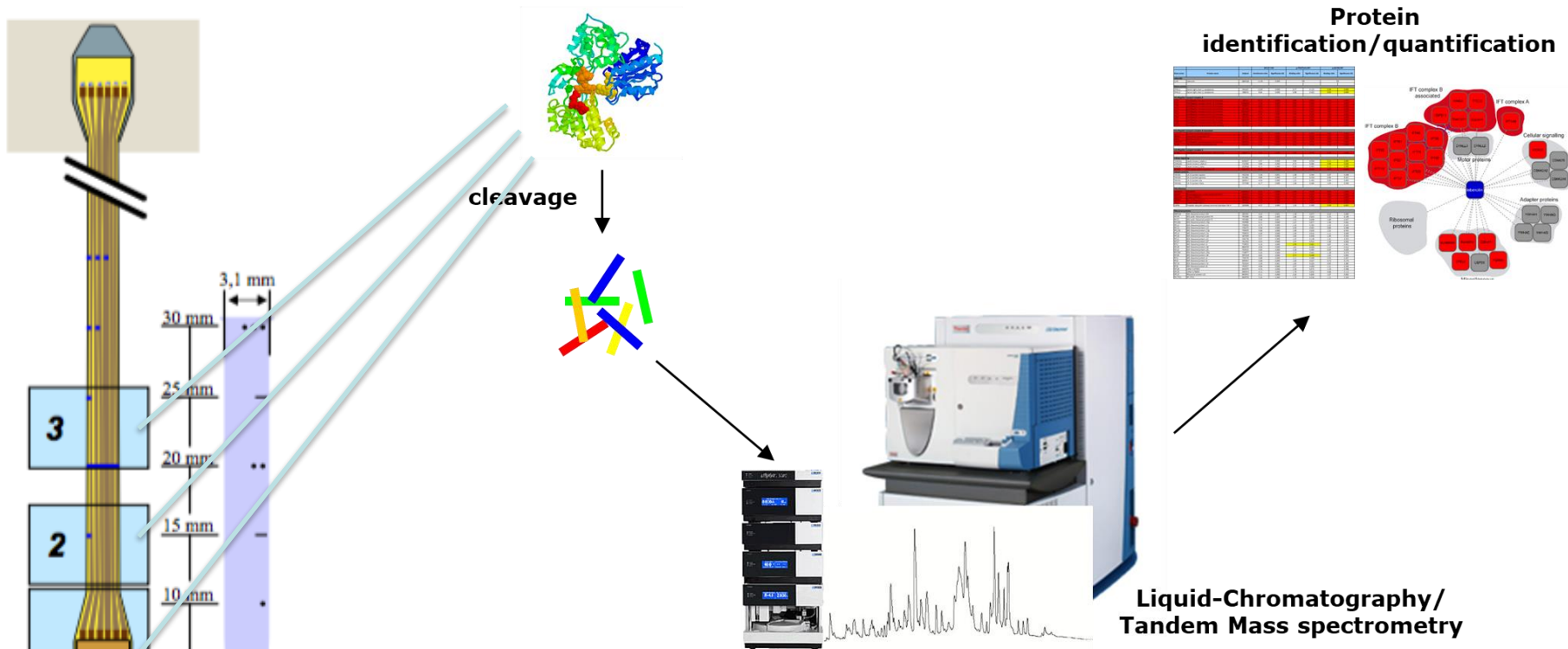
Zrenner E., Sci Transl Med. 2013 Nov 6;5(210):210ps16

Explants sections



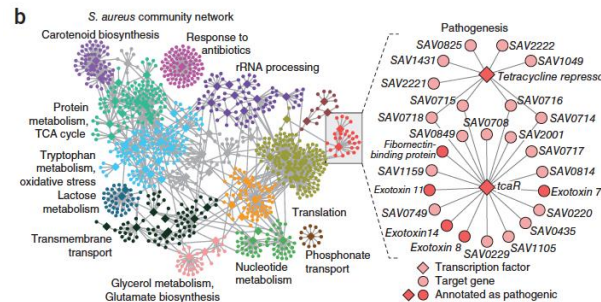
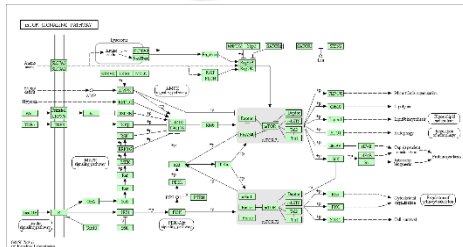
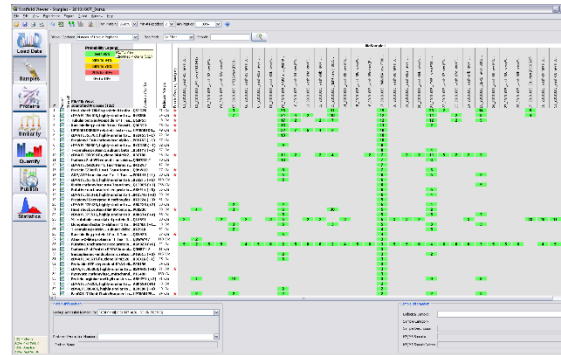
Section	Image	Description
1		Contains the chip, Localization: subretinal
2		Part of the transmitting wire Localization: subretinal
3		Part of the transmitting wire Localization: subretinal, peripheral

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Getting to the biological processes

List of proteins *Relative or quantitative*



<https://www.genome.jp/kegg/pathway.html>

<http://geneontology.org/>





Questions?