Functional Genomics and Proteomics
National Centre for Biomolecular Research
Faculty of Science Masaryk University

CEITEC

Protein characterization by mass spectrometry

C7250

Part V

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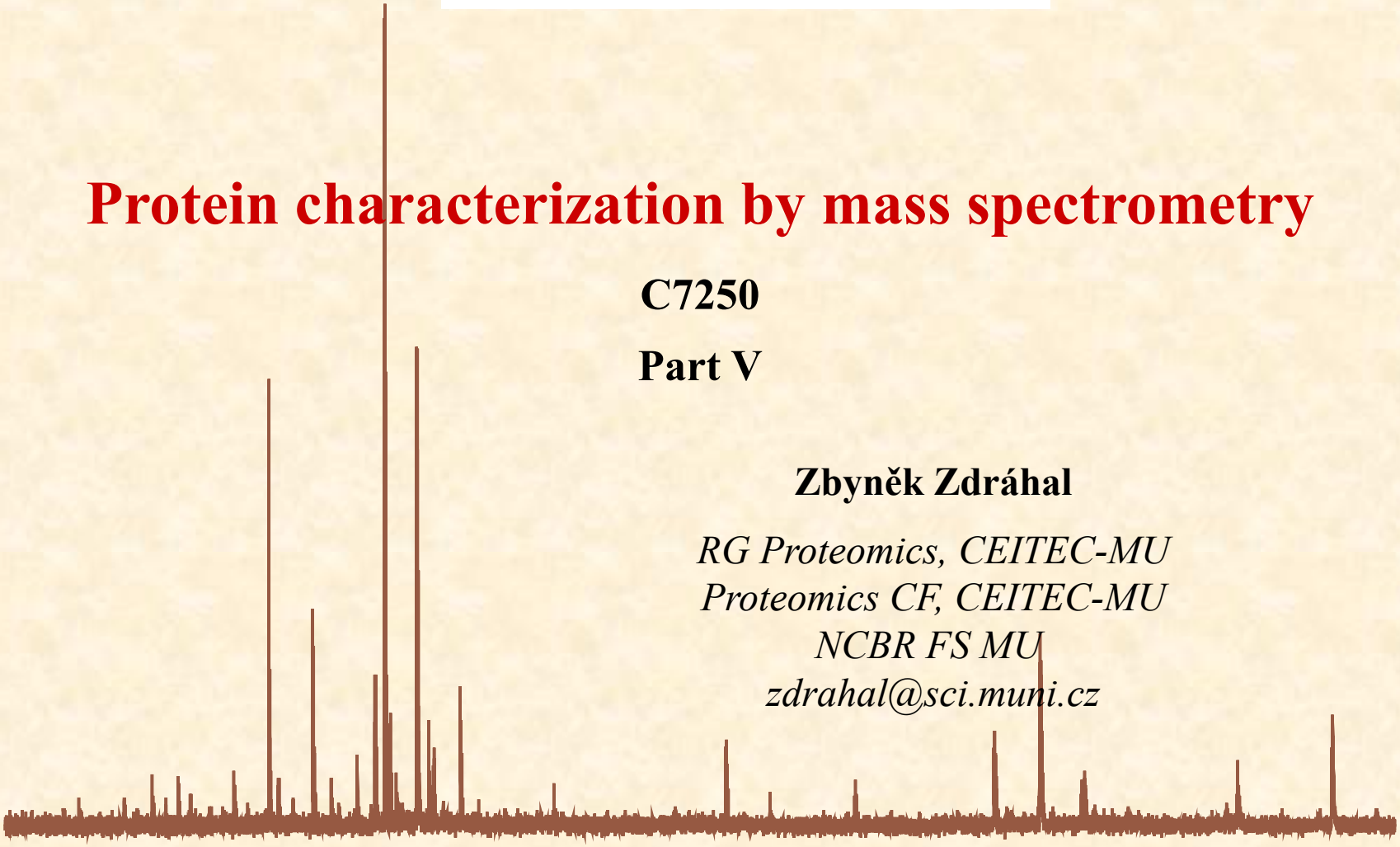
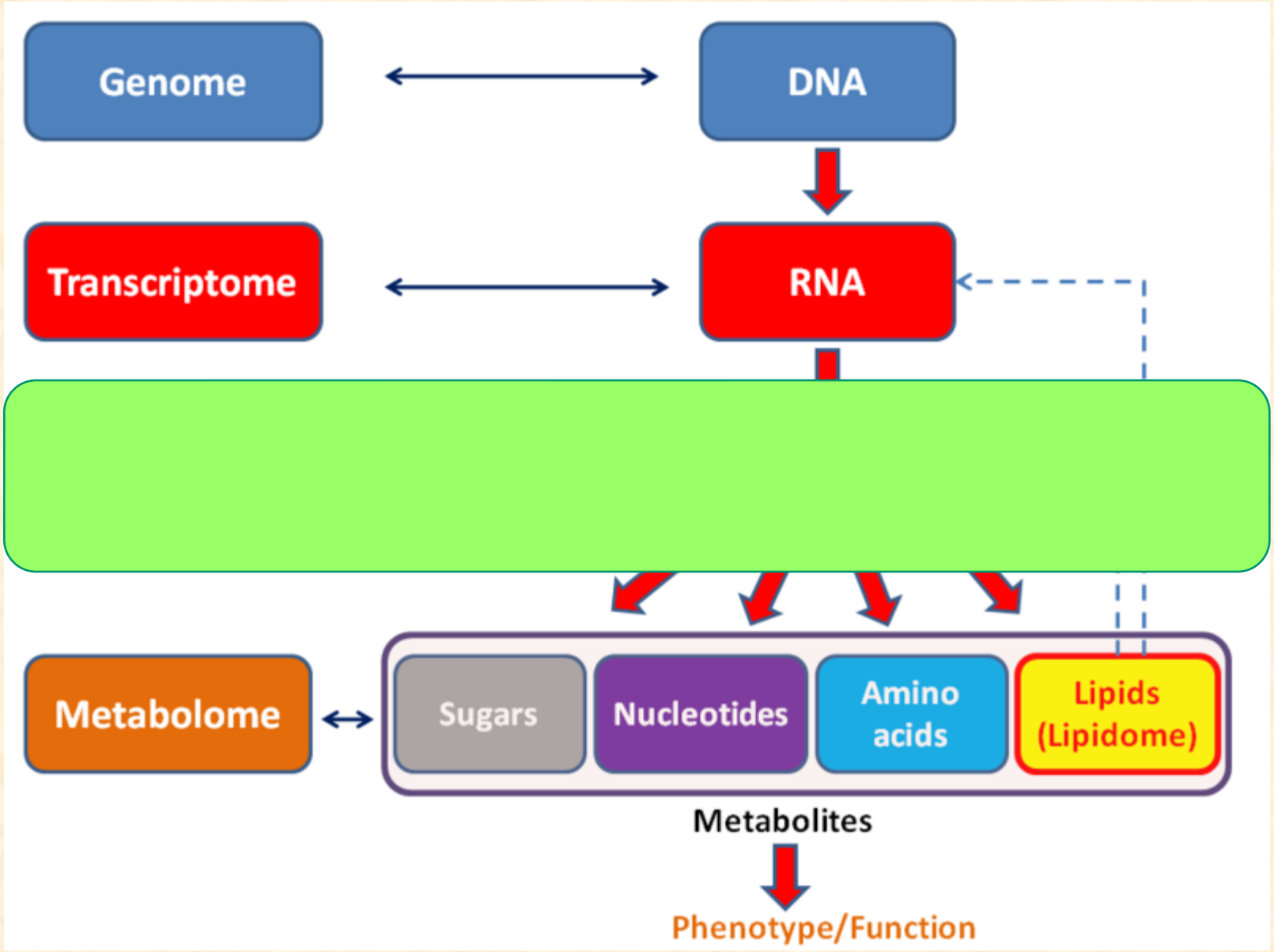
Proteomics CF, CEITEC-MU
NCBR FS MU
zdrahal@sci.muni.cz

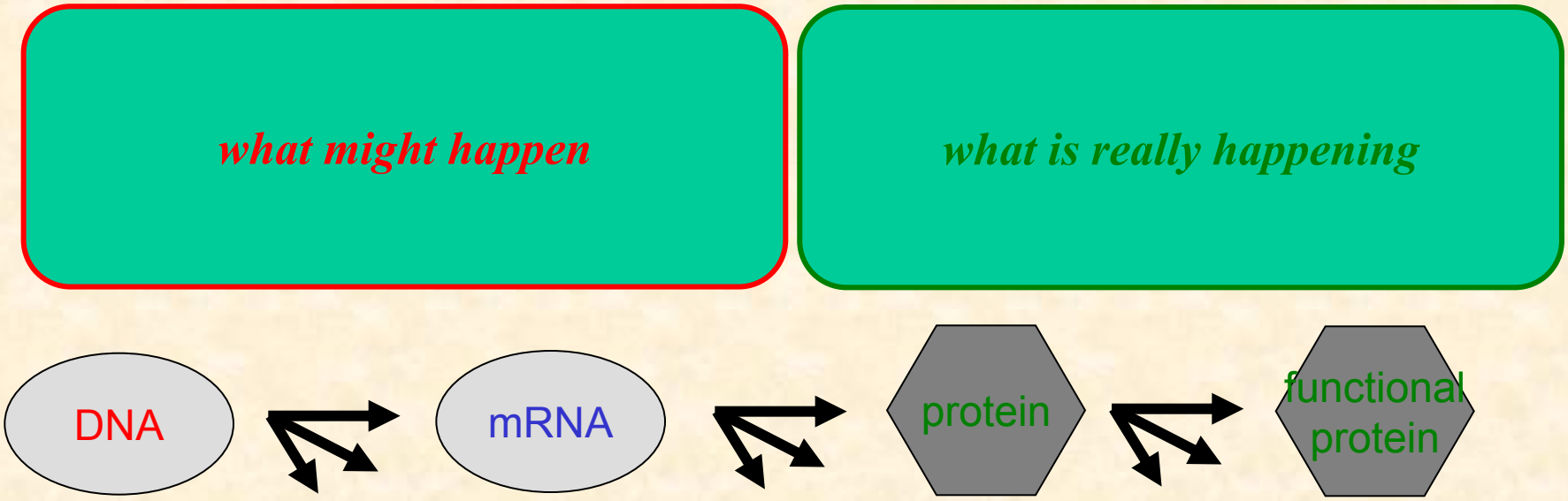


Proteomic MS applications



http://en.wikipedia.org/wiki/File:Metabolomics_schema.png

Proteomics – discipline dealing with proteome analysis



transcription
posttranscriptional modifications
(alternative splicing etc.)

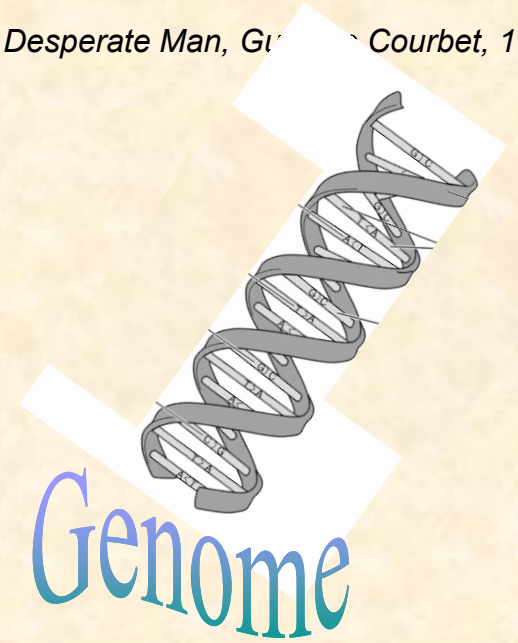
translation

posttranslational mods
Protein complexes

Proteomics - Why?

