

Proteomics for Biocompatibility Testing of Implant Materials

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Overview

- **Implants**

- The Retina Implant
- Organism-implant interplay
- Implant material types

- **Proteomics and its use in implantology**

- What is proteomics
- How can we detect proteins
- Protein quantification
- Application of proteomics for implantology

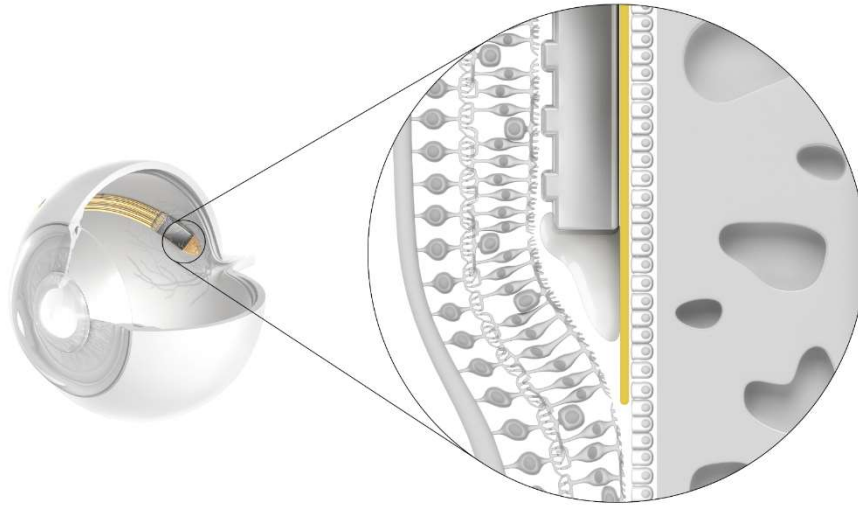
Organism –Implant interplay

Types of Biomaterial (concerning biocompatibility aspects)

Classification	Definition
Biotolerant Materials	• Have a retention time of a few months up to several years in the body. • Are recognized as a foreign material from the surrounding tissue. • There will be no decomposition, no cell changes and no toxic effects during the intervention time.
Bioinert Materials	• There will be no chemical or biological interplay between the tissue and the material. • Low to no release of toxic substances and initiation of a native inflammation. • Development of a connective capsule around the implant.
Bioactive Materials	• The implants will be able to interplay with the surrounding tissue (e.g. an adhesion from the bone to the surface of the implant material, the surface of the material will be changed into bone material) and implants heal into the surrounding tissue. • Based on the coating of the implants the interaction between implant and tissue could be modulated.

The Retina Implant

Retina implant



Transmitter

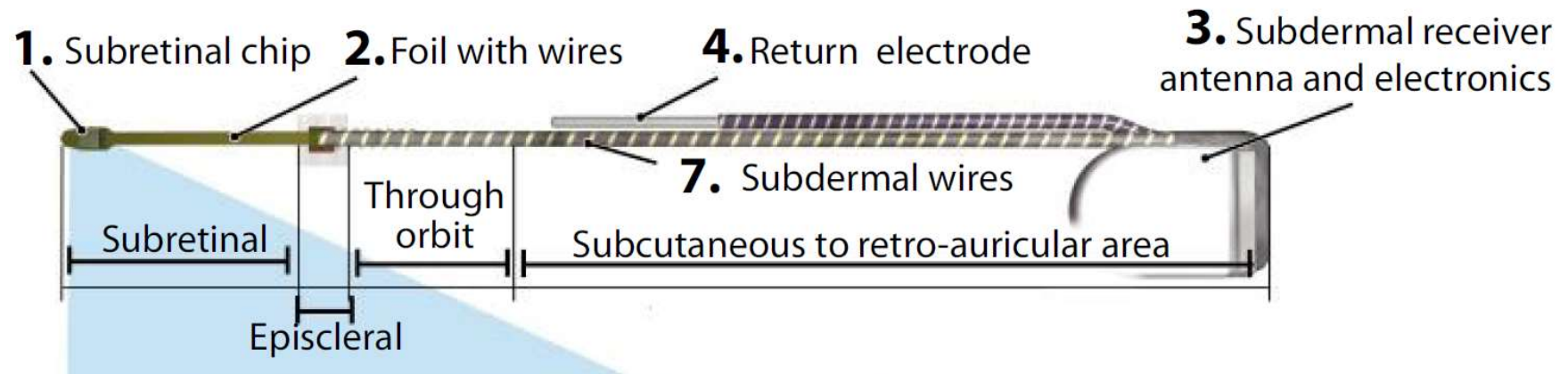


Control unit



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

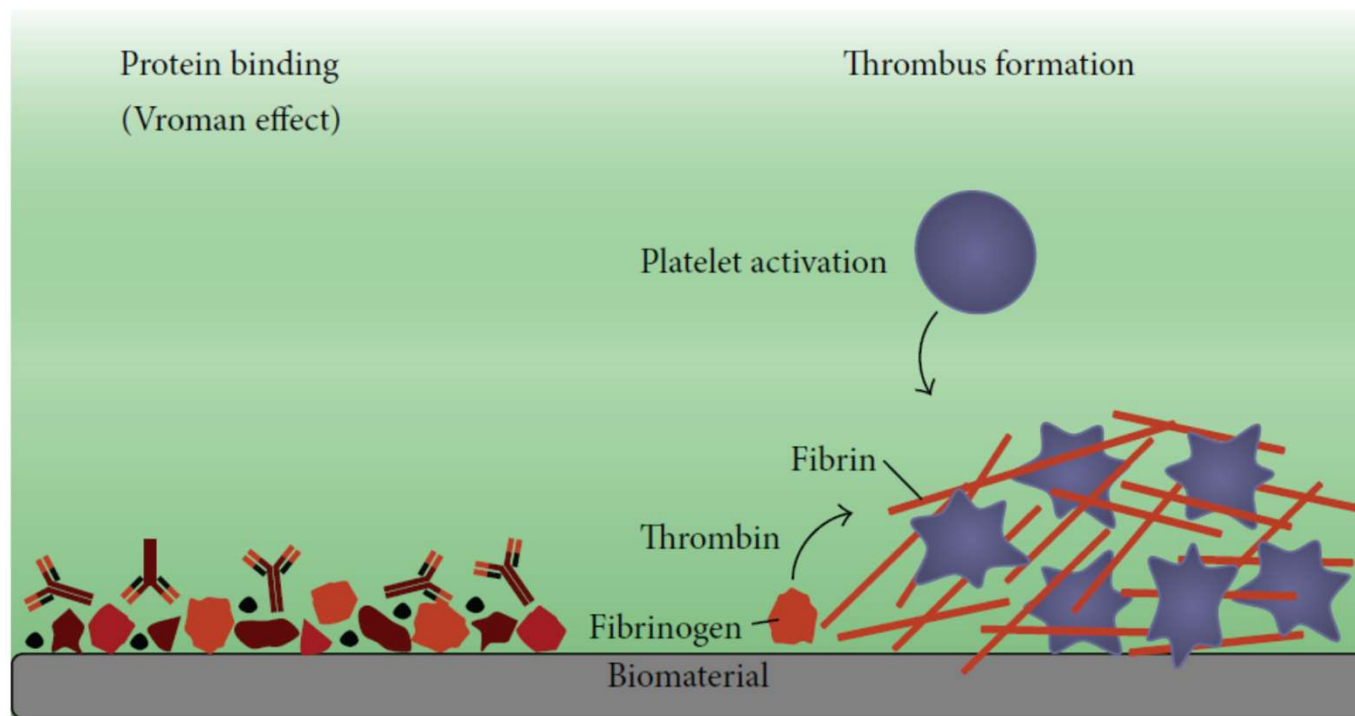
The Retina Implant



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

Foreign Body Reaction (FBR) Phase 1

Protein adsorption / deadsorption

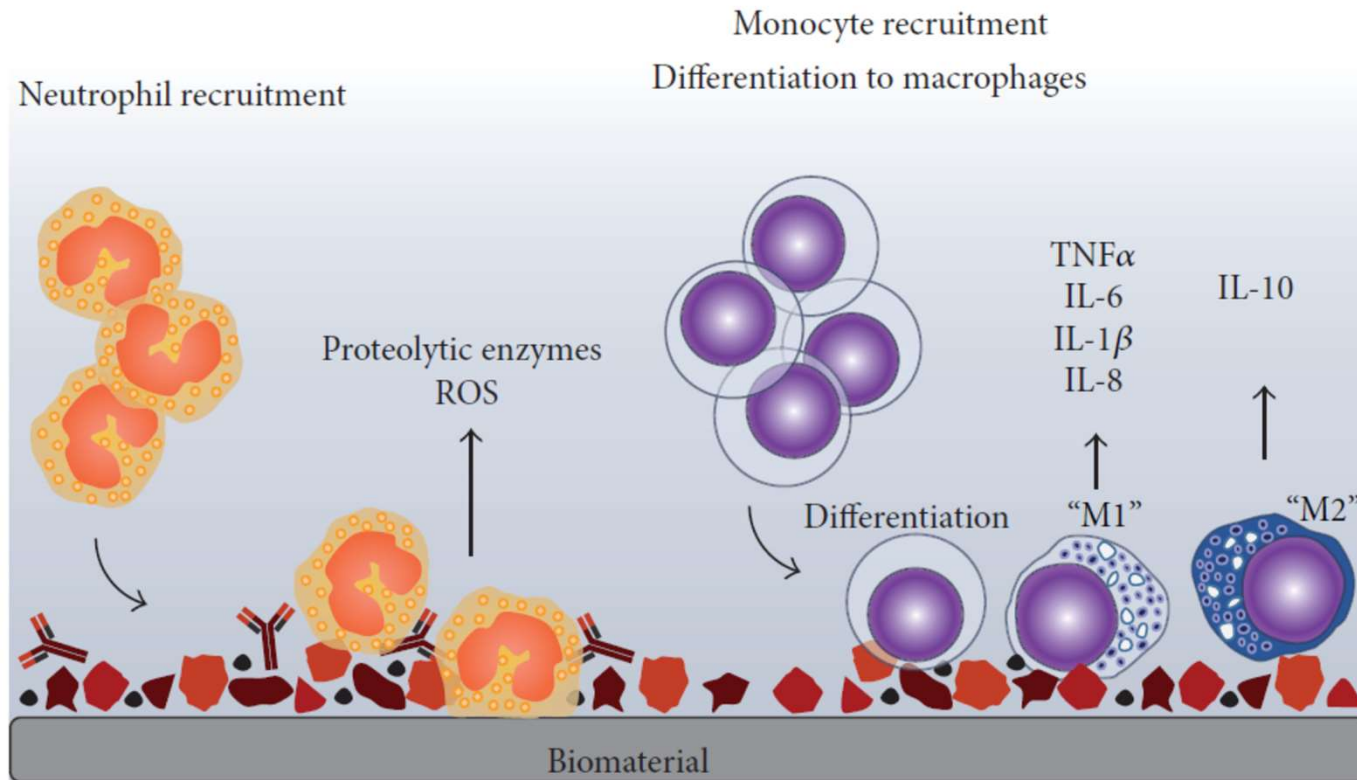


albumin, fibrinogen, fibronectin, vitronectin, gamma globulin, complement, immunomodulatory proteins

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

Foreign Body Reaction (FBR) Phase 2

Acute inflammation



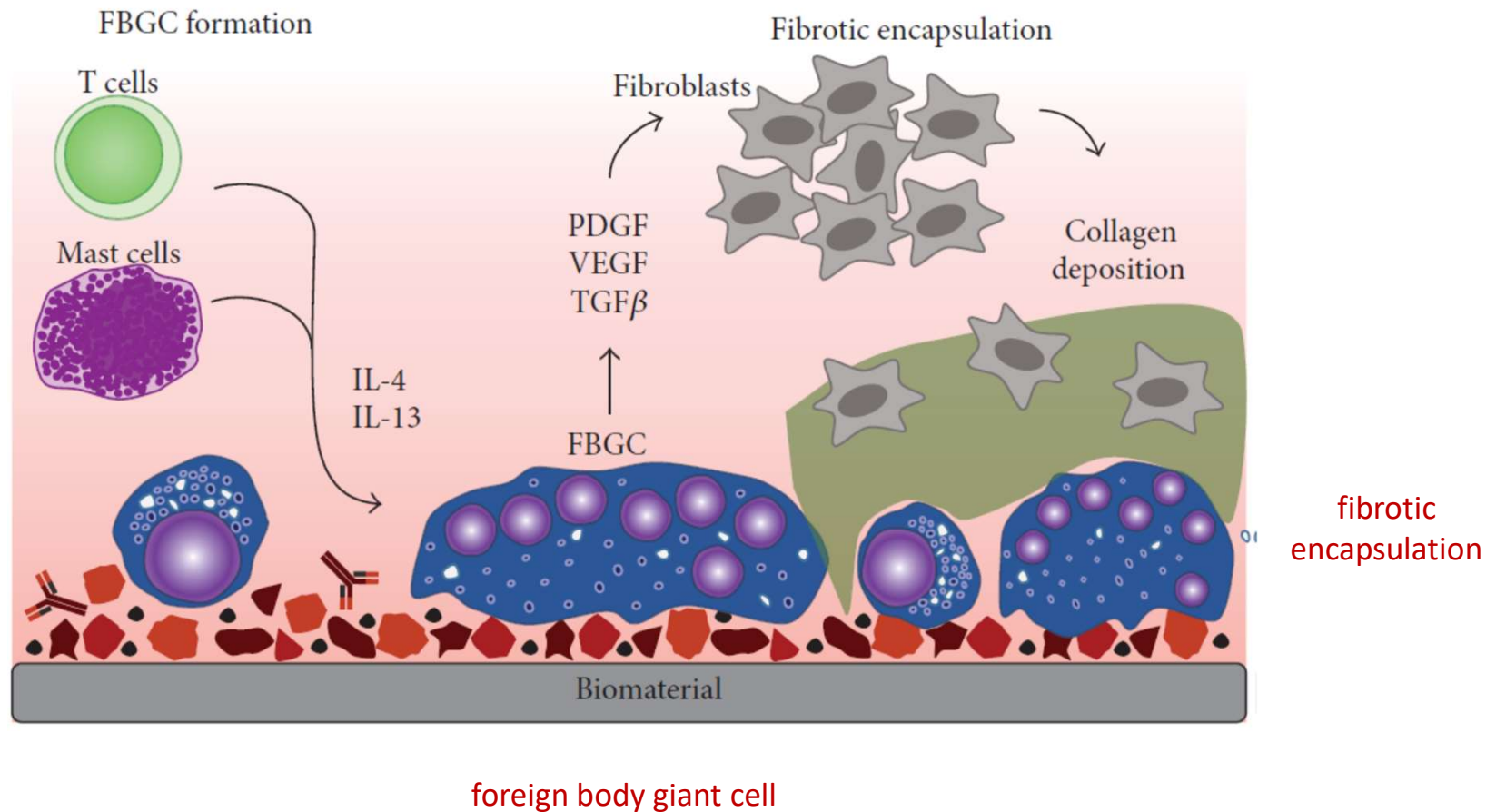
neutrophil extracellular traps
(NETs)