- several proteins/proteoforms might form from each gene, not possible to indicate them by DNA/RNA analysis
- there no direct correlation between mRNA content and final content of proteins
- functionality of protein depends frequently on its interaction with other proteins or DNA/RNA
- only at protein level epigenetics factors of gene expression regulation are detectable

The Desperate Man, Gu… Courbet, 1844-45



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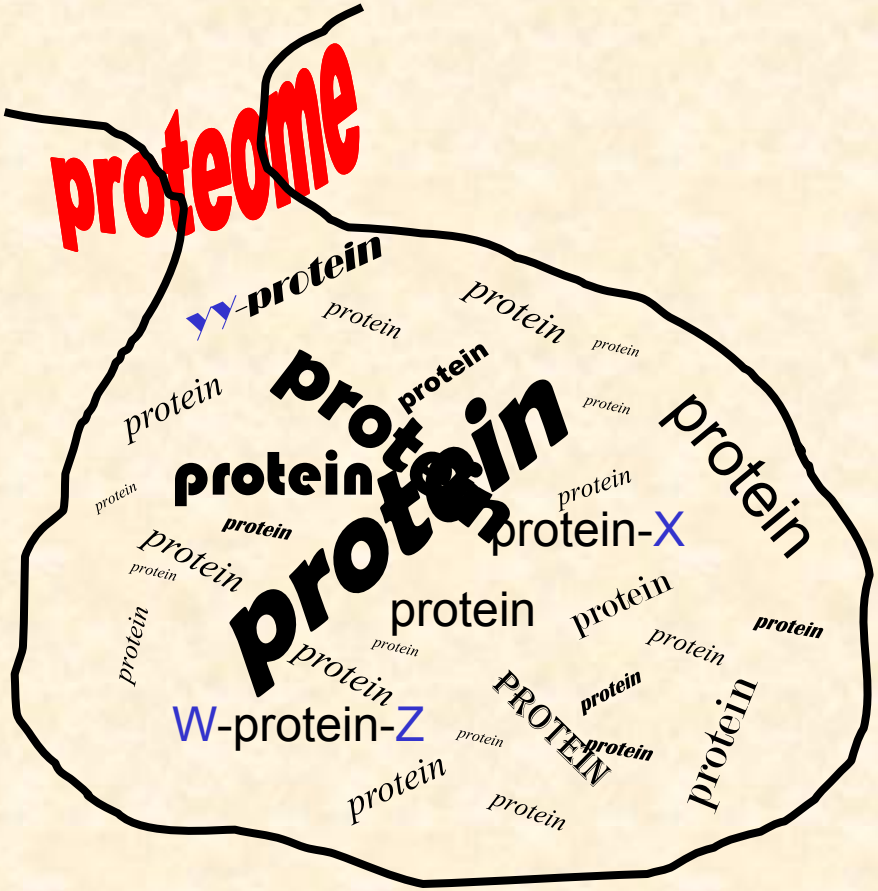
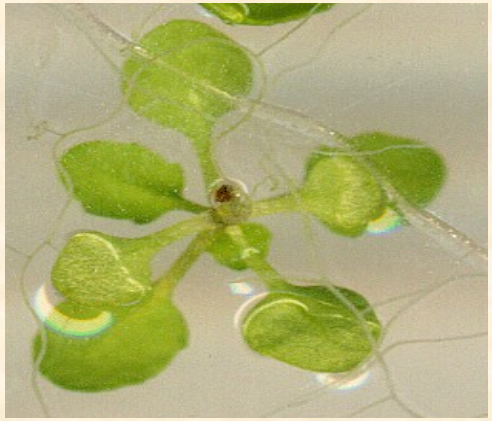
Proteome analysis

characterization of all proteins including all their forms in cell (tissue, organisms) at given time under given conditions



Genome	*versus*	**Proteome**
relatively stable DNA sequence		all proteins including all their forms in cell (tissue, organisms) at given time under given conditions
4 basic building units		20 basic building units
efficient analytical techniques developed (PCR, NGS)		necessity of development of sensitive and reliable techniques for identification and quantitation

Arabidopsis thaliana



Mouse-ear cress

Genome 0.135 x 10^9 bp, ~ 27 000 geness

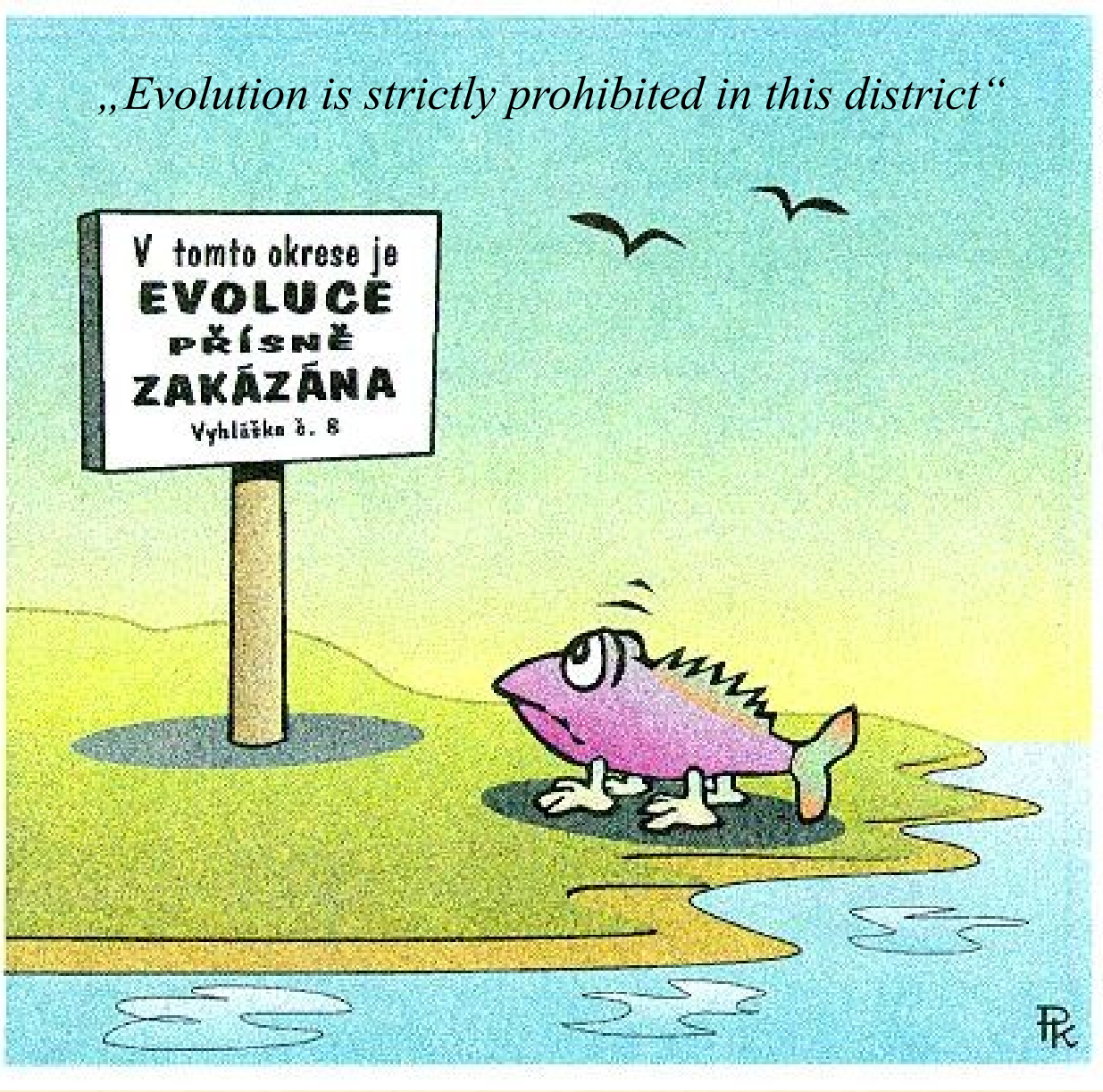
32 000 proteins

human genome - 3.3 x 10^9 bp, ~ 21 000 genes

http://www.uniprot.org/taxonomy/complete-proteomes

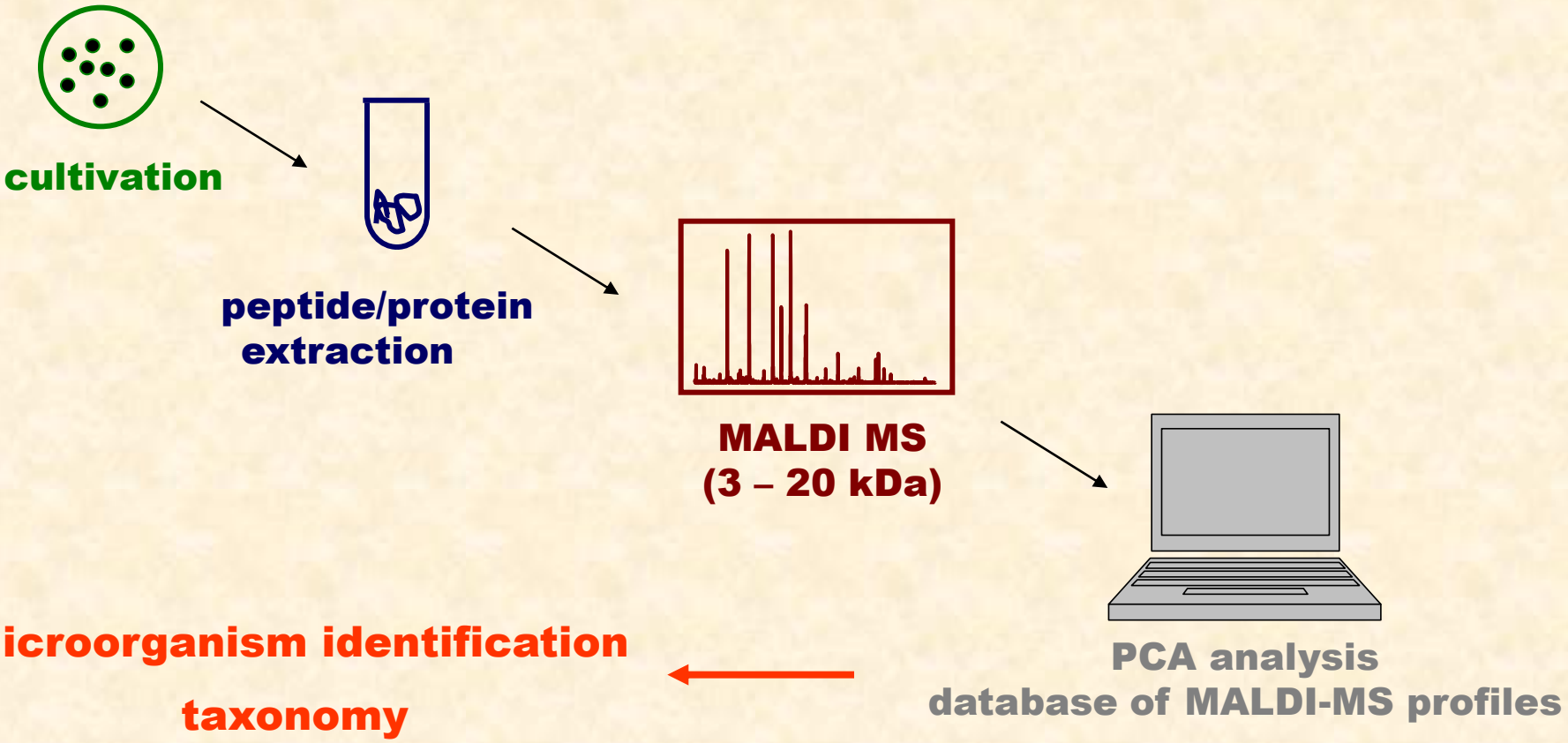
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/G/GenomeSizes.html

„Evolution is strictly prohibited in this district“



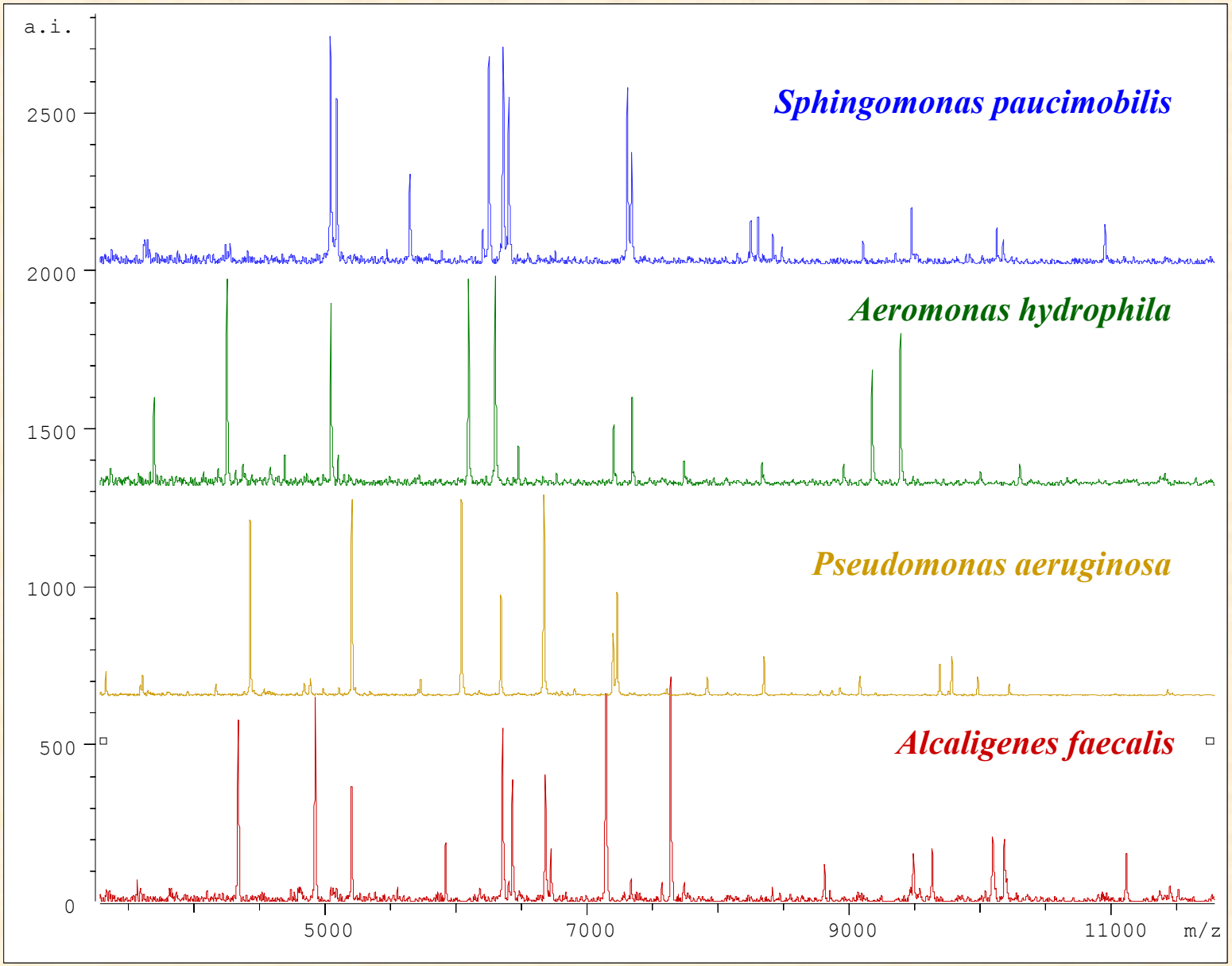
MALDI-MS profiling

Identification of microorganisms by MALDI-MS

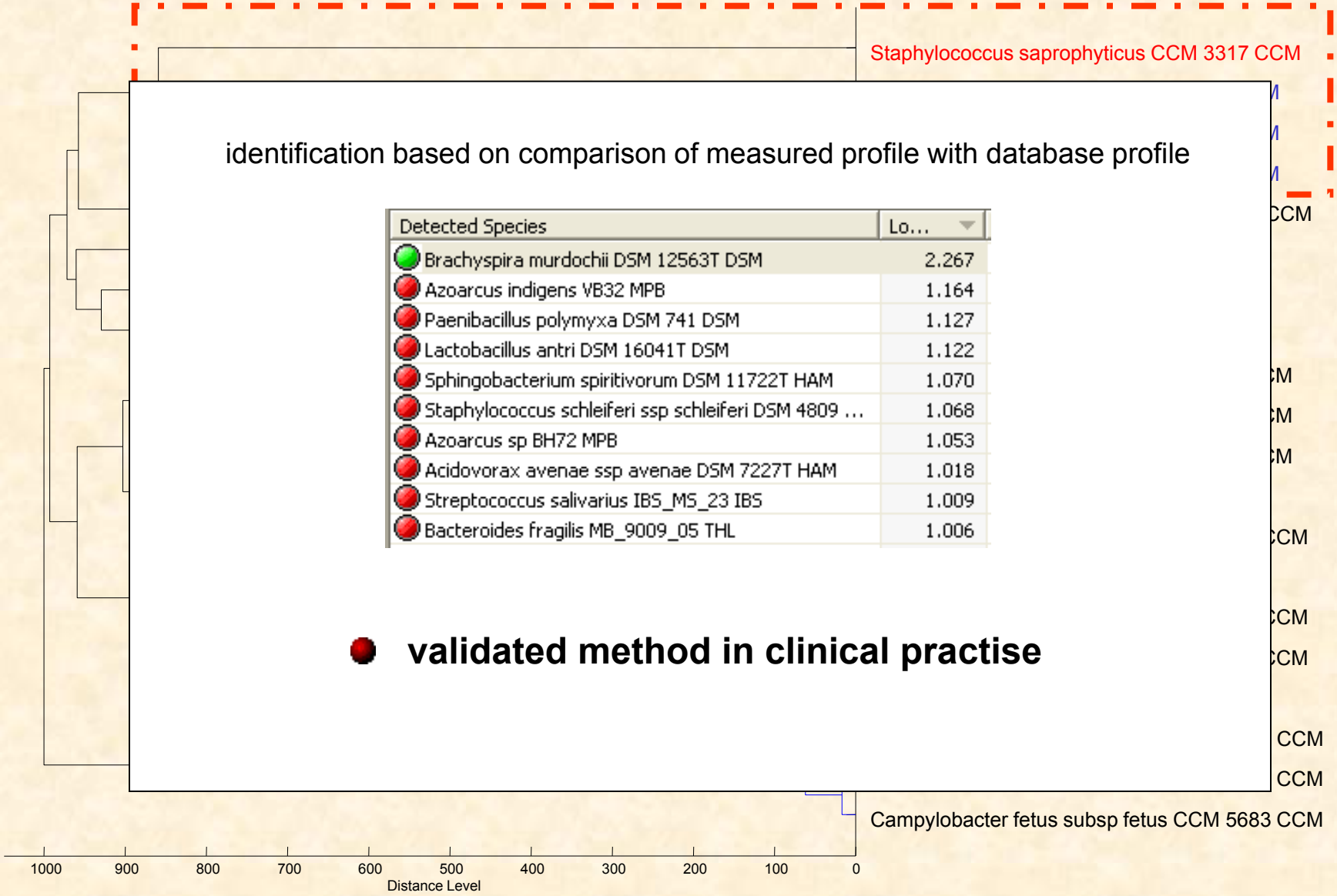


MALDI-MS spectra (profiles) of selected bacteria

C7250

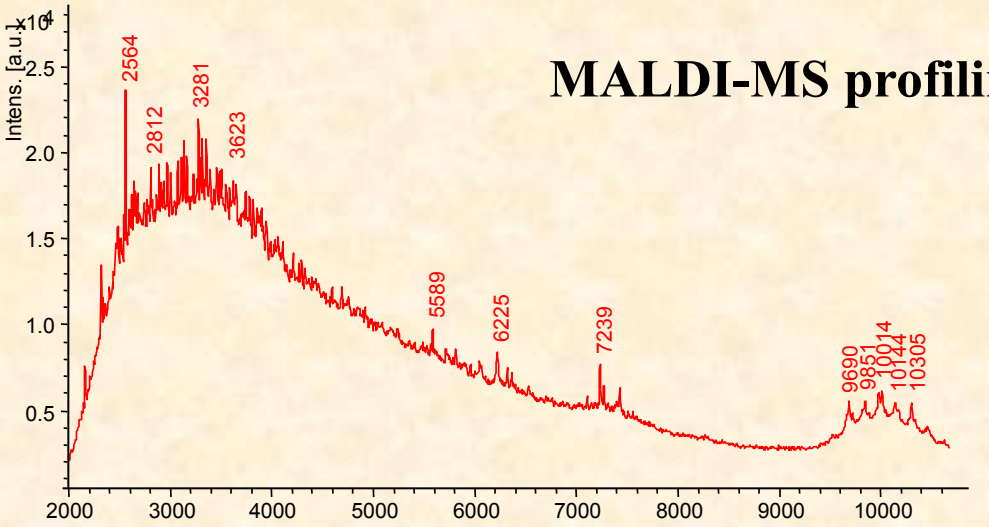


Graphical expression of MALDI-MS bacteria profile similarity

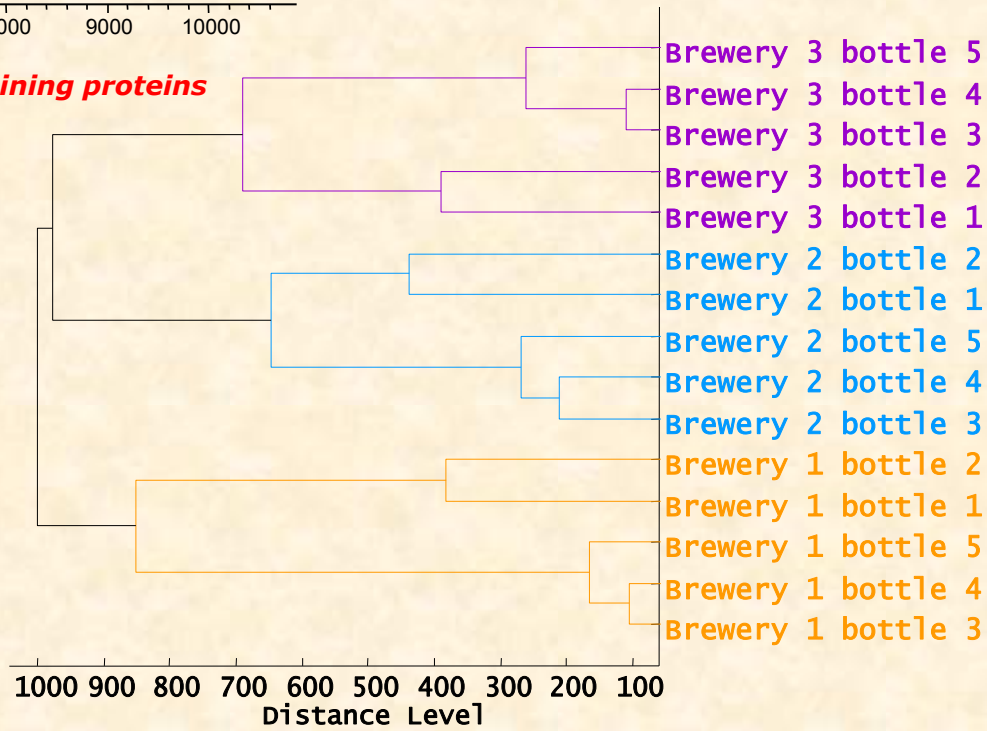


L. Tvrzová, A. Teshim, I. Sedláček, M. Lexa, A. Voráč, O. Šedo,, A. Kostrzewa, T. Meier

MALDI-MS profiling of beer



MALDI-TOF MS fingerprint containing proteins

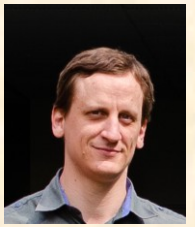


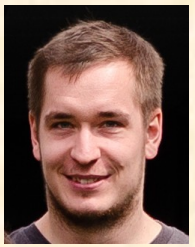
cooperation with FCH BUT Brno
prof. Márová