"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



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"

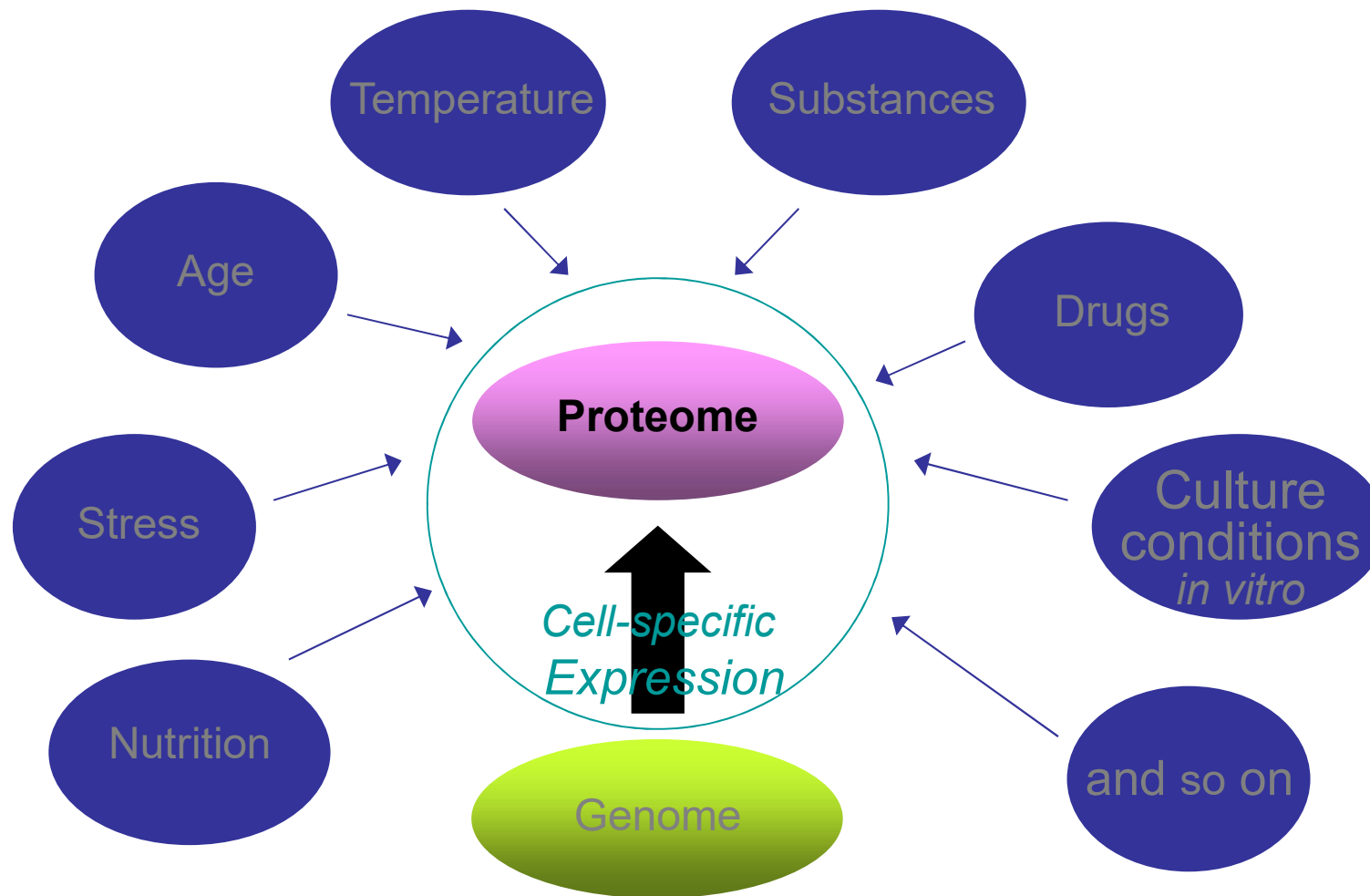
The proteome describes the **entirety of proteins** of an organism at a **certain point in time and in a certain state**.

*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



Analysing the proteome

➤ **Methods for proteome analysis:**

➤ **Gel based approaches**

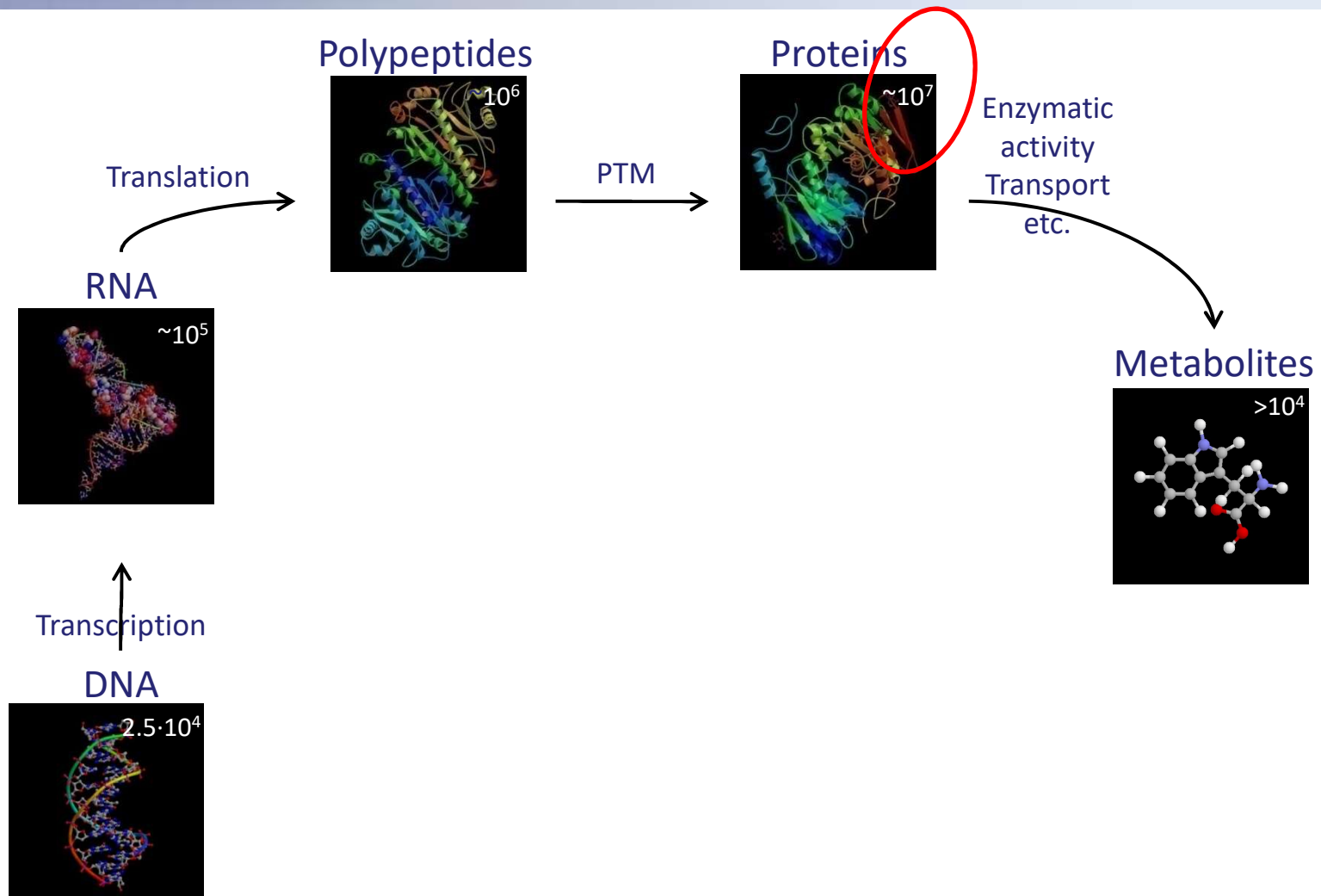
- *Labour intensive, hard to standardize*
- *Mass spectrometry (MS) necessary for protein identification*

➤ **Gel-free, MS-based approaches**

- *Standardization possible*
- *Automation possible*
- *Great improvement in sensitivity, speed and resolution within the last 10 years*
- *Automated quantification possible*

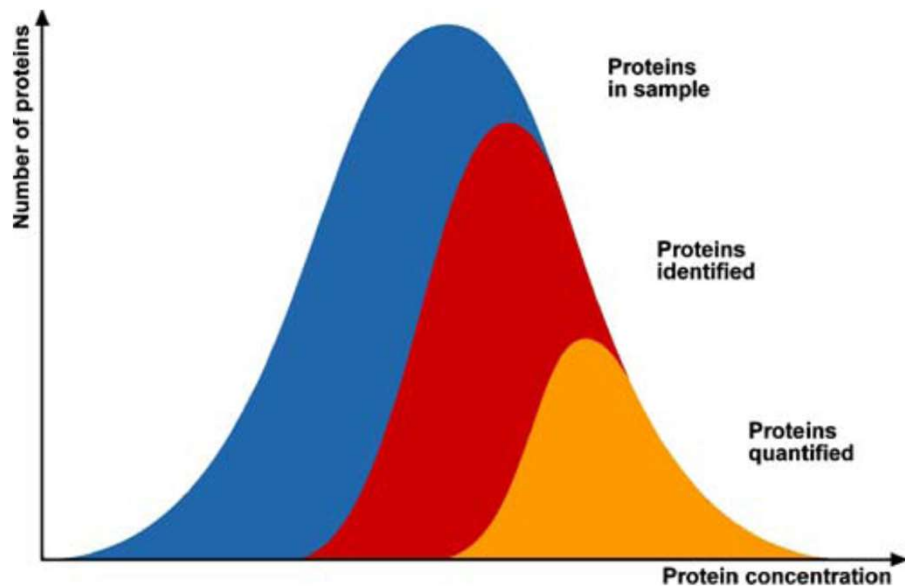
→ **Gel-free, MS-based approaches are highly suitable for proteome analysis**

The Analytical Challenge

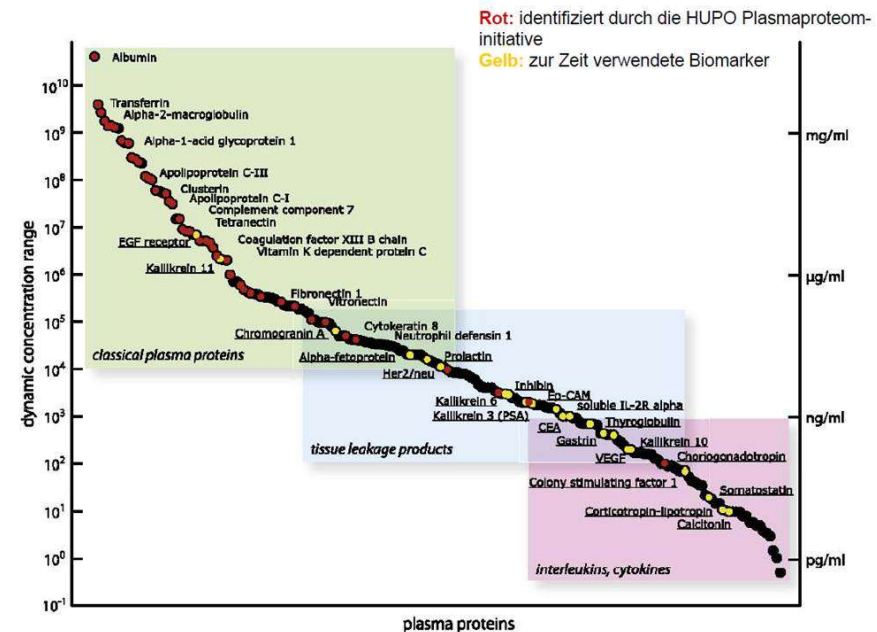


More analytical challenges

- **High complexity:**
 - ~ 20 000 genes
 - 50 000 - 100 000 proteins (w/o PTM)
- **Modifications** lead to even higher complexity ($>> 10^6$)
- **Huge dynamic range** (10 orders of magnitude)