MALDI-MS profiling of spider venoms

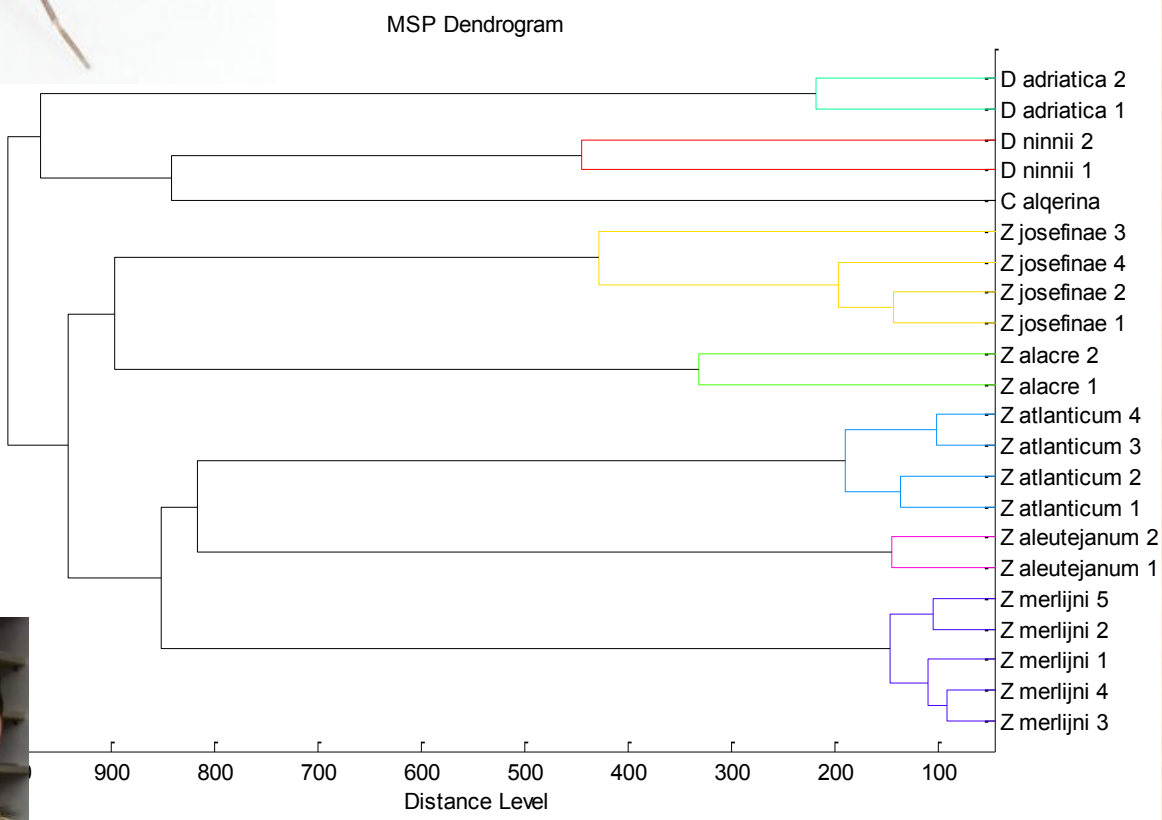
Z. merlijni

- evolution of food specialisation in spiders
- species adaptations
- ant-eating spiders





cooperation with prof. Pekar, FS MU

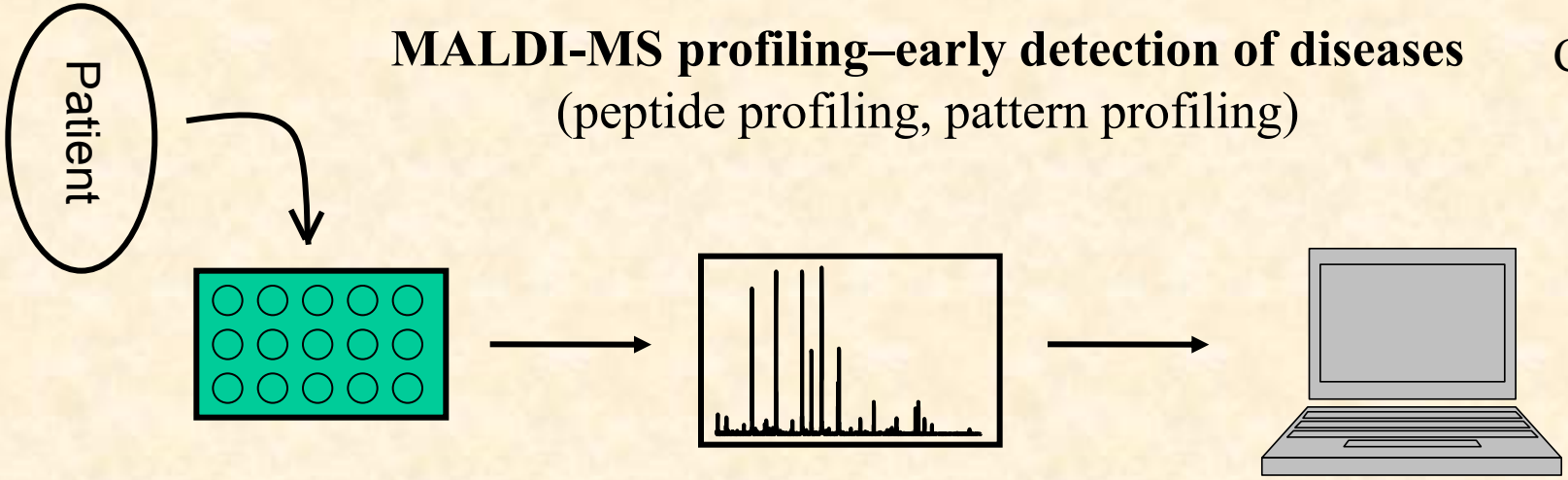


Pekár S. et al., J. Anim. Ecol., 81 (4), 838-848 (2012)
Bočánek O. et al., Toxicon, 133, 18-25 (2017)
Pekár S. et al. Mol. Ecol., 27 (4), 1053-1064 (2018)

MALDI-MS profiling–early detection of diseases

(peptide profiling, pattern profiling)

C7250

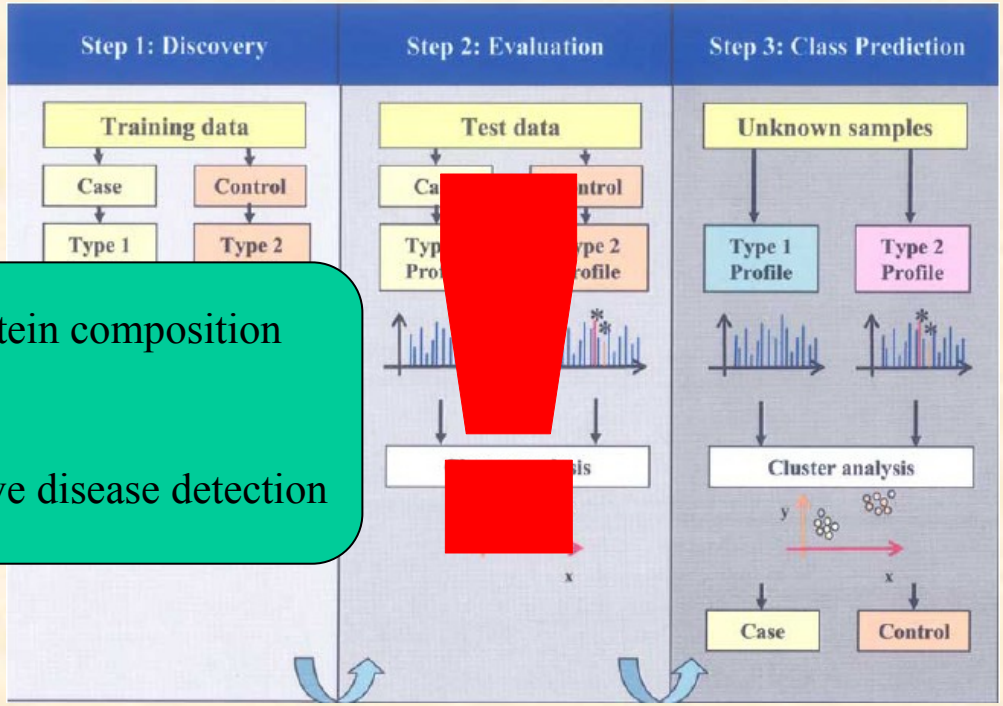


no or minimal sample prep

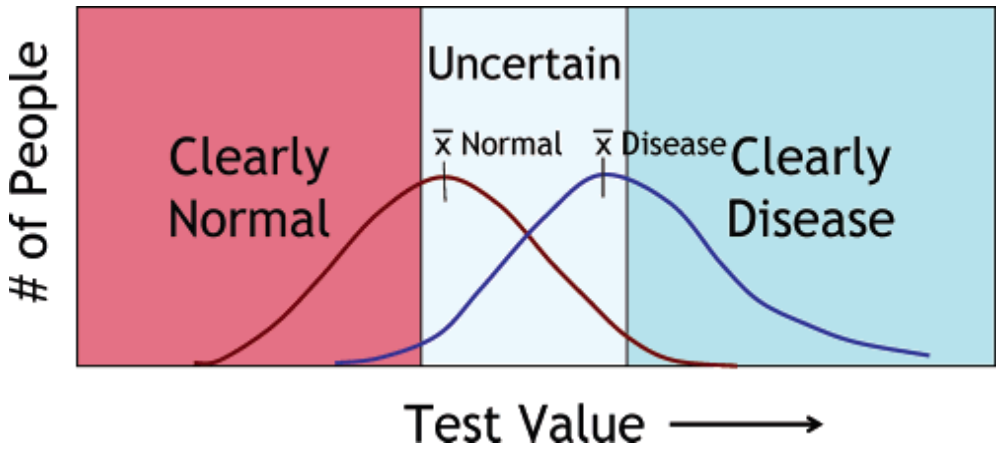
(ionex, IMAC, affinity sorbents)

MALDI MS, SELDI MS (3 – 20 kDa)

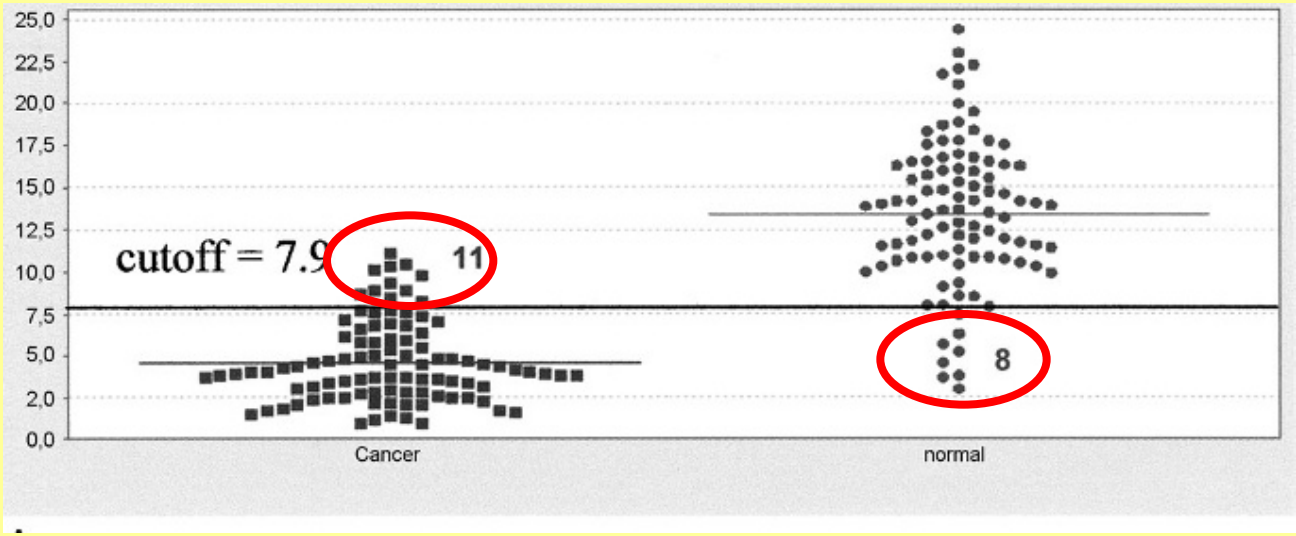
profile analysis



- high number of factors influencing protein composition
  not related with diagnosed disease
  high profile variability
- no strict limits for clear positive/negative disease detection