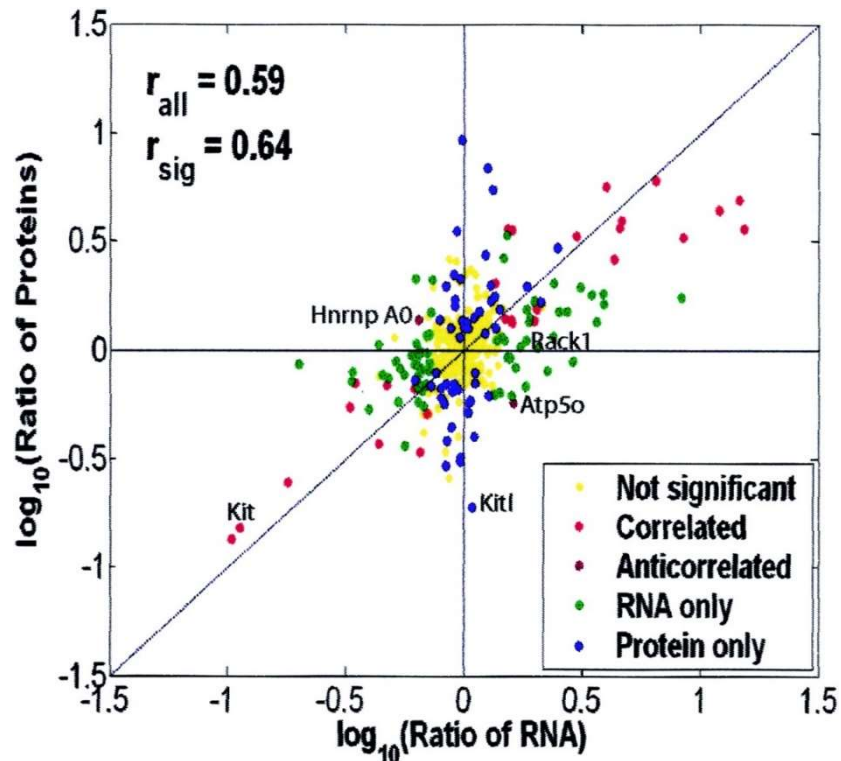


Banscheff et al., Anal. Bioanal. Chem., 2007



RNA versus protein analysis

mRNA versus protein expression



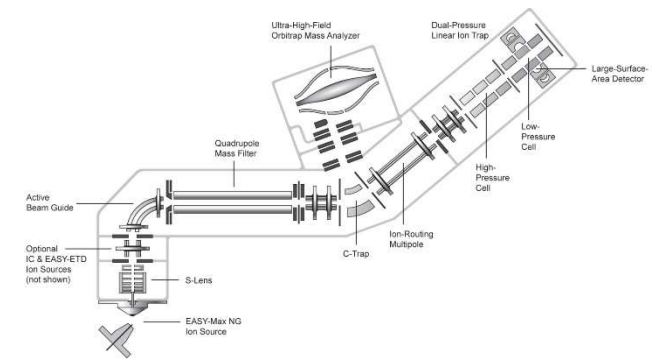
Tian Q et al. Mol Cell Proteomics
2004;3:960-969

Sub-proteome analysis:

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

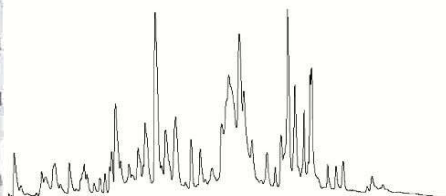
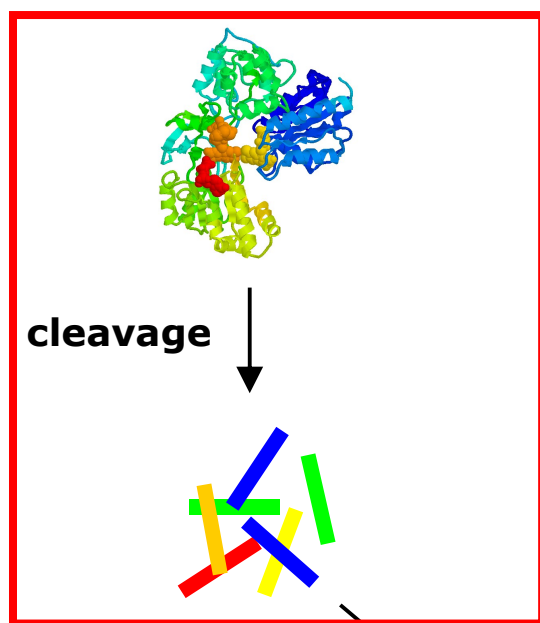
Post-translational modifications:

- Phosphorylation
- Acetylation
- Sumoylation
- ...