J. LaBaer et al., J. Proteome Res., ***4*** *(4) 1053-1059 (*2005).



M. Ehmann et al., Pancreas, ***34*** *(2) 205-214 (*2007).

MS-based approaches for biomarker searching

direct MS analysis
(MALDI MS, SELDI MS)

Pattern profiling

comparison of peptide (protein) profile of sample „healthy“ vs. patient
(bez identifikace, statistická analýza)

early disease detection

biomarker specifity!!!

biomarker identification

Separation
GE. LC

comparison of protein (peptide) content of sample „healthy“ vs patient

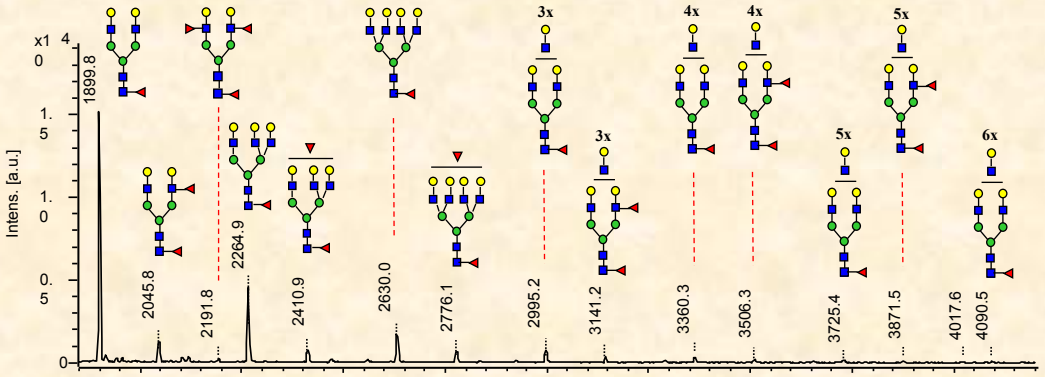
MS
MS/MS

identification of differentialy regulated proteins

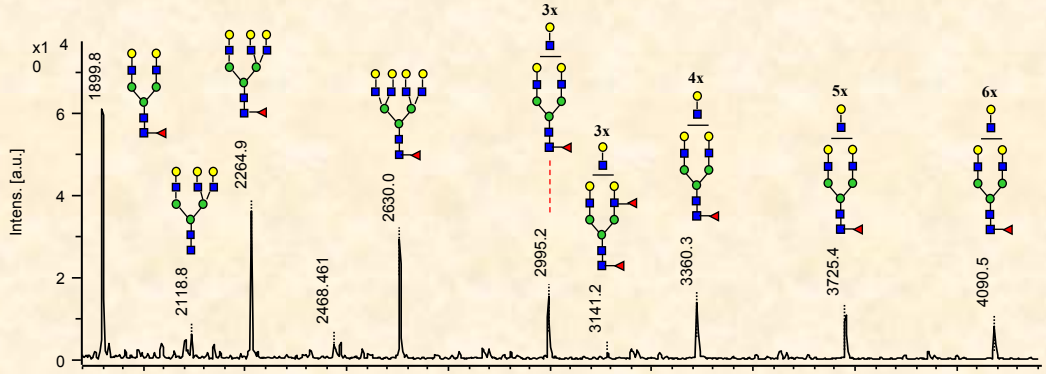
specific antibody
Immunodetection
SRM, SWATH/Protein arrays

Glycan profiling and structural analysis of glycans

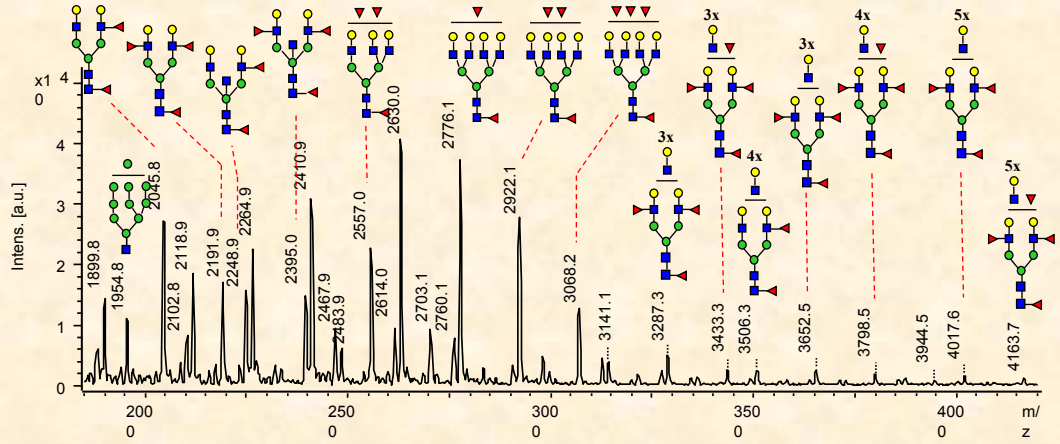
C7250



NSCLC - Bronchoalveolar Carcinoma



Bronchoalveolar Adenocarcinoma



Large Cell Carcinoma

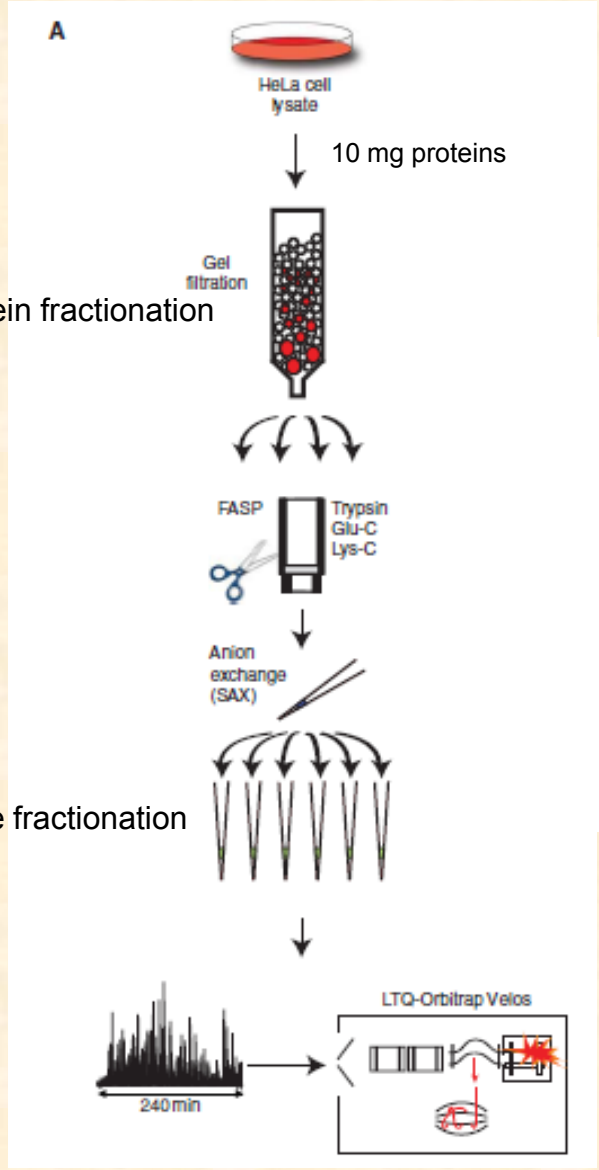
MALDI-TOF-MS spectra of N-glycans after desialylation

Man; Gal; GlcNAc; Fuc

Lattová E., J. Proteome Res., 15 (8), 2777-2786 (2016)


BERLINBEAIRBUS

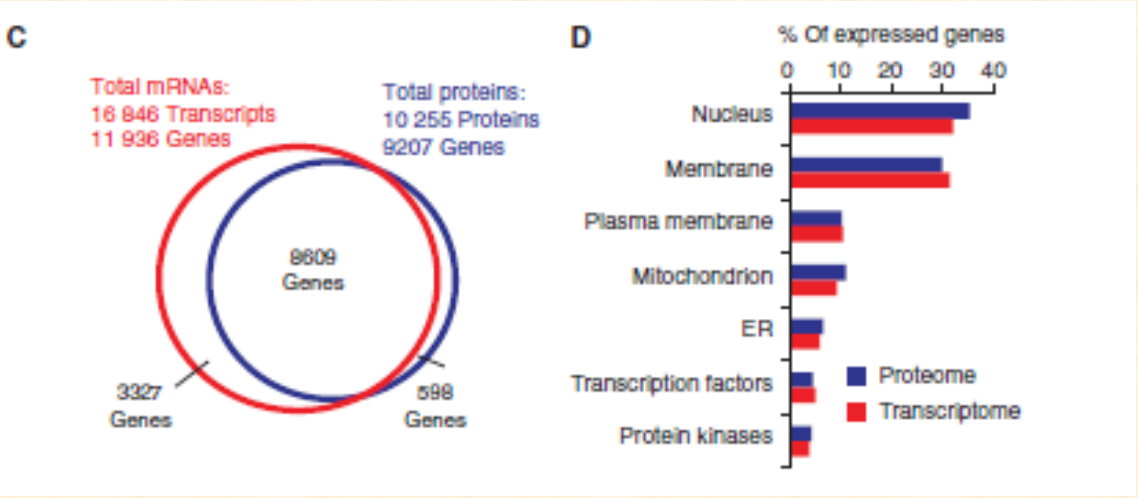
Comparison of human cancer cell line proteome and transcriptome



126 frakcí
72 frakcí

B

Experiment	Proteins	Peptides	MS measurement time
Exp 1	*10 596*	*187 006*	*21 days*
Exp 2	10 255	163 784	12 days



*N. Nagaraj et al., Mol. Syst. Biol., 7, 548 (*2011).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Relative quantification by MS

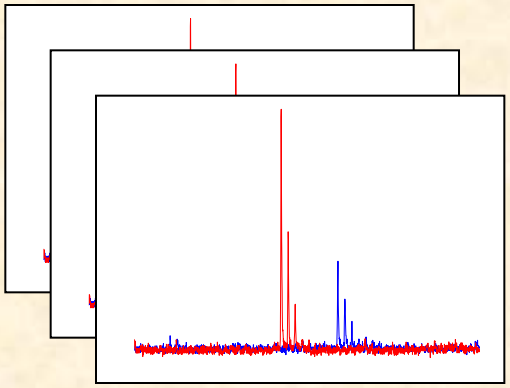
- **isotopically labeled tag approaches**
  (comparison of limited number of samples, up to 10)