Mass spectrometry based proteomics

Mass spectrometry based proteomics workflow



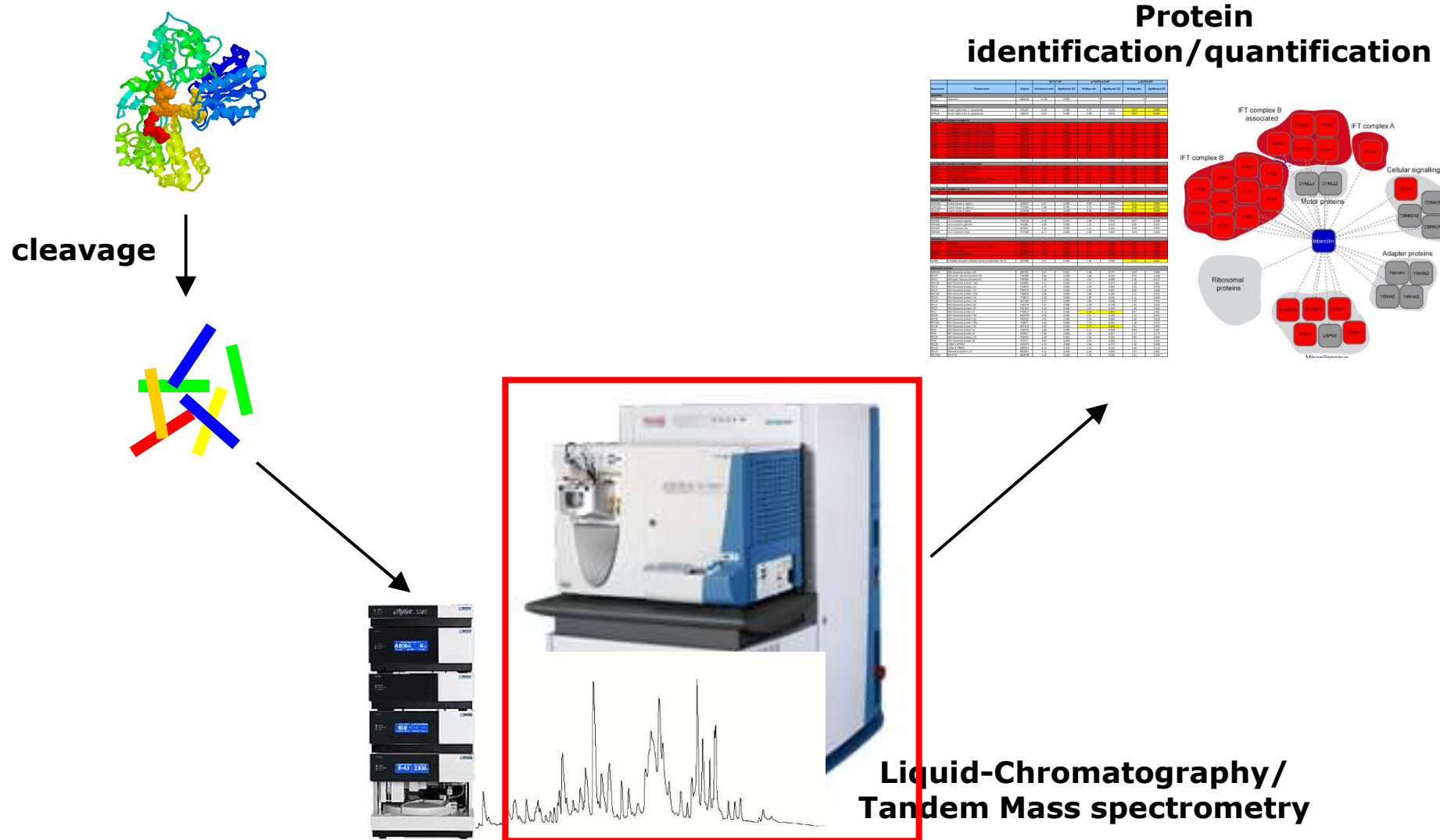
Protein identification/quantification

Accession	Protein name	Size of			Number of			Starting site	Length
		Residue	Sequence	Sequence	Sequence	Sequence	Sequence		
Protein A	Protein A	100	1000	1000	1000	1000	1000	1000	
Protein B	Protein B	100	1000	1000	1000	1000	1000	1000	
Protein C	Protein C	100	1000	1000	1000	1000	1000	1000	
Protein D	Protein D	100	1000	1000	1000	1000	1000	1000	
Protein E	Protein E	100	1000	1000	1000	1000	1000	1000	
Protein F	Protein F	100	1000	1000	1000	1000	1000	1000	
Protein G	Protein G	100	1000	1000	1000	1000	1000	1000	
Protein H	Protein H	100	1000	1000	1000	1000	1000	1000	
Protein I	Protein I	100	1000	1000	1000	1000	1000	1000	
Protein J	Protein J	100	1000	1000	1000	1000	1000	1000	
Protein K	Protein K	100	1000	1000	1000	1000	1000	1000	
Protein L	Protein L	100	1000	1000	1000	1000	1000	1000	
Protein M	Protein M	100	1000	1000	1000	1000	1000	1000	
Protein N	Protein N	100	1000	1000	1000	1000	1000	1000	
Protein O	Protein O	100	1000	1000	1000	1000	1000	1000	
Protein P	Protein P	100	1000	1000	1000	1000	1000	1000	
Protein Q	Protein Q	100	1000	1000	1000	1000	1000	1000	
Protein R	Protein R	100	1000	1000	1000	1000	1000	1000	
Protein S	Protein S	100	1000	1000	1000	1000	1000	1000	
Protein T	Protein T	100	1000	1000	1000	1000	1000	1000	
Protein U	Protein U	100	1000	1000	1000	1000	1000	1000	
Protein V	Protein V	100	1000	1000	1000	1000	1000	1000	
Protein W	Protein W	100	1000	1000	1000	1000	1000	1000	
Protein X	Protein X	100	1000	1000	1000	1000	1000	1000	
Protein Y	Protein Y	100	1000	1000	1000	1000	1000	1000	
Protein Z	Protein Z	100	1000	1000	1000	1000	1000	1000	
Protein AA	Protein AA	100	1000	1000	1000	1000	1000	1000	
Protein AB	Protein AB	100	1000	1000	1000	1000	1000	1000	
Protein AC	Protein AC	100	1000	1000	1000	1000	1000	1000	
Protein AD	Protein AD	100	1000	1000	1000	1000	1000	1000	
Protein AE	Protein AE	100	1000	1000	1000	1000	1000	1000	
Protein AF	Protein AF	100	1000	1000	1000	1000	1000	1000	
Protein AG	Protein AG	100	1000	1000	1000	1000	1000	1000	
Protein AH	Protein AH	100	1000	1000	1000	1000	1000	1000	
Protein AI	Protein AI	100	1000	1000	1000	1000	1000	1000	
Protein AJ	Protein AJ	100	1000	1000	1000	1000	1000	1000	
Protein AK	Protein AK	100	1000	1000	1000	1000	1000	1000	
Protein AL	Protein AL	100	1000	1000	1000	1000	1000	1000	
Protein AM	Protein AM	100	1000	1000	1000	1000	1000	1000	
Protein AN	Protein AN	100	1000	1000	1000	1000	1000	1000	
Protein AO	Protein AO	100	1000	1000	1000	1000	1000	1000	
Protein AP	Protein AP	100	1000	1000	1000	1000	1000	1000	
Protein AQ	Protein AQ	100	1000	1000	1000	1000	1000	1000	
Protein AR	Protein AR	100	1000	1000	1000	1000	1000	1000	
Protein AS	Protein AS	100	1000	1000	1000	1000	1000	1000	
Protein AT	Protein AT	100	1000	1000	1000	1000	1000	1000	
Protein AU	Protein AU	100	1000	1000	1000	1000	1000	1000	
Protein AV	Protein AV	100	1000	1000	1000	1000	1000	1000	
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Protein AZ	Protein AZ	100	1000	1000	1000	1000	1000	1000	
Protein BA	Protein BA	100	1000	1000	1000	1000	1000	1000	
Protein BB	Protein BB	100	1000	1000	1000	1000	1000	1000	
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Protein BE	Protein BE	100	1000	1000	1000	1000	1000	1000	
Protein BF	Protein BF	100	1000	1000	1000	1000	1000	1000	
Protein BG	Protein BG	100	1000	1000	1000	1000	1000	1000	
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Protein BJ	Protein BJ	100	1000	1000	1000	1000	1000	1000	
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Protein BL	Protein BL	100	1000	1000	1000	1000	1000	1000	
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Protein BN	Protein BN	100	1000	1000	1000	1000	1000	1000	
Protein BO	Protein BO	100	1000	1000	1000	1000	1000	1000	
Protein BP	Protein BP	100	1000	1000	1000	1000	1000	1000	
Protein BQ	Protein BQ	100	1000	1000	1000	1000	1000	1000	
Protein BR	Protein BR	100	1000	1000	1000	1000	1000	1000	
Protein BS	Protein BS	100	1000	1000	1000	1000	1000	1000	
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Protein BU	Protein BU	100	1000	1000	1000	1000	1000	1000	
Protein BV	Protein BV	100	1000	1000	1000	1000	1000	1000	
Protein BW	Protein BW	100	1000	1000	1000	1000	1000	1000	
Protein BX	Protein BX	100	1000	1000	1000	1000	1000	1000	
Protein BY	Protein BY	100	1000	1000	1000	1000	1000	1000	
Protein BZ	Protein BZ	100	1000	1000	1000	1000	1000	1000	
Protein CA	Protein CA	100	1000	1000	1000	1000	1000	1000	
Protein CB	Protein CB	100	1000	1000	1000	1000	1000	1000	
Protein CC	Protein CC	100	1000	1000	1000	1000	1000	1000	
Protein CD	Protein CD	100	1000	1000	1000	1000	1000	1000	
Protein CE	Protein CE	100	1000	1000	1000	1000	1000	1000	
Protein CF	Protein CF	100	1000	1000	1000	1000	1000	1000	
Protein CG	Protein CG	100	1000	1000	1000	1000	1000	1000	
Protein CH	Protein CH	100	1000	1000	1000	1000	1000	1000	
Protein CI	Protein CI	100	1000	1000	1000	1000	1000	1000	
Protein CJ	Protein CJ	100	1000	1000	1000	1000	1000	1000	
Protein CK	Protein CK	100	1000	1000	1000	1000	1000	1000	
Protein CL	Protein CL	100	1000	1000	1000	1000	1000	1000	
Protein CM	Protein CM	100	1000	1000	1000	1000	1000	1000	
Protein CN	Protein CN	100	1000	1000	1000	1000	1000	1000	
Protein CO	Protein CO	100	1000	1000	1000	1000	1000	1000	
Protein CP	Protein CP	100	1000	1000	1000	1000	1000	1000	
Protein CQ	Protein CQ	100	1000	1000	1000	1000	1000	1000	
Protein CR	Protein CR	100	1000	1000	1000	1000	1000	1000	
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Protein CT	Protein CT	100	1000	1000	1000	1000	1000	1000	
Protein CU	Protein CU	100	1000	1000	1000	1000	1000	1000	
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Protein DT	Protein DT	100	1000	1000	1000	1000	1000	1000	
Protein DU	Protein DU	100	1000	1000	1000	1000	1000	1000	
Protein DV	Protein DV	100	1000	1000	1000	1000	1000	1000	
Protein DW	Protein DW	100	1000	1000	1000	1000	1000	1000	
Protein DX	Protein DX	100	1000	1000	1000	1000	1000	1000	
Protein DY	Protein DY	100	1000	1000	1000	1000	1000	1000	
Protein DZ	Protein DZ	100	1000	1000	1000	1000	1000	1000	
Protein EA	Protein EA	100	1000	1000	1000	1000	1000	1000	
Protein EB	Protein EB	100	1000	1000	1000	1000	1000	1000	
Protein EC	Protein EC	100	1000	1000	1000	1000	1000	1000	
Protein ED	Protein ED	100	1000	1000	1000	1000	1000	1000	
Protein EE	Protein EE	100	1000	1000	1000	1000	1000	1000	
Protein EF	Protein EF	100	1000	1000	1000	1000	1000	1000	
Protein EG	Protein EG	100	1000	1000	1000	1000	1000	1000	
Protein EH	Protein EH	100	1000	1000	1000	1000	1000	1000	
Protein EI	Protein EI	100	1000	1000	1000	1000	1000	1000	
Protein EJ	Protein EJ	100	1000	1000	1000	1000	1000	1000	
Protein EK	Protein EK	100	1000	1000	1000	1000	1000	1000	
Protein EL	Protein EL	100	1000	1000	1000	1000	1000	1000	
Protein EN	Protein EN	100	1000	1000	1000	1000	1000	1000	
Protein EO	Protein EO	100	1000	1000	1000	1000	1000	1000	
Protein EP	Protein EP	100	1000	1000	1000	1000	1000	1000	
Protein EQ	Protein EQ	100	1000	1000	1000	1000	1000	1000	
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Protein EU	Protein EU	100	1000	1000	10				

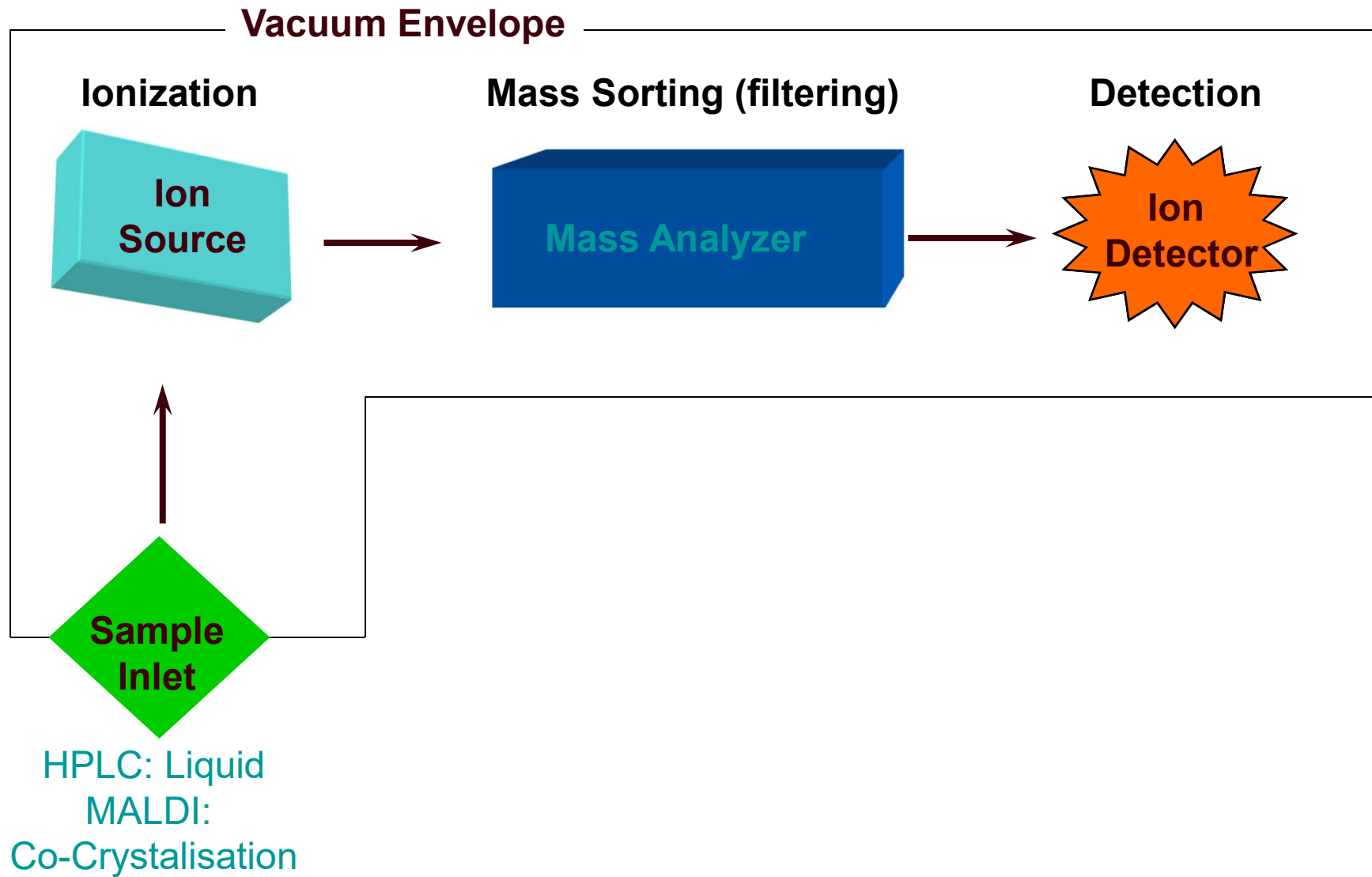
Why do we need proteolytic cleavage

- 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

Mass spectrometry based proteomics workflow



Essential components of a mass spectrometer



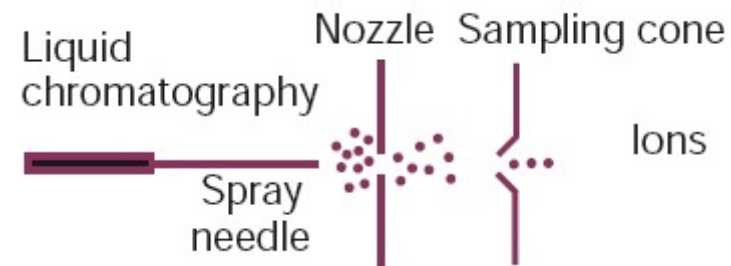
Ion sources

Ion source

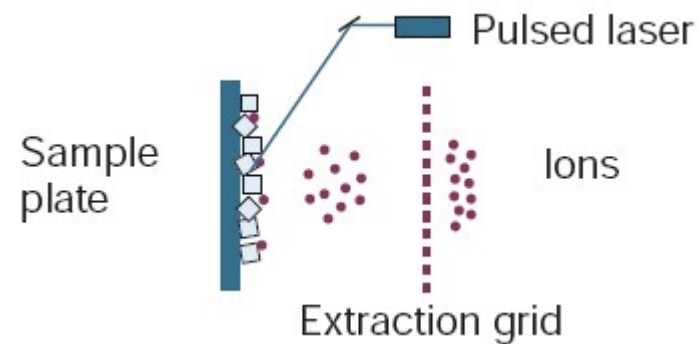
Mass analyser

Detector

Electro-Spray Ionization
ESI



Matrix-Assisted laser
desorption/ionization
(MALDI)

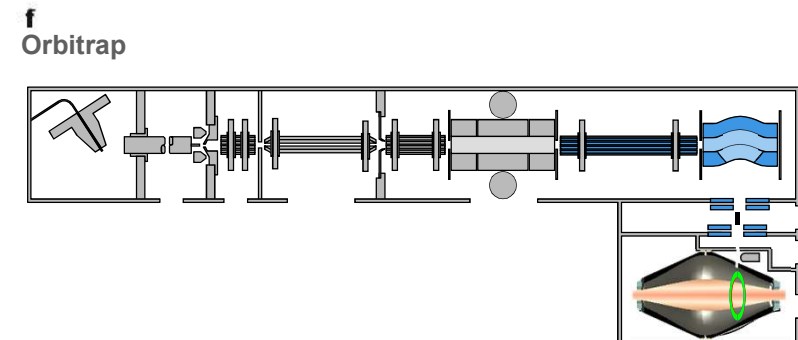
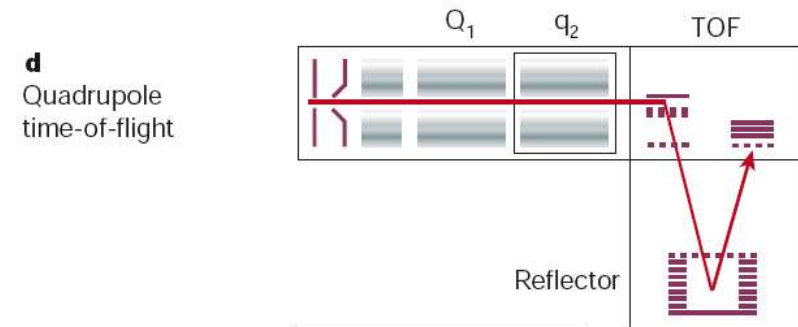
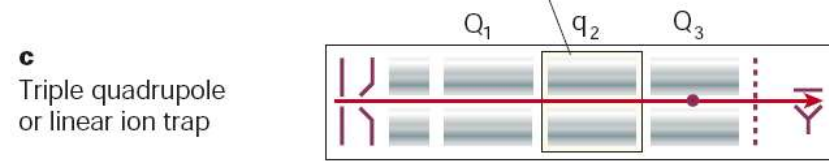
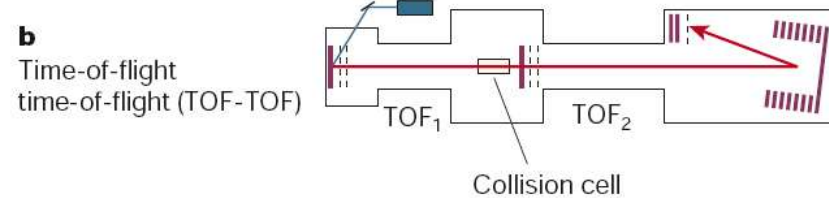
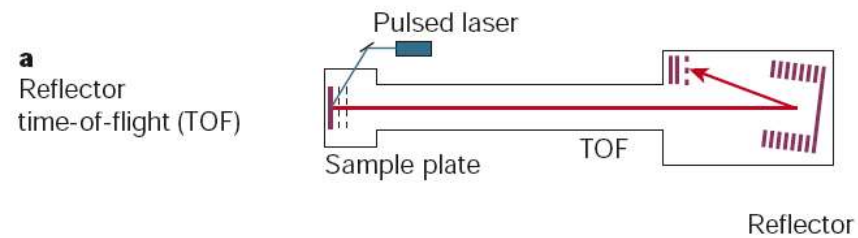