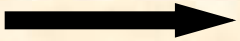
protein quantification

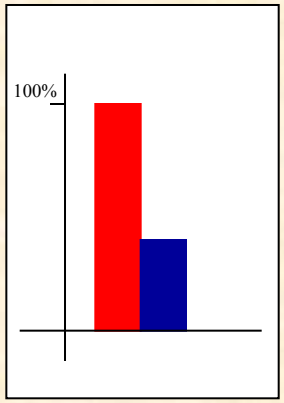
replica 1

replica 2

replica 3







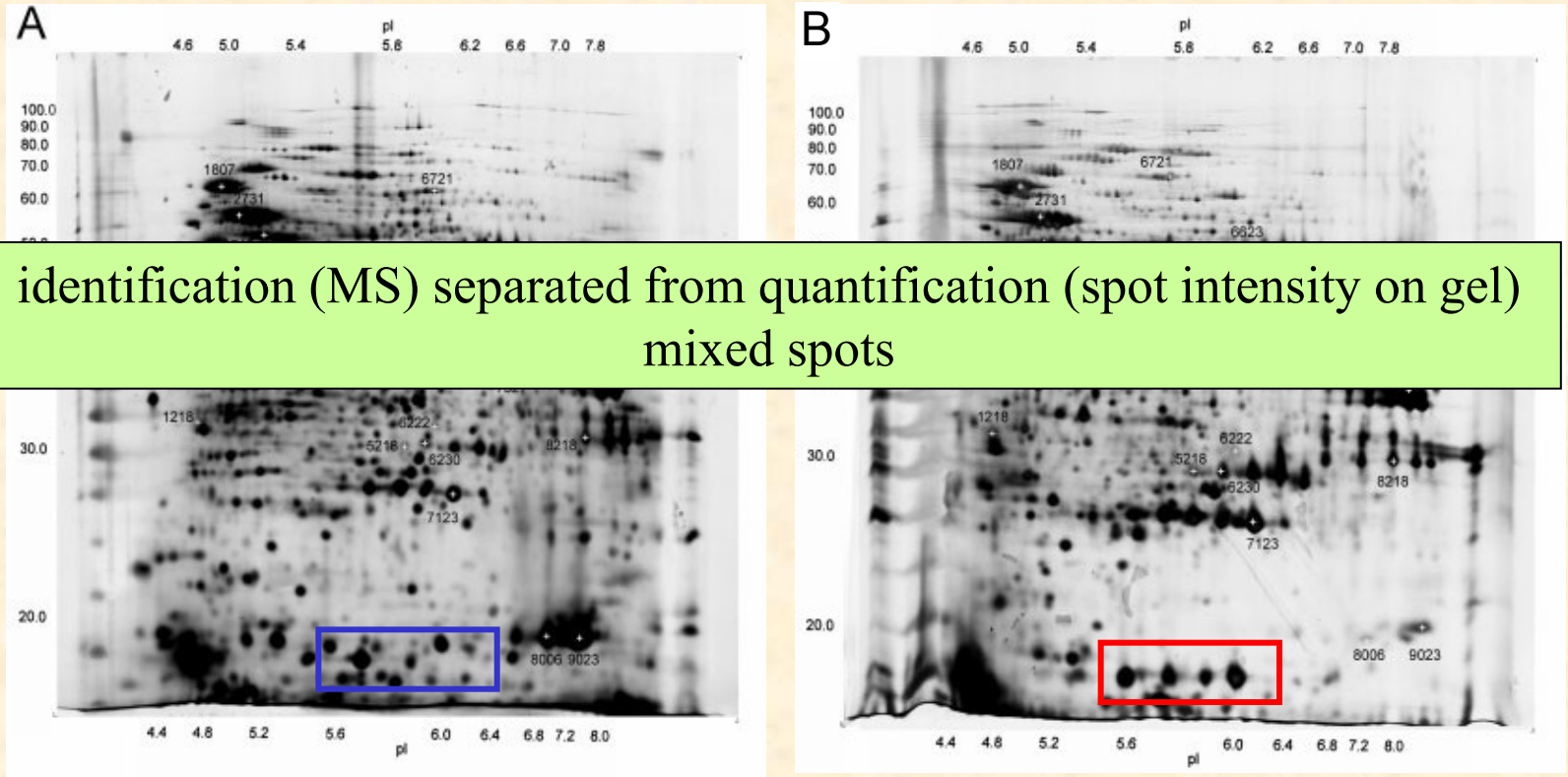
- **label-free approaches**
  (comparison of unlimited number of samples, lower accuracy)

Targeted quantification of selected proteins by MS

- MRM, SWATH

Characterization of proteome changes
differential (expression) proteomics

image analysis of 2-D gels
LC-MS/MS of selected spots with different intensity



identification (MS) separated from quantification (spot intensity on gel)
mixed spots

Acidithiobacilus ferrooxidans grown on ferrous iron (A) and elemental sulfur (B)

cooperation with Department of Biochemistry, FS MU
P. Bouchal et al., Proteomics 2006, *6,* 4278–4285.

Search for marker proteins for chicken salmonella infection
relative quantification

control

infected

gut wall sample

protein isolates

tryptic digestion

reductive alkylation

LC-MS/MS
data processing

- identification more than 2300 protein
- quantification for more than 1900

Accession	Description	infected/ control
363741657	PREDICTED: syntenin-2-like [Gallus gallus]	41.032
118095649	]	34.036
4927286		33.575
112491068		30.221
56118294	ribonuclease homolog precursor [Gallus gallus]	25.497
363741459	PREDICTED: protein-glutamine gamma-glutamyltransferase E [Gallus gallus]	24.786

clarifying of mechanisms of molecular processes

search for marker proteins for early detection

confirmation by real-time PCR

cooperation with VRI Brno

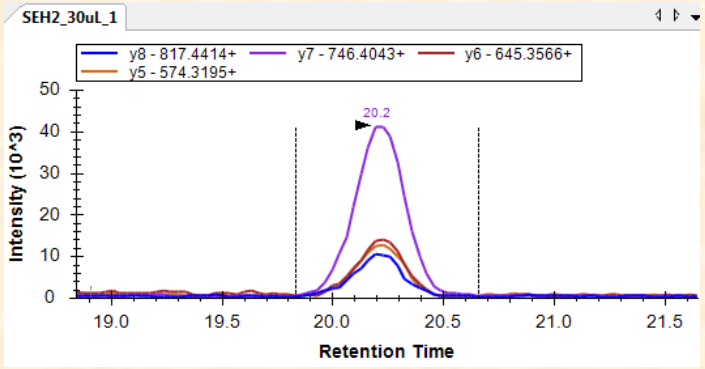
Matulova M. et al., Vet. Res., 44:37 (2013)

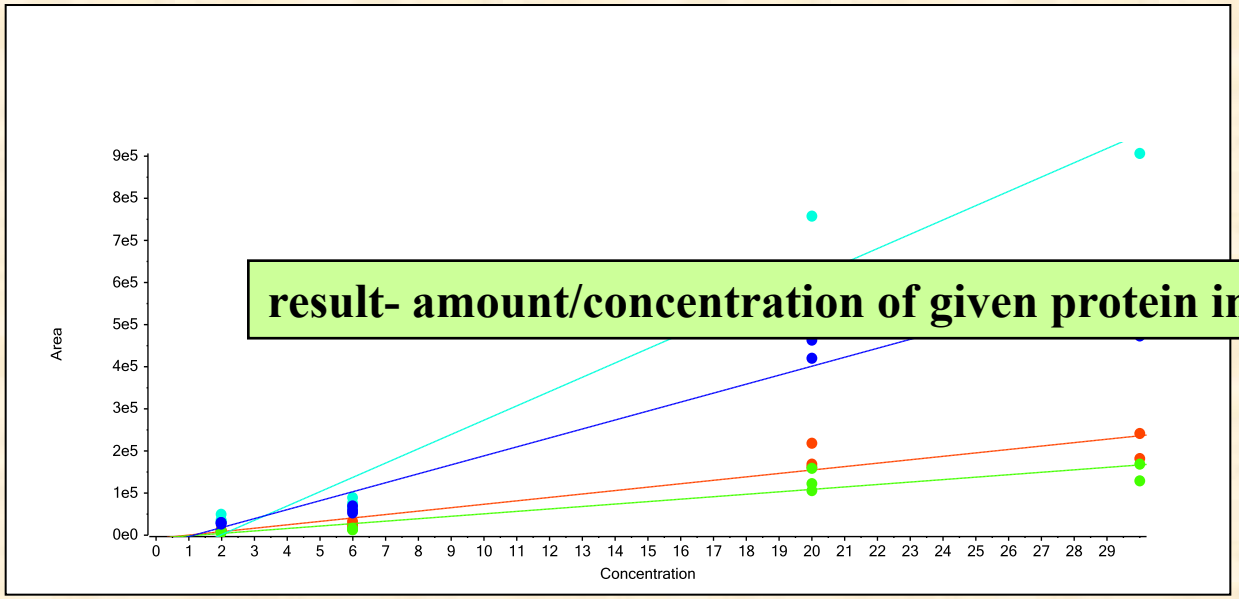
Quantification of enterotoxins

targeted analysis of selected protein

MRM

- selection of peptides suitable for MRM
- absolute quantification by AQUA peptides



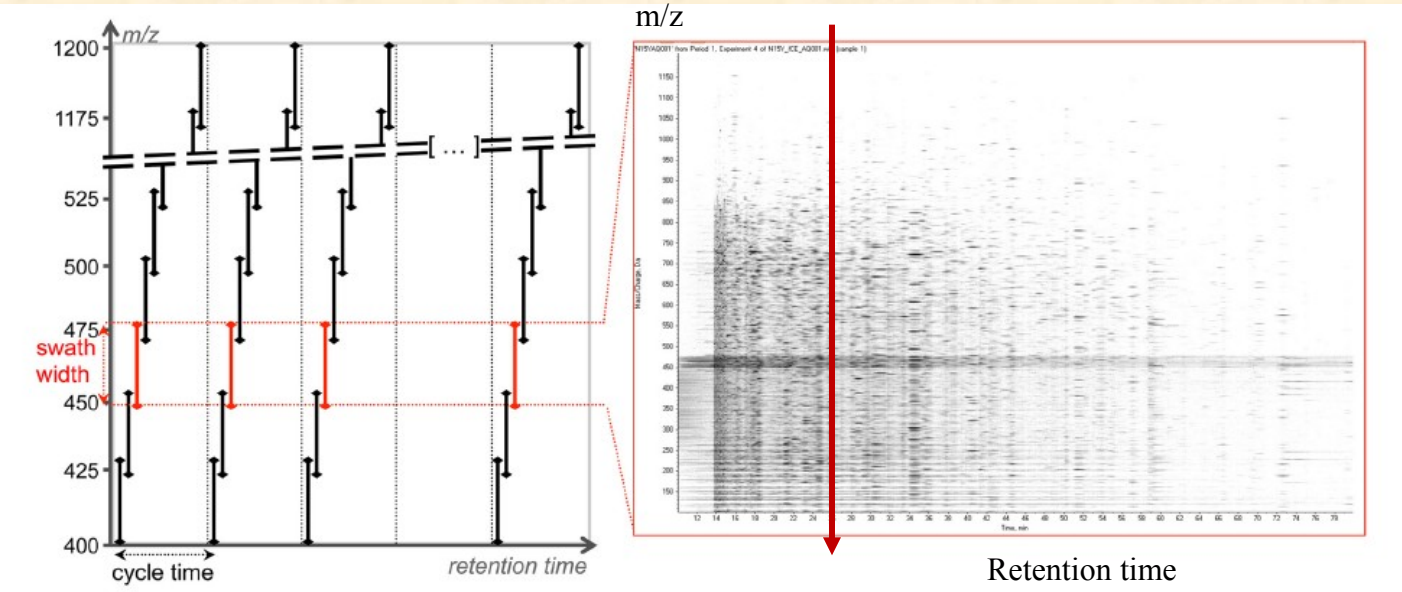


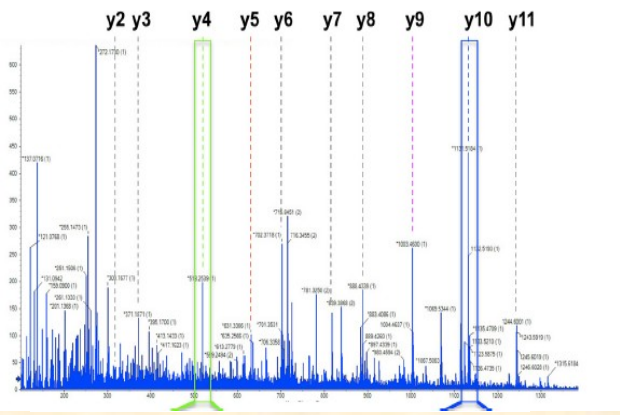
result- amount/concentration of given protein in sample

SWATH MS

C7250

Q-TOF, MS/MS < 10 ppm



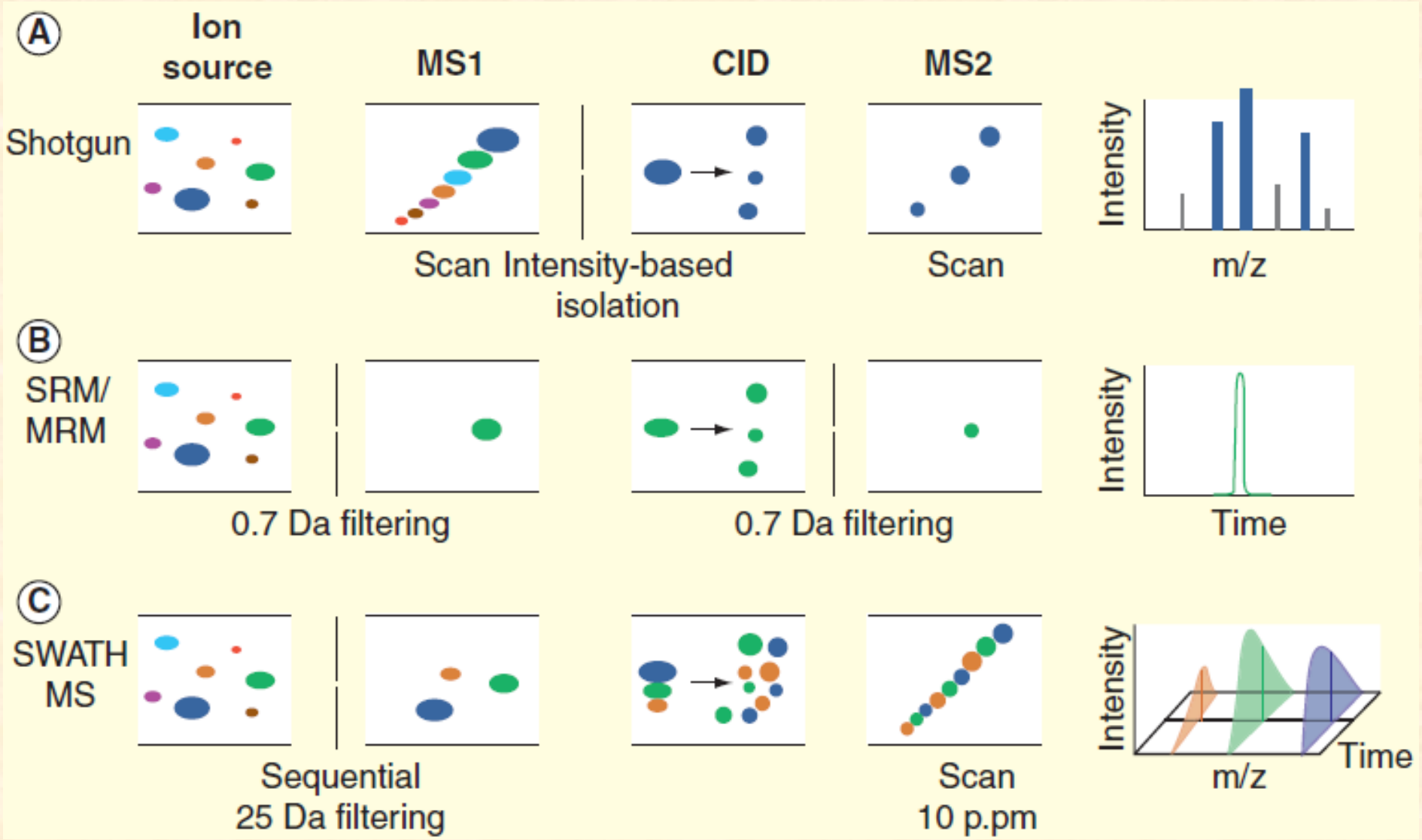


all MS/MS spectrum

- classic DB searching **not possible**
- comparison with libraries of ref. MS/MS spectra
- Relative and absolute quantification

Gillet et al, MCP, 11, 1-17 (2012)

Shot gun vs SRM vs SWATH



Liu et al, Expert Rev. Mol. Diagn., 13(8), 811 (2013)

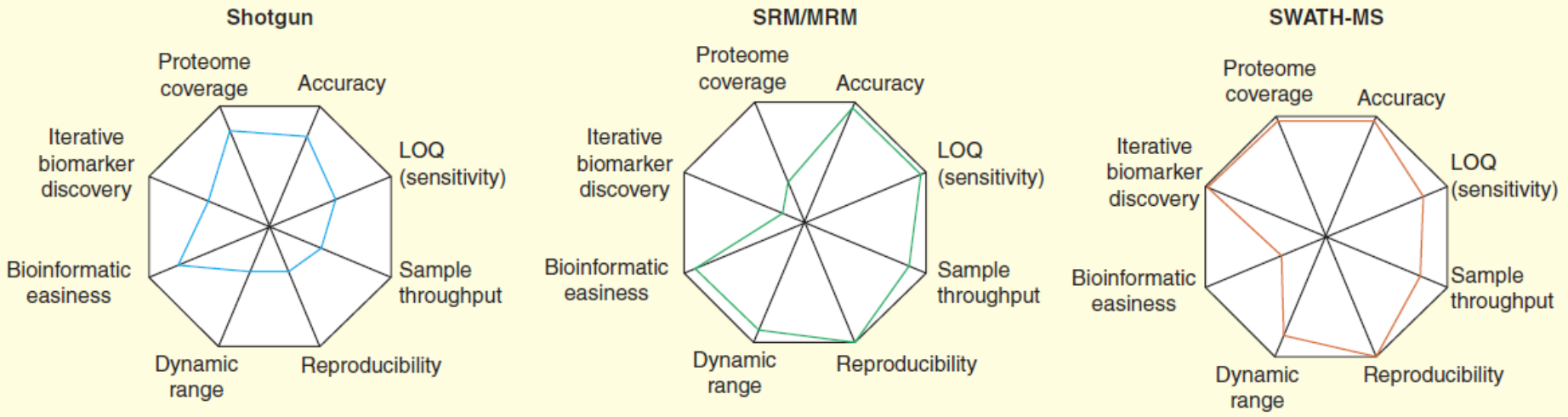
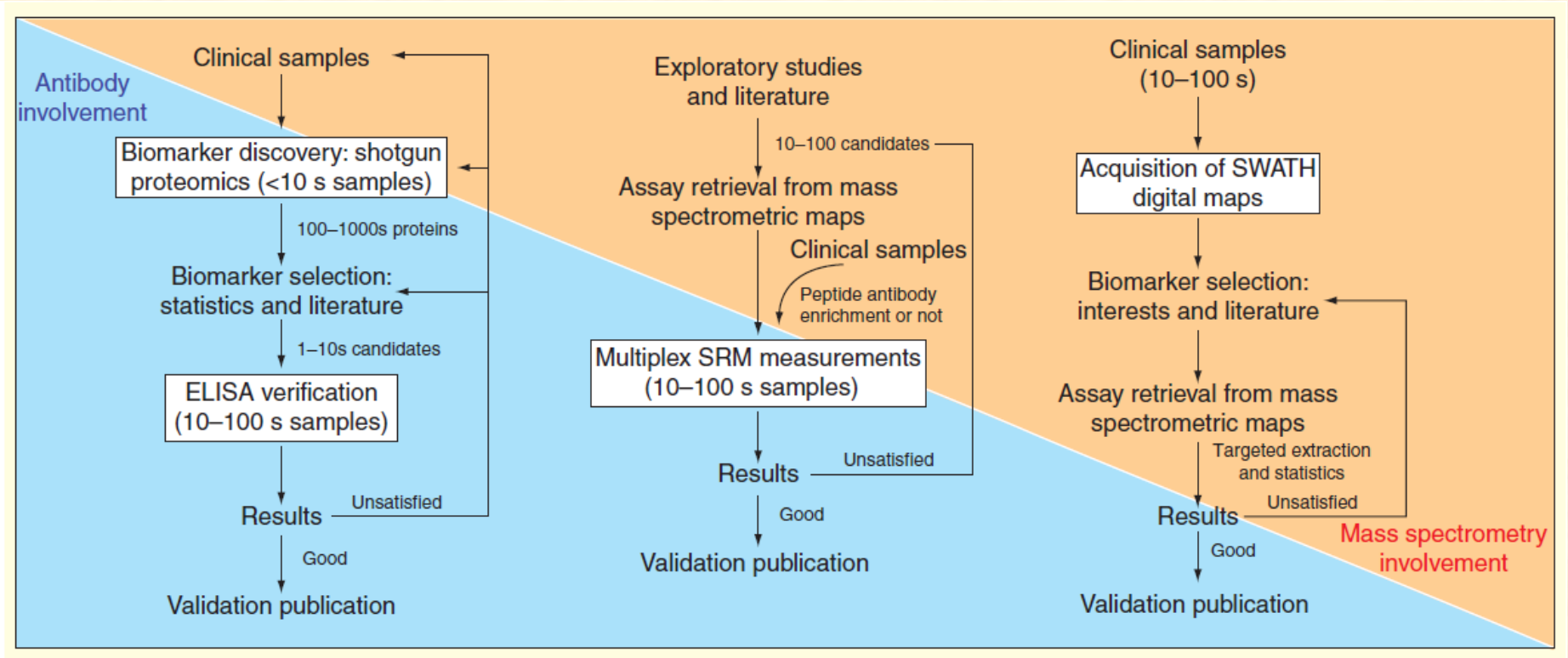


Figure 3. Performance profiles comparing technical advantages and disadvantages of shotgun proteomics, SRM and SWATH MS. In the radar chart, analytical variables are presented on axes staring from the same point and each variable is represented by a spoke. The length of a spoke indicates the magnitude of the variables. Note that SWATH-MS combines the strengths of shotgun and SRM technologies; however, requires more powerful bioinformatic tools for data analysis.
LOQ: Limit of quantification; MRM: Multiple reaction monitoring; MS: Mass spectrometry; SRM: Selected reaction monitoring.

Liu et al, Expert Rev. Mol. Diagn., 13(8), 811 (2013)

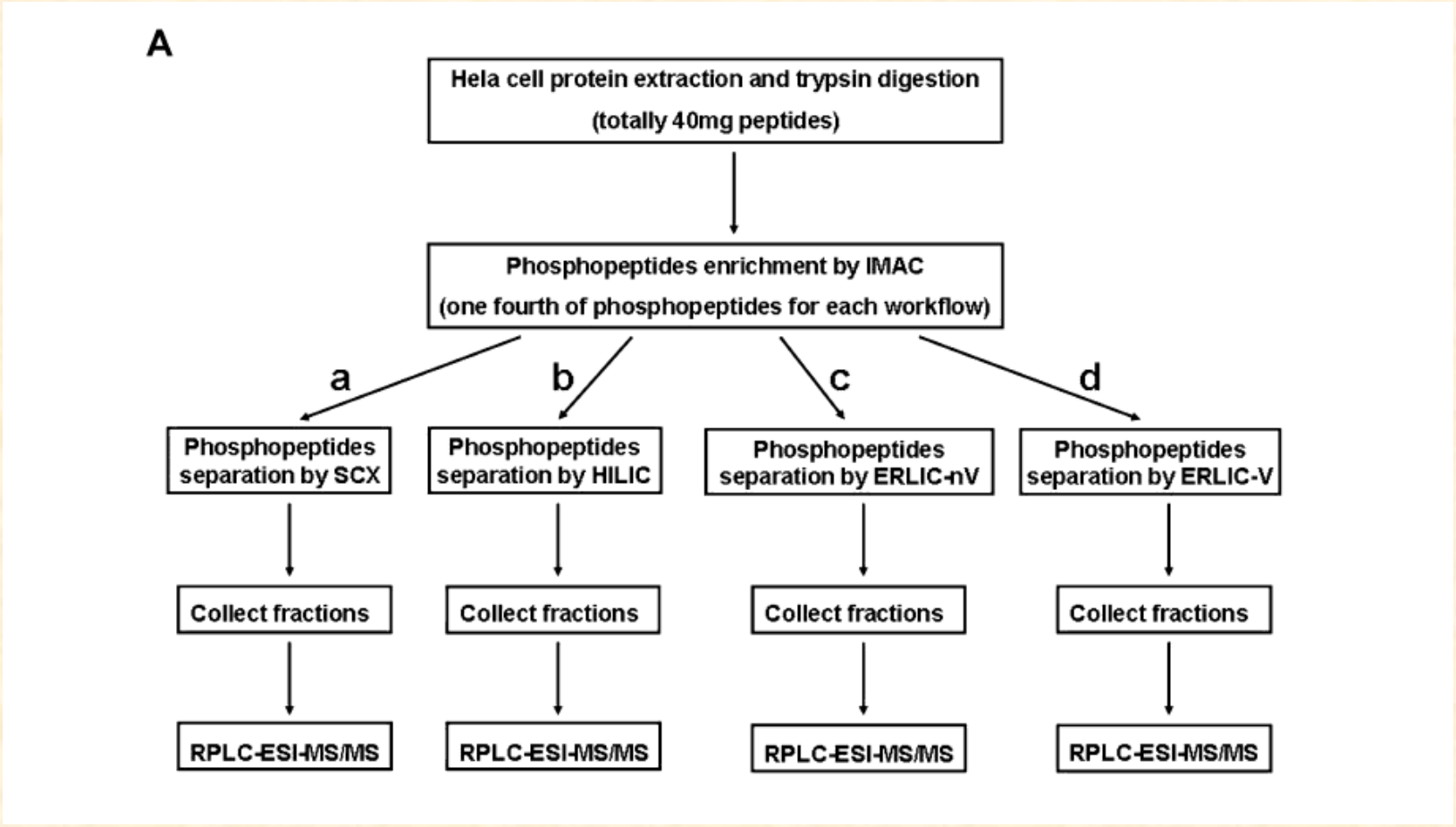
C7250



Liu et al, Expert Rev. Mol. Diagn., 13(8), 811 (2013)