Mass analysers

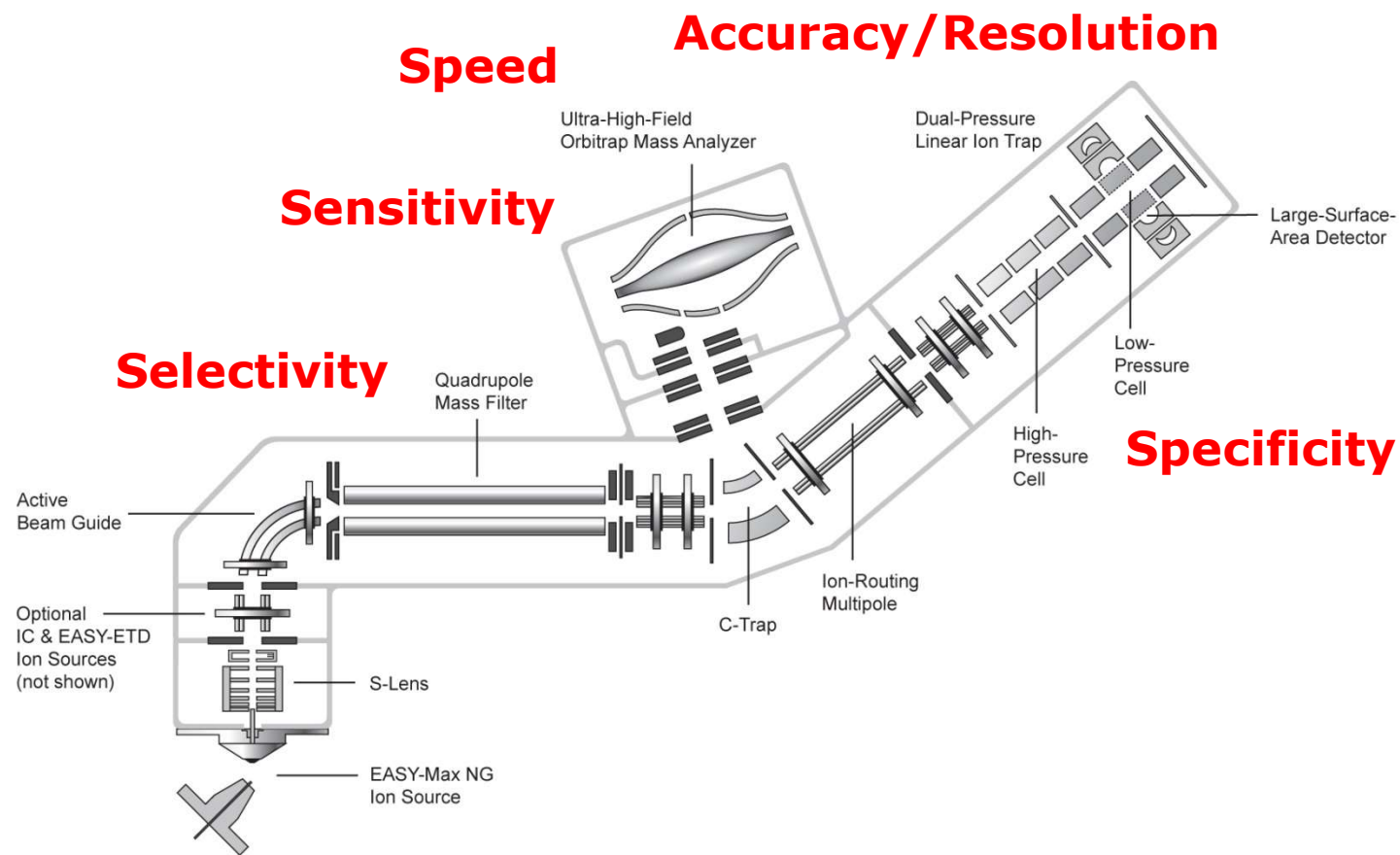
Ion source

Mass analyser

Detector

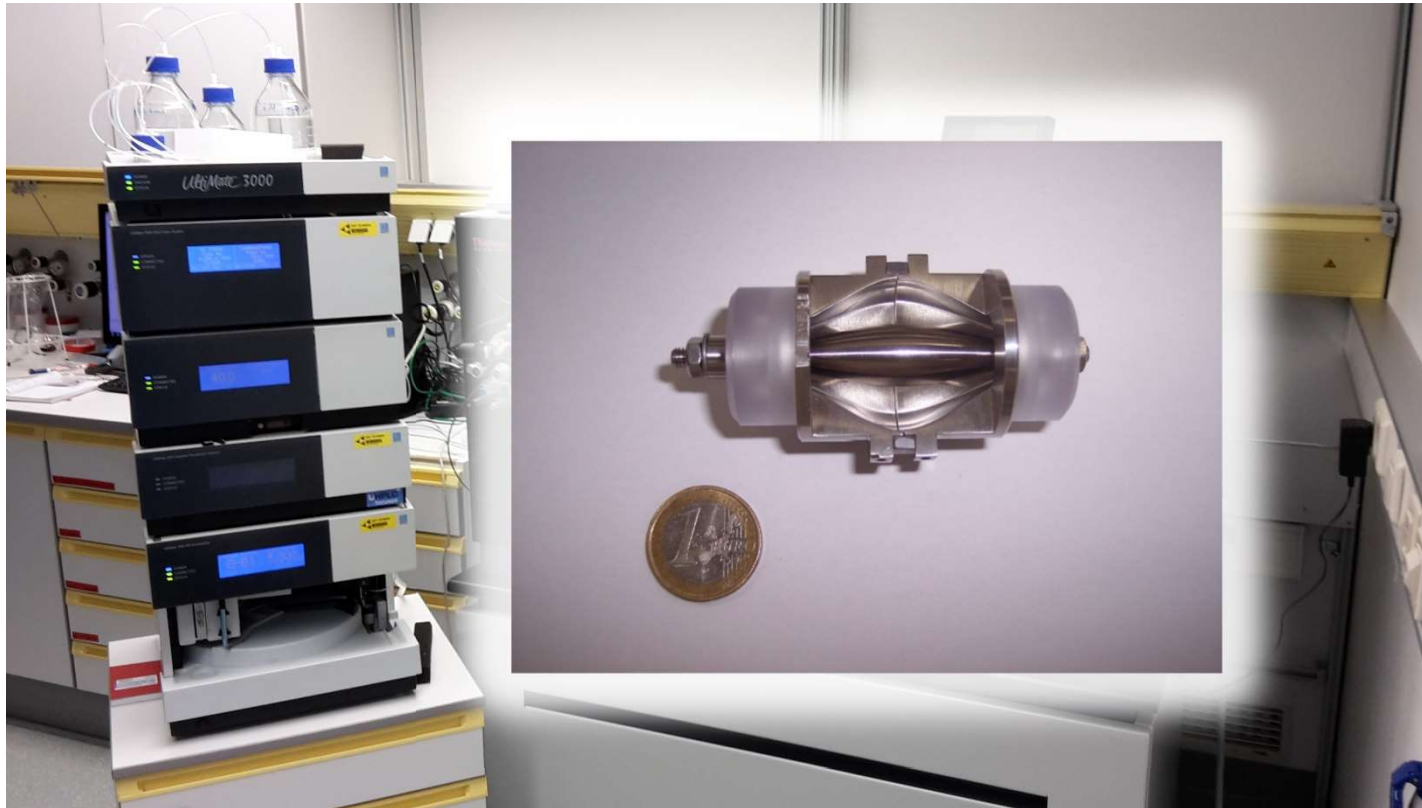


High end MS: Orbitrap Fusion

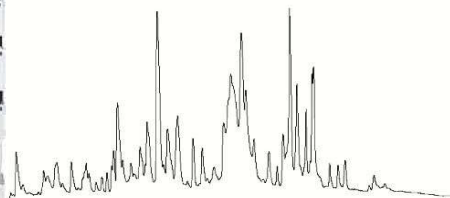
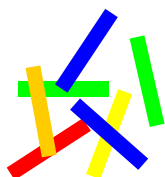
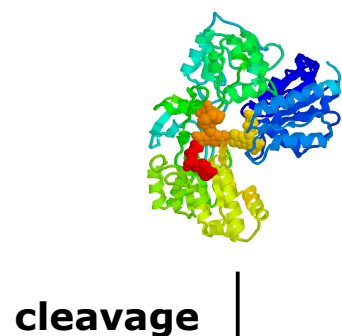


Source: Thermo Scientific

High end MS: Orbitrap Fusion



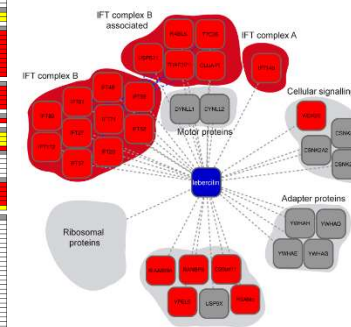
Mass spectrometry based proteomics workflow



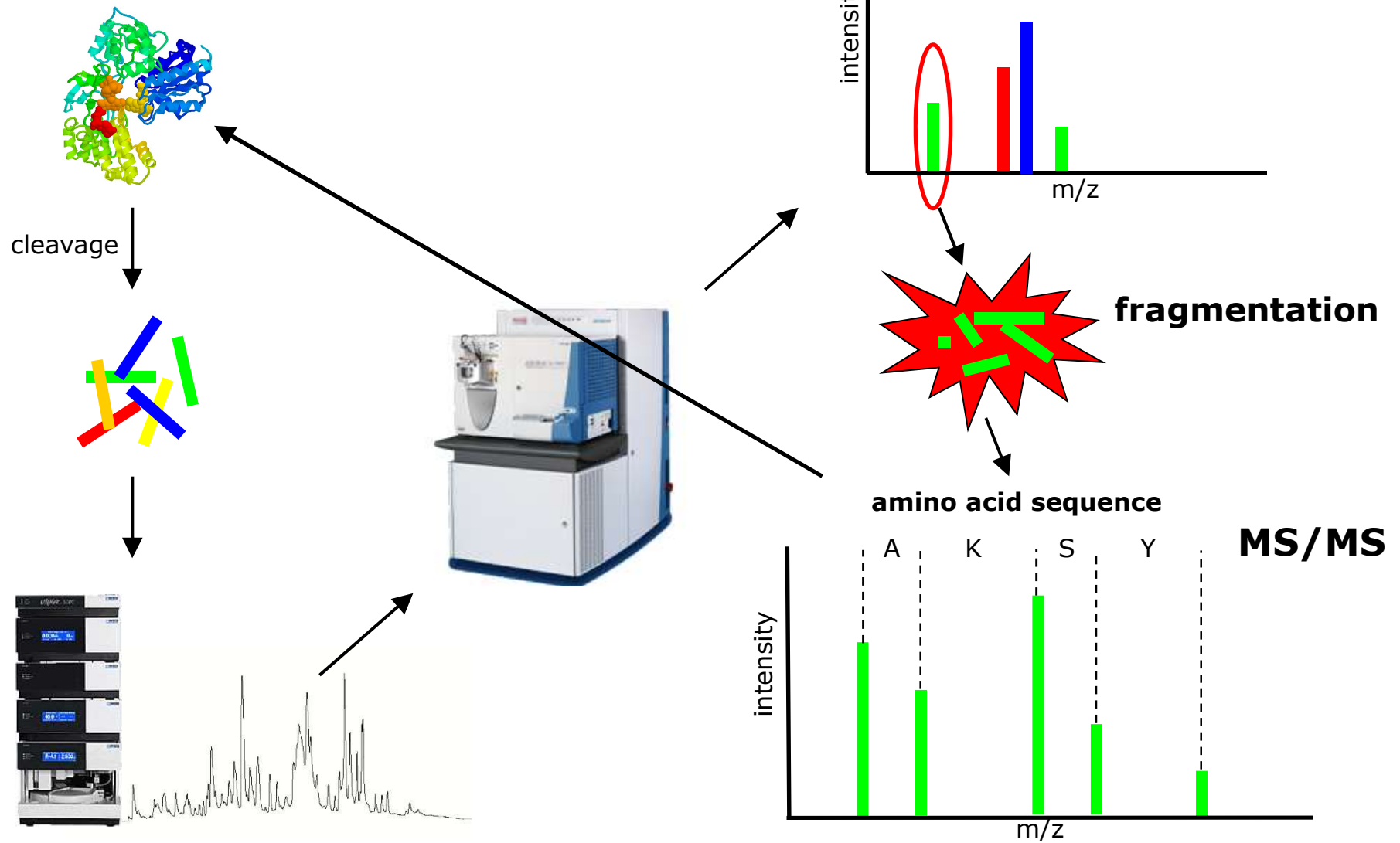
**Liquid-Chromatography/
Tandem Mass spectrometry**

Protein identification/quantification

Protein Name	Accession	Length	MW (kDa)	pI	Score	Rank	Identified	Quantified
Protein A	P00001	100	10.0	10.0	1000	1	Yes	Yes
Protein B	P00002	100	10.0	10.0	1000	2	Yes	Yes
Protein C	P00003	100	10.0	10.0	1000	3	Yes	Yes
Protein D	P00004	100	10.0	10.0	1000	4	Yes	Yes
Protein E	P00005	100	10.0	10.0	1000	5	Yes	Yes
Protein F	P00006	100	10.0	10.0	1000	6	Yes	Yes
Protein G	P00007	100	10.0	10.0	1000	7	Yes	Yes
Protein H	P00008	100	10.0	10.0	1000	8	Yes	Yes
Protein I	P00009	100	10.0	10.0	1000	9	Yes	Yes
Protein J	P00010	100	10.0	10.0	1000	10	Yes	Yes

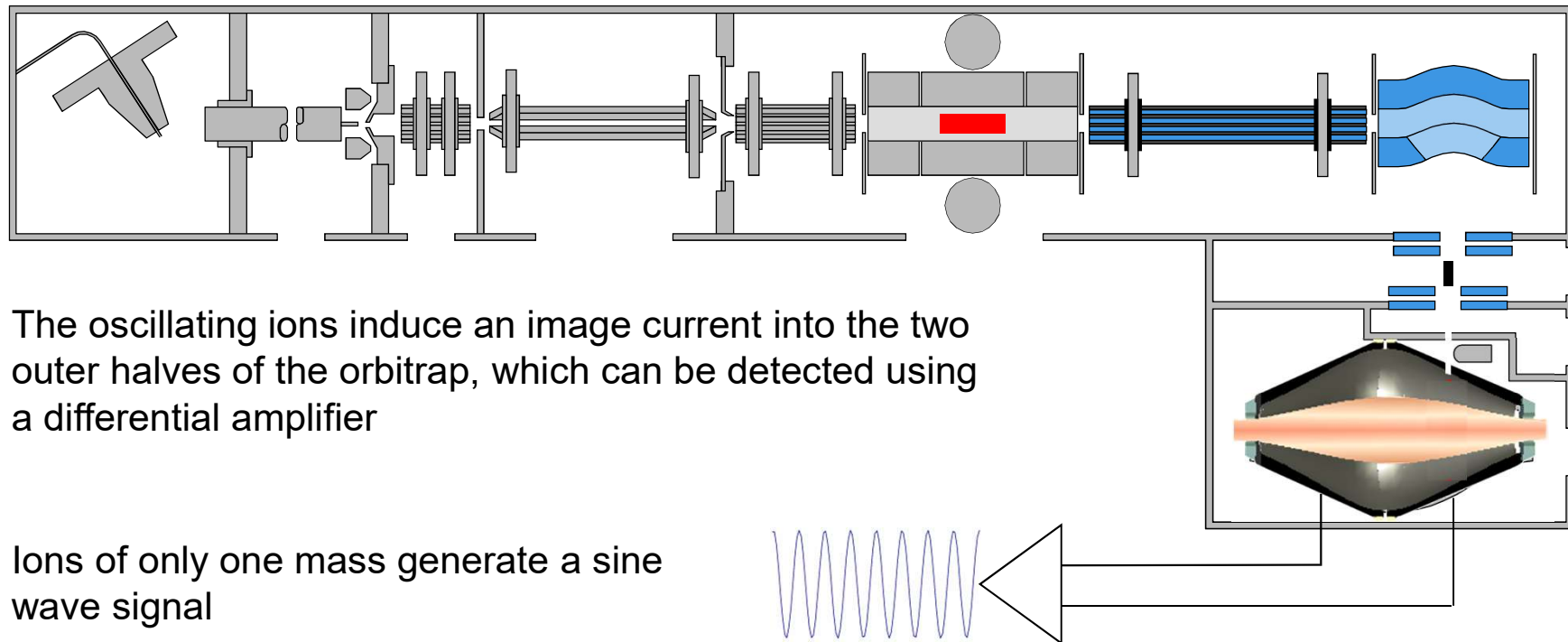


Protein identificatio by MS

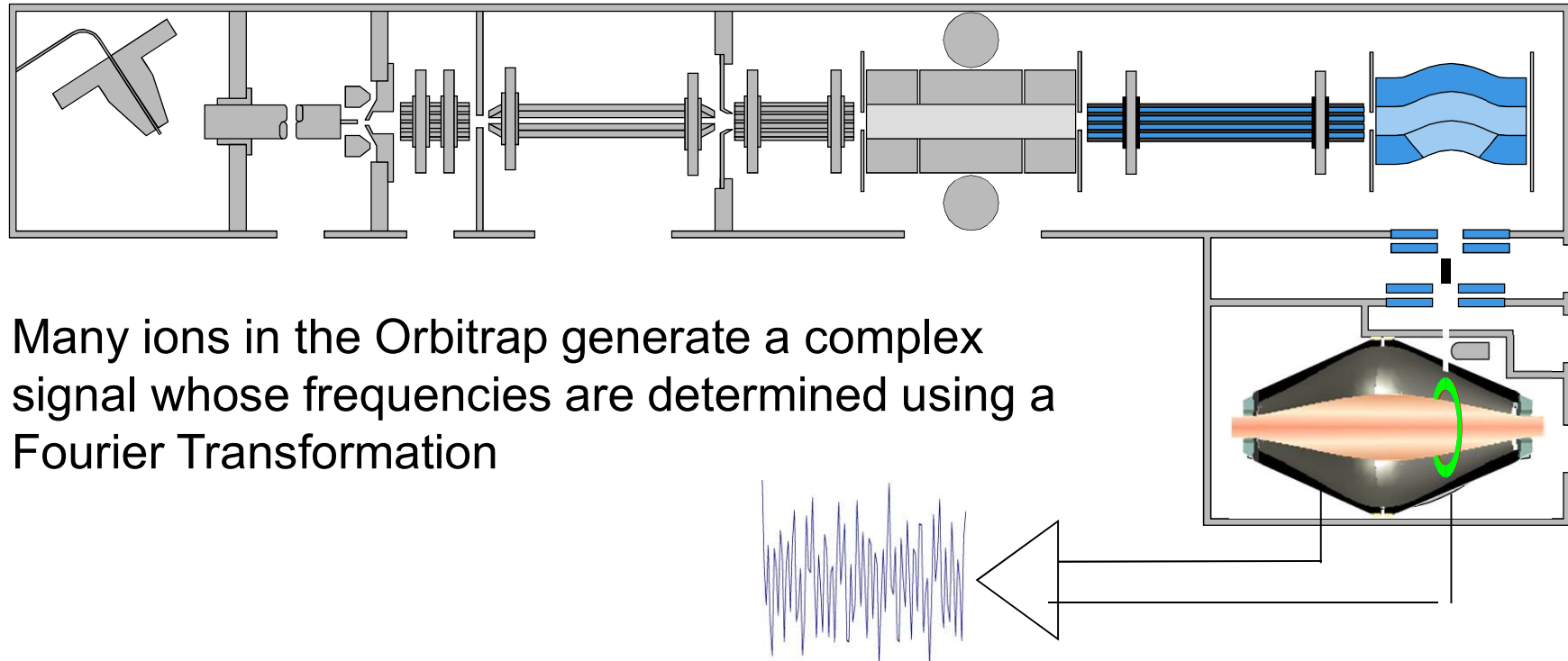


LC-MSMS on an Orbitrap

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

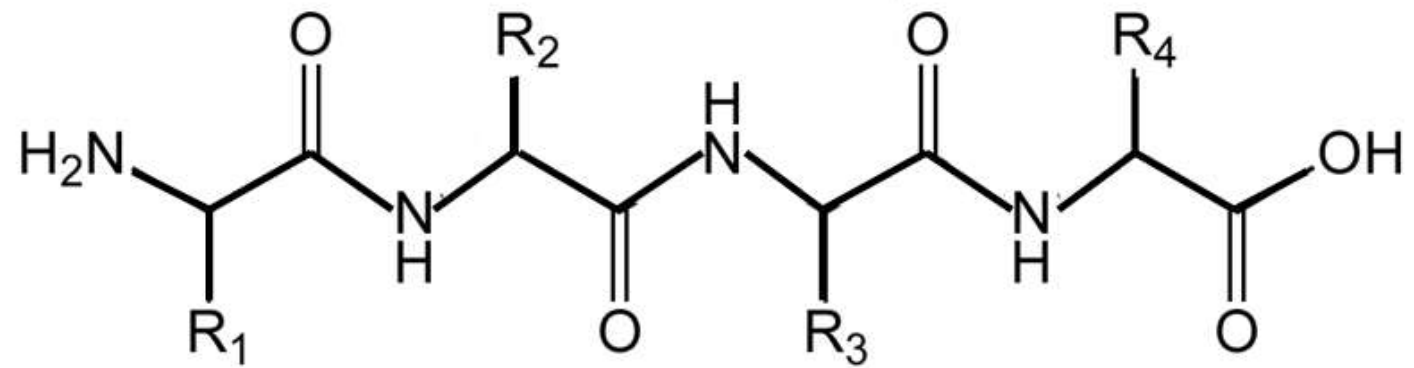


LC-MSMS on an Orbitrap

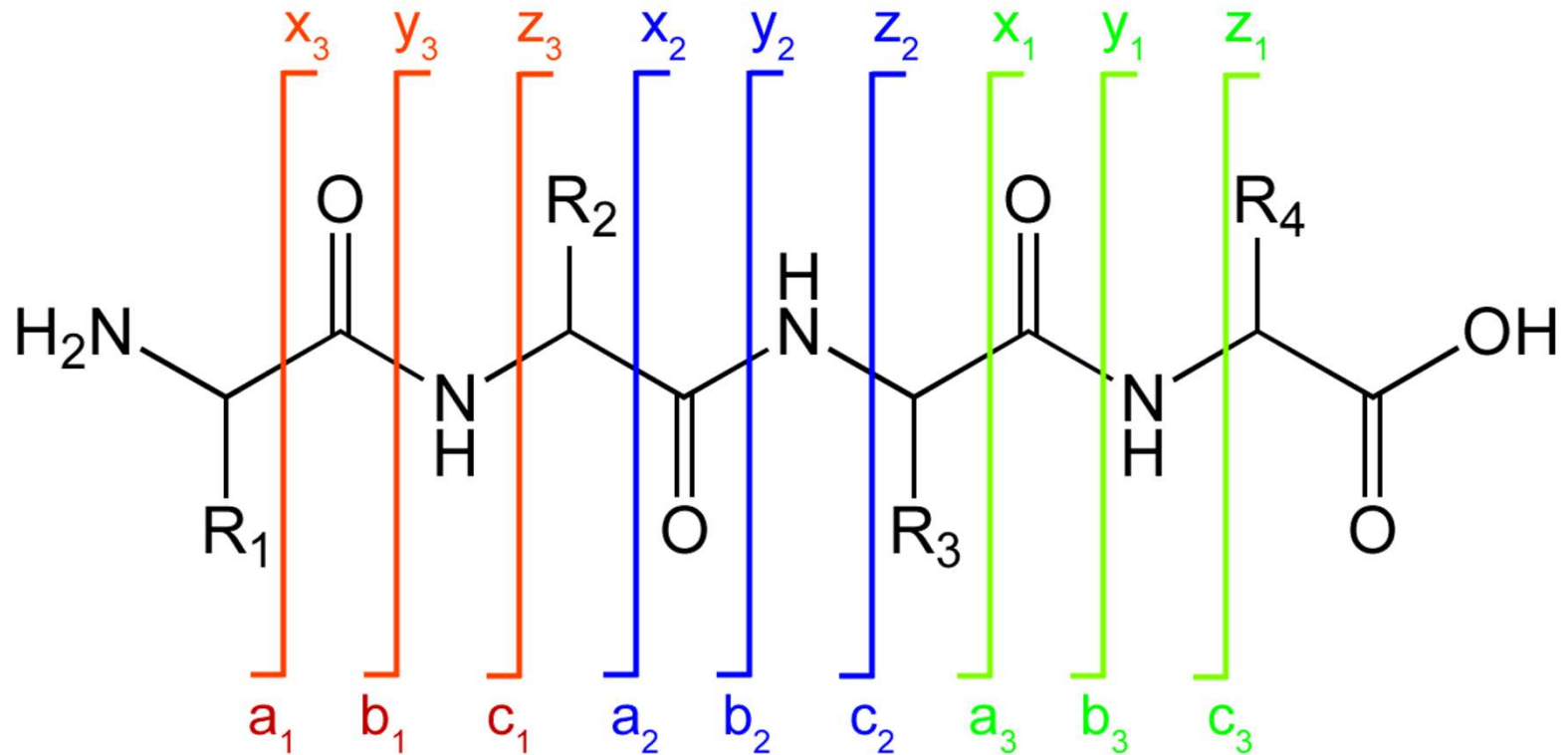


Many ions in the Orbitrap generate a complex signal whose frequencies are determined using a Fourier Transformation

Peptide Fragmentation



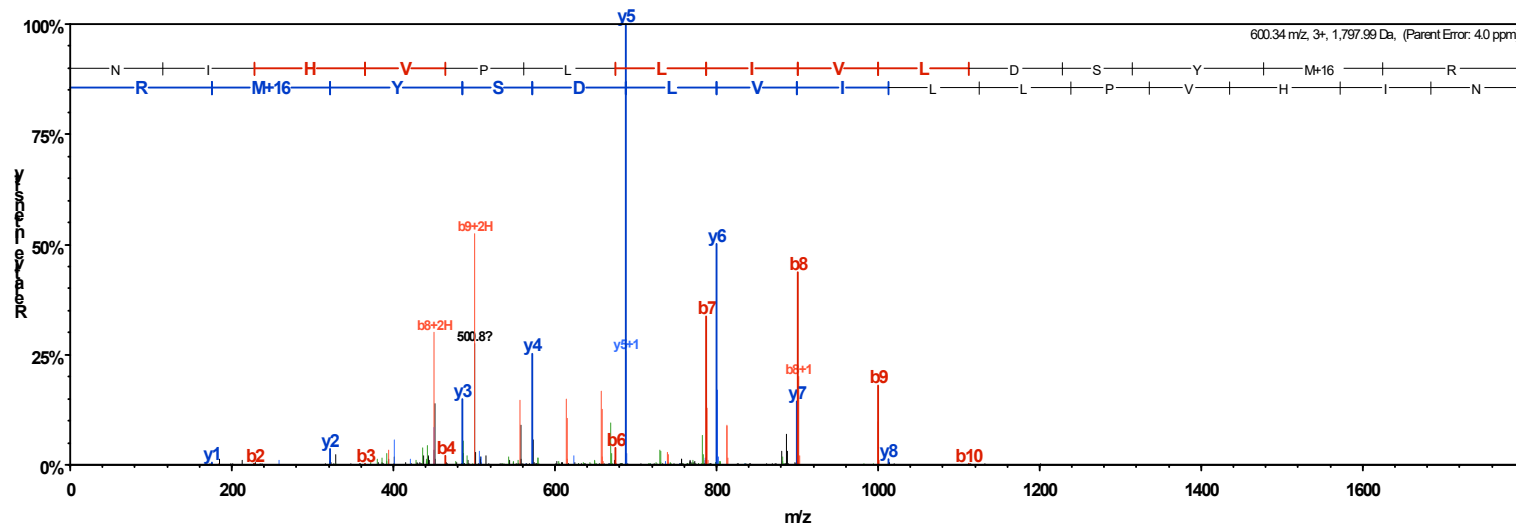
Peptide Fragmentation



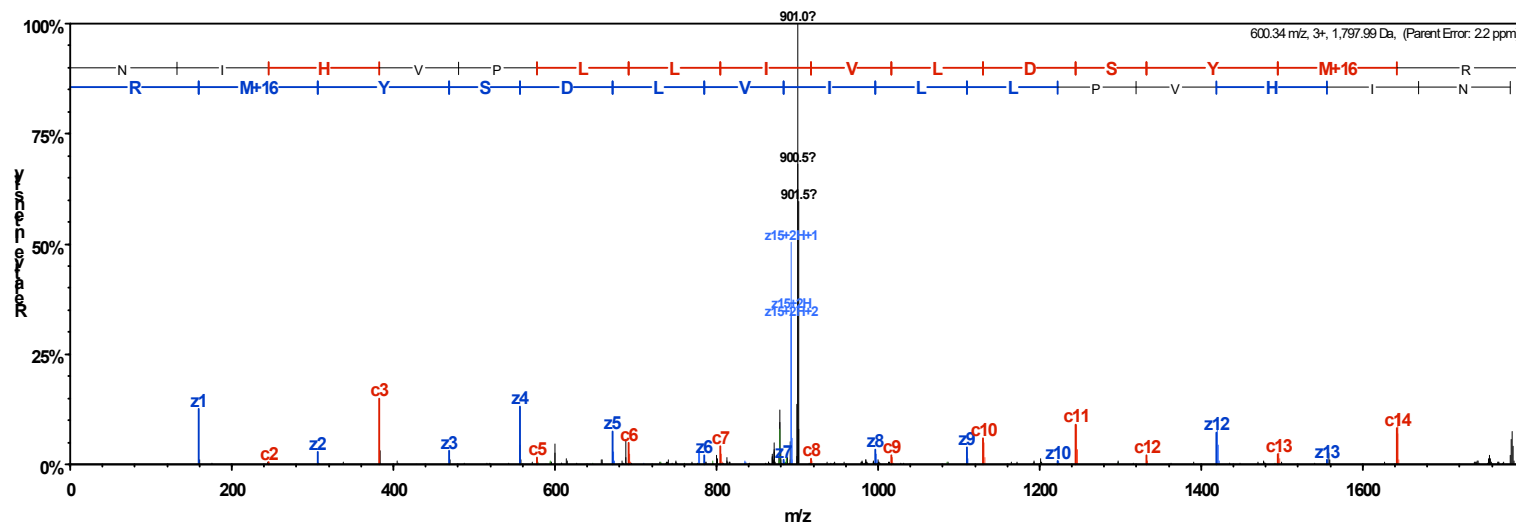
Source: Wikipedia

Peptide Fragmentation: Effect of fragmentation types

CID

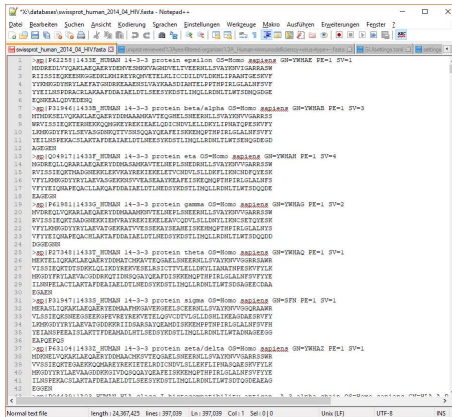


ETD



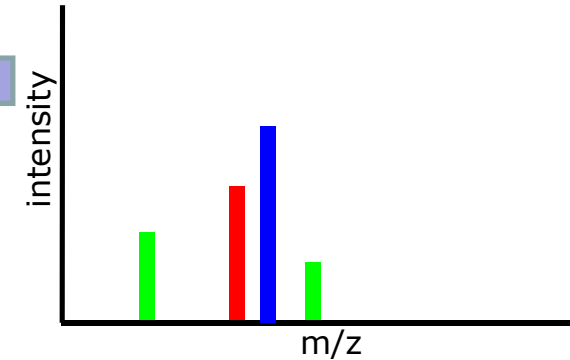
Identification by sequence tags

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



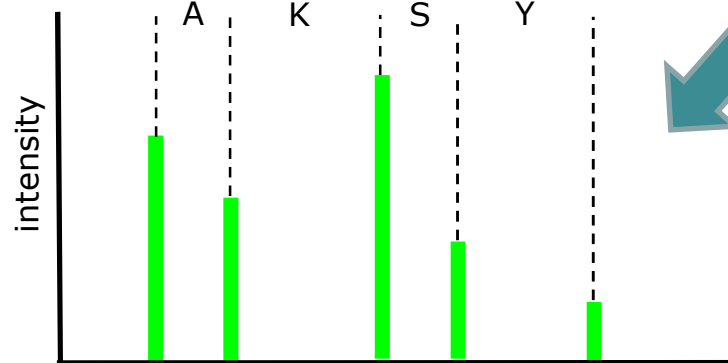
Search engines
(Mascot, MaxQuant,
OMSSA, X!tandem,...)

Measured
molecular weight

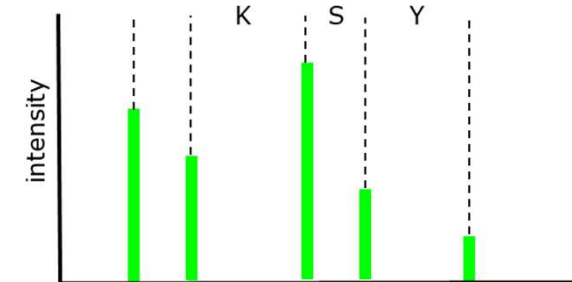


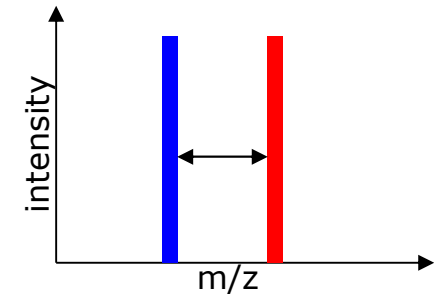
List of potential peptides
P1, P2, ...

Peptide ID and annotation



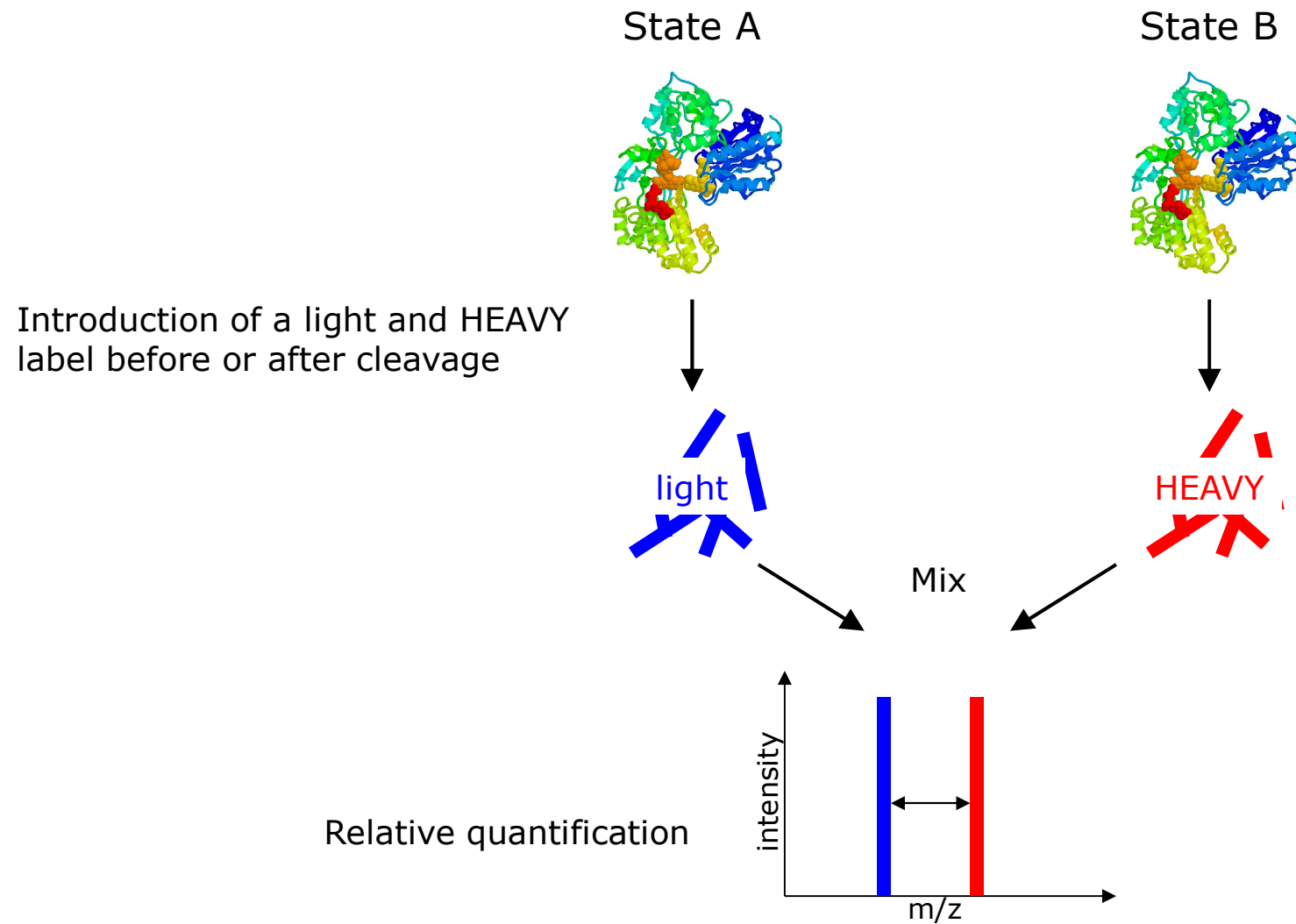
Sequence tag



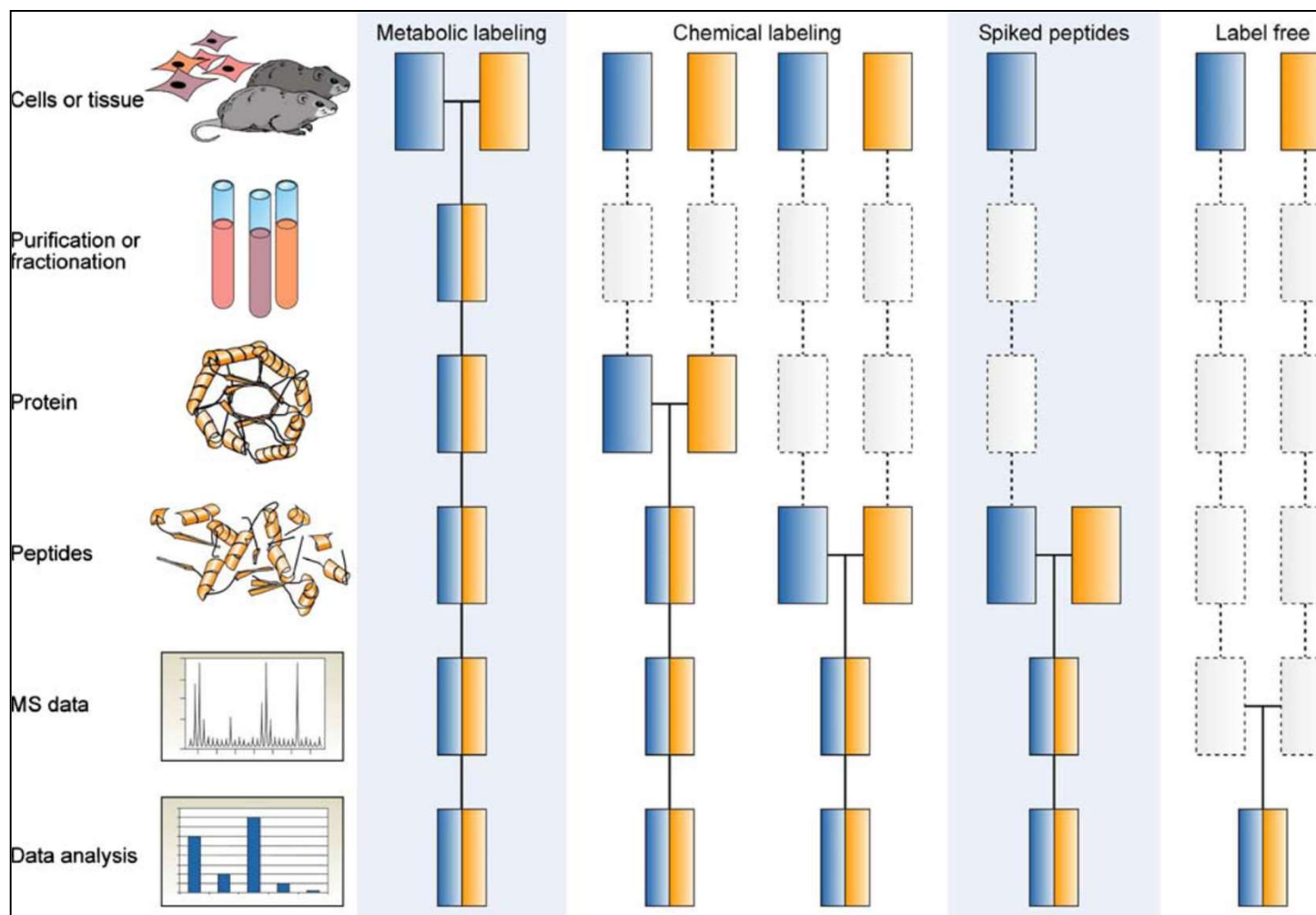


Protein Quantification

MS-based quantification: Principle



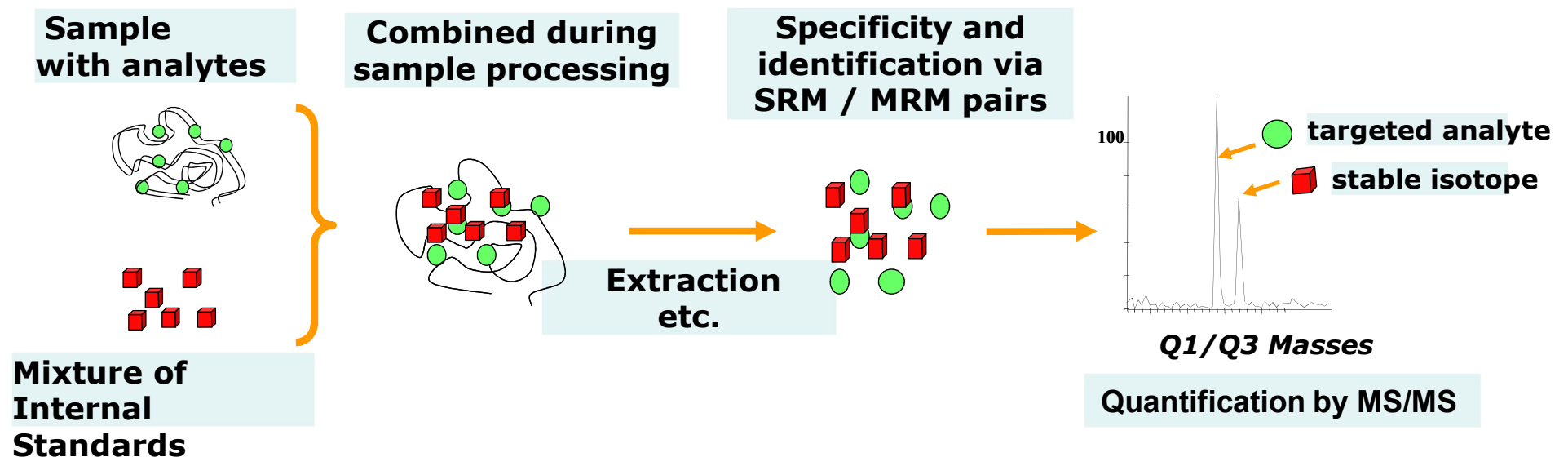
MS-based quantification: An overview



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

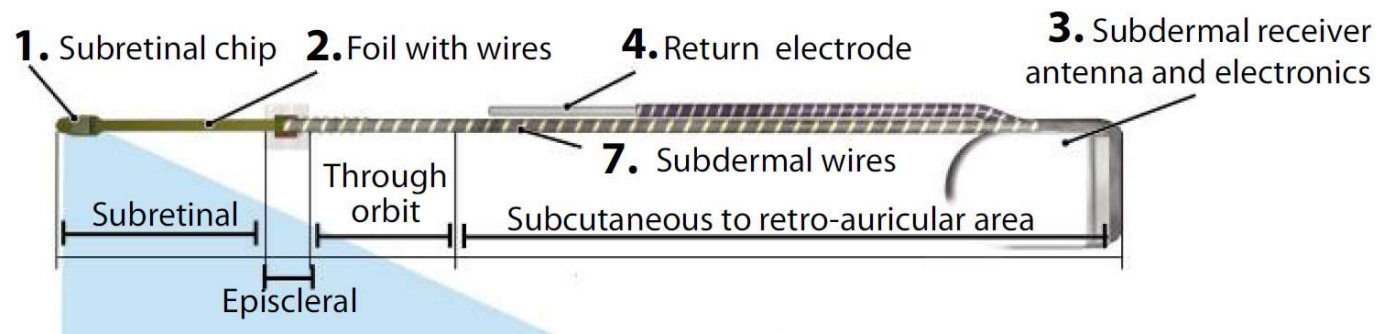
MS-based quantification: Absolute quantification

Quantification is achieved using stable isotope dilution (SID)



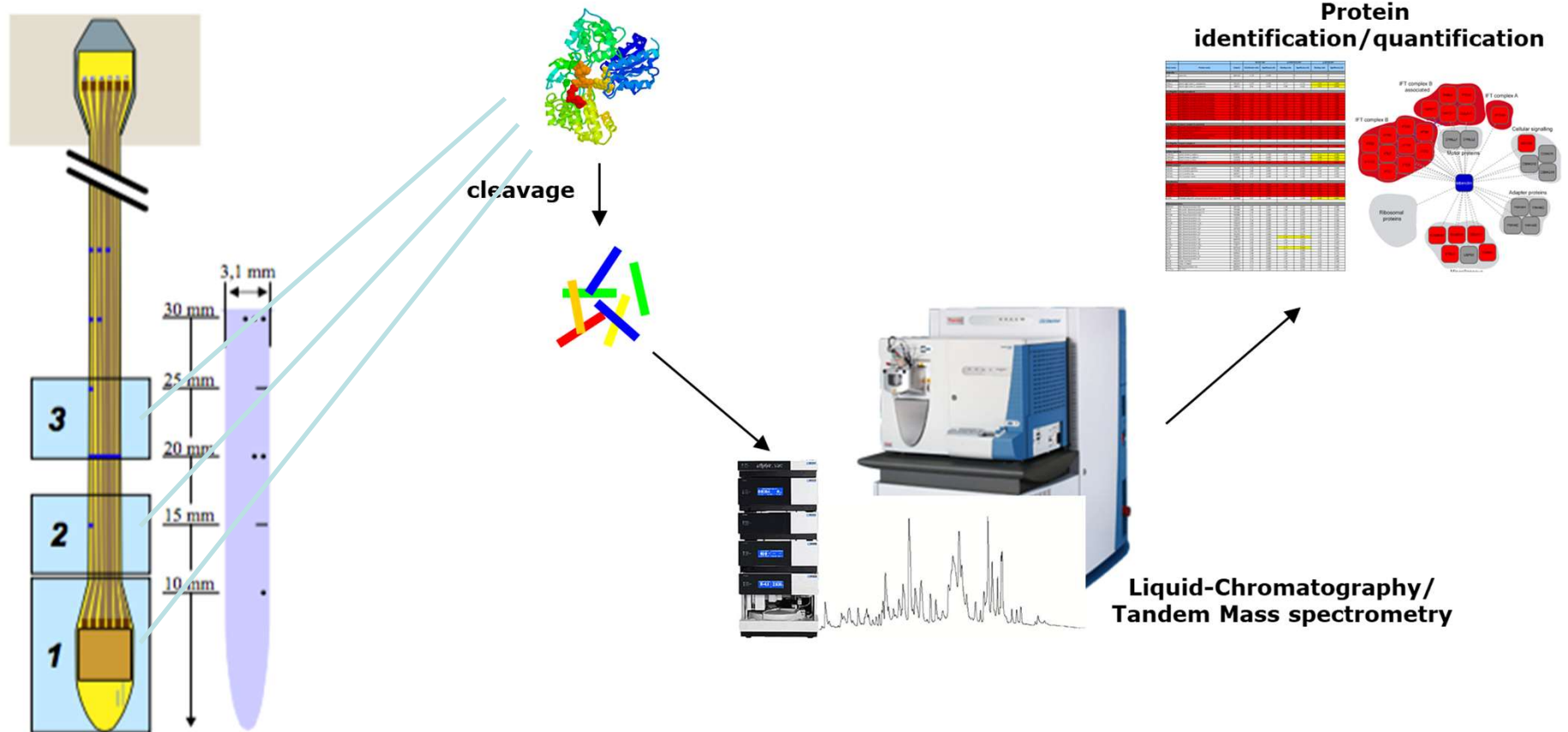
Application to Implantology

Explants sections



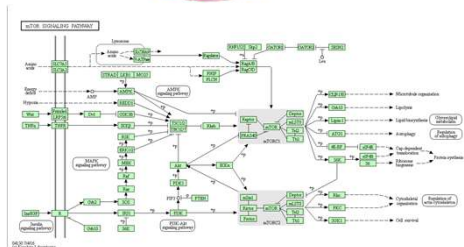
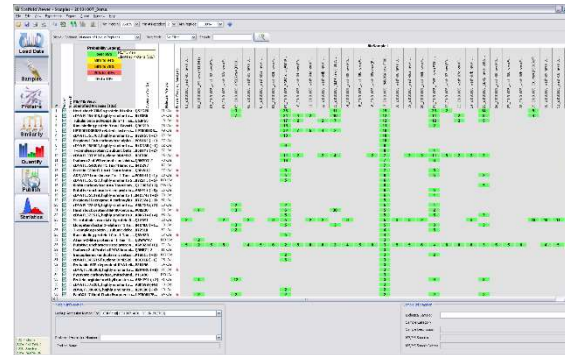
Section	Image	Description
1		Contains the chip, Localization: subretinal
2		Part of the transmitting wire Localization: subretinal
3		Part of the transmitting wire Localization: subretinal, peripheral

Surface analysis of proteins

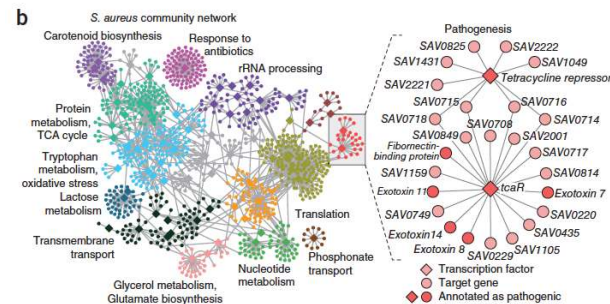


Getting to the biological processes

List of proteins *Relative or quantitative*



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



<http://geneontology.org/>





Questions?