G-ZAPH
Copyright John Gregory
AIRLINERS.NET

Phosphoproteome analysis – four fractionation approaches

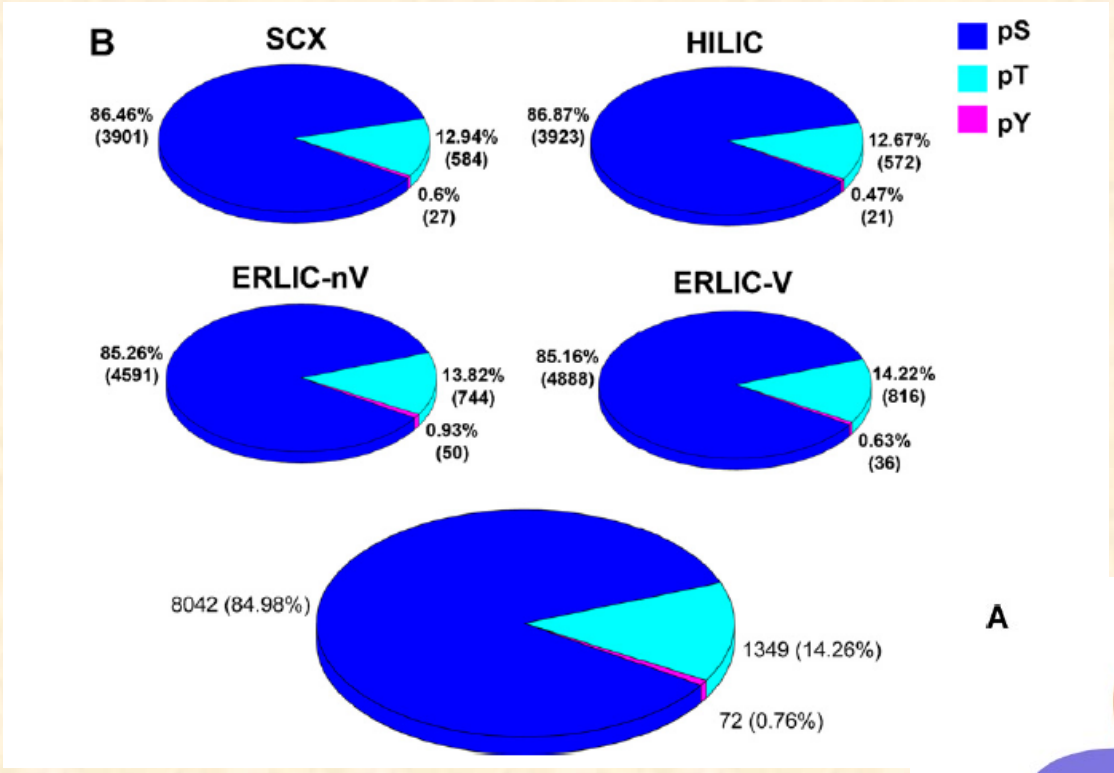


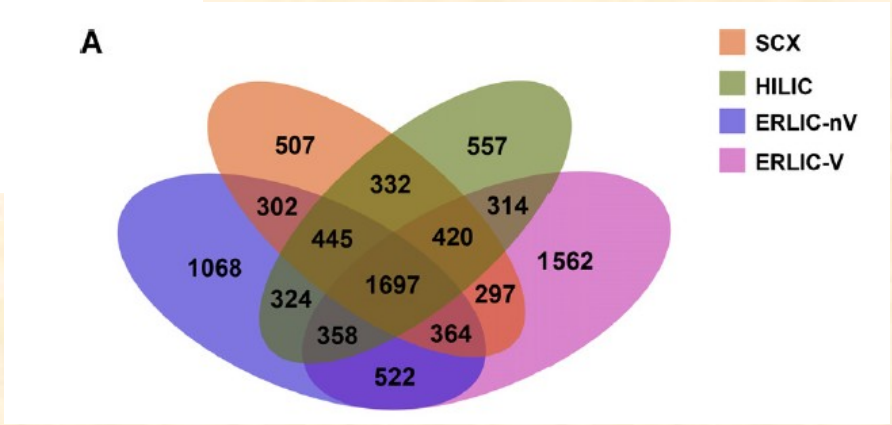
ERLIC - Electrostatic Repulsion-Hydrophilic Interaction Chromatography

Chen et al., J. Chromatogr. B, 879, 25 (2011)

Phosphoproteome analysis – four fractionation approaches

C7250



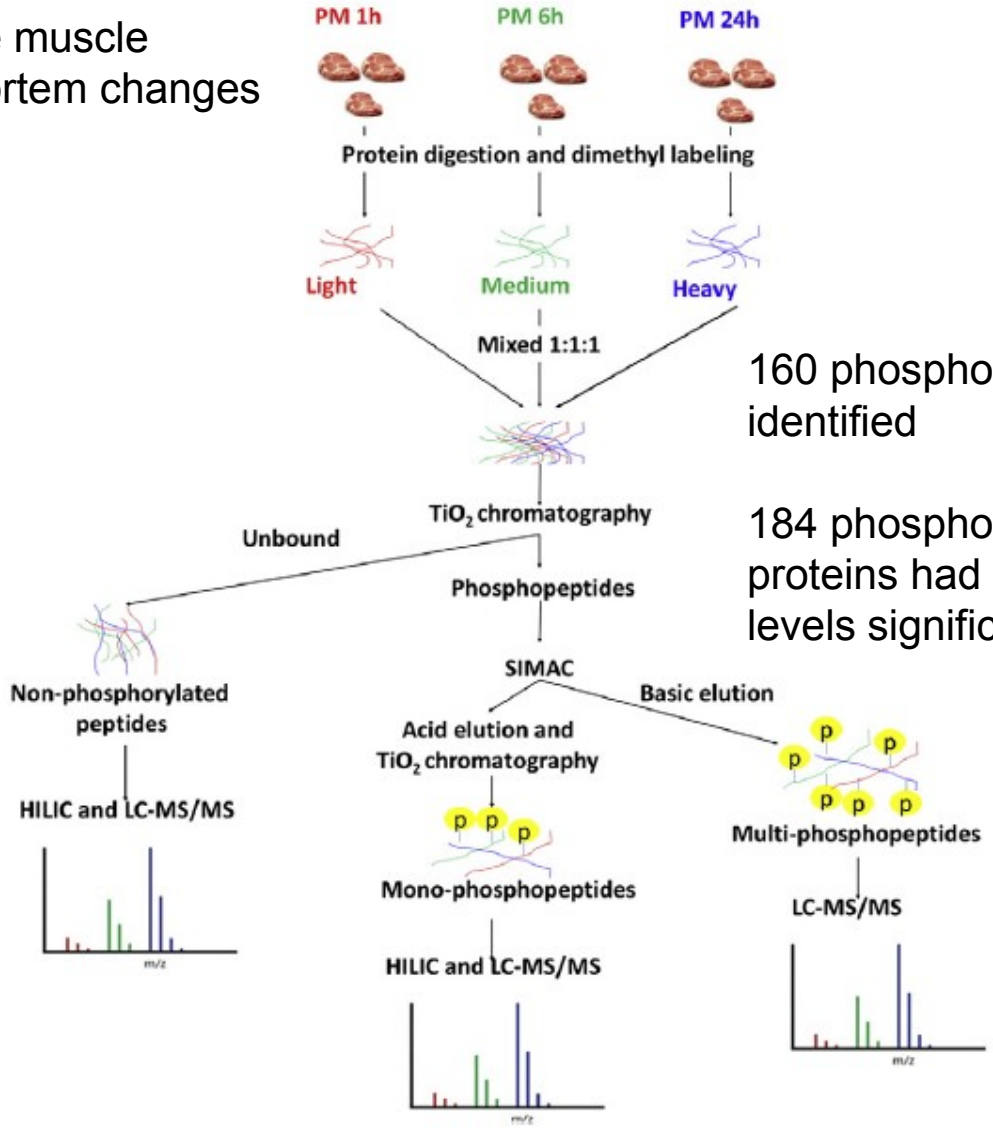


Each method – over 4000 phoshopeptides
In total – 9069 phosphopeptides – 9463 sites / 3260 proteins

Chen et al., J. Chromatogr. B, 879, 25 (2011)

Phosphoproteome analysis– quality and quantity

Porcine muscle
Postmortem changes



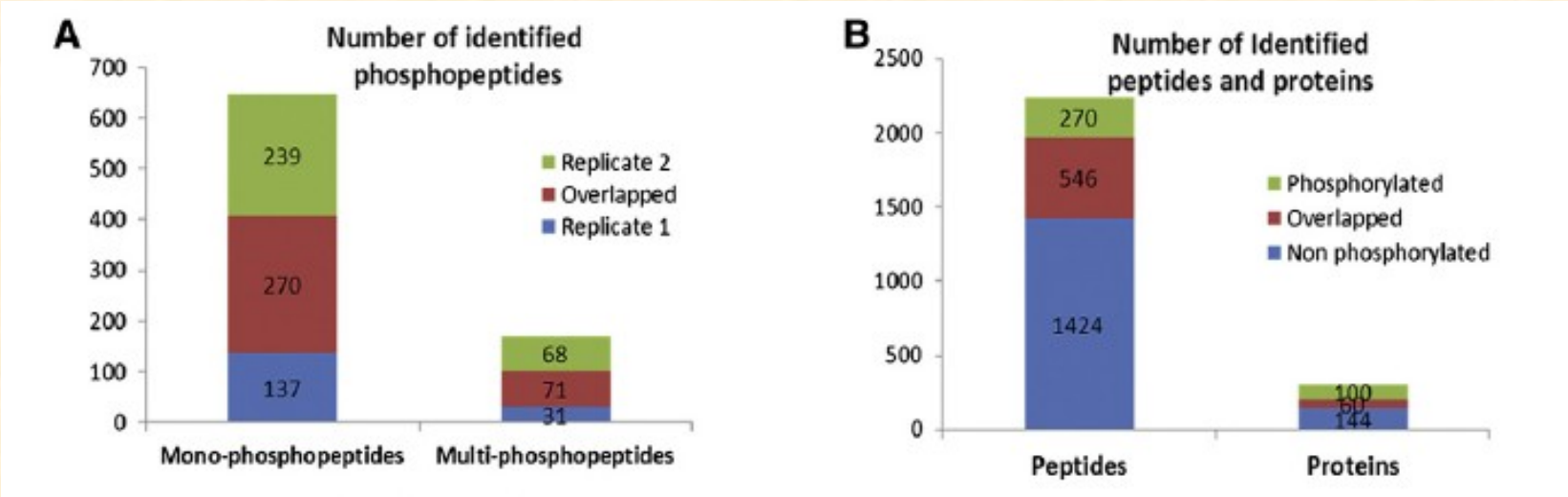
160 phosphoproteins with 784 sites identified

184 phosphorylation sites on 93 proteins had their phosphorylation levels significantly changed.

Huang et al., J. Proteomics, 106, 125 (2014)

Phosphoproteome analysis– quality

Comparison of identified peptides in replicas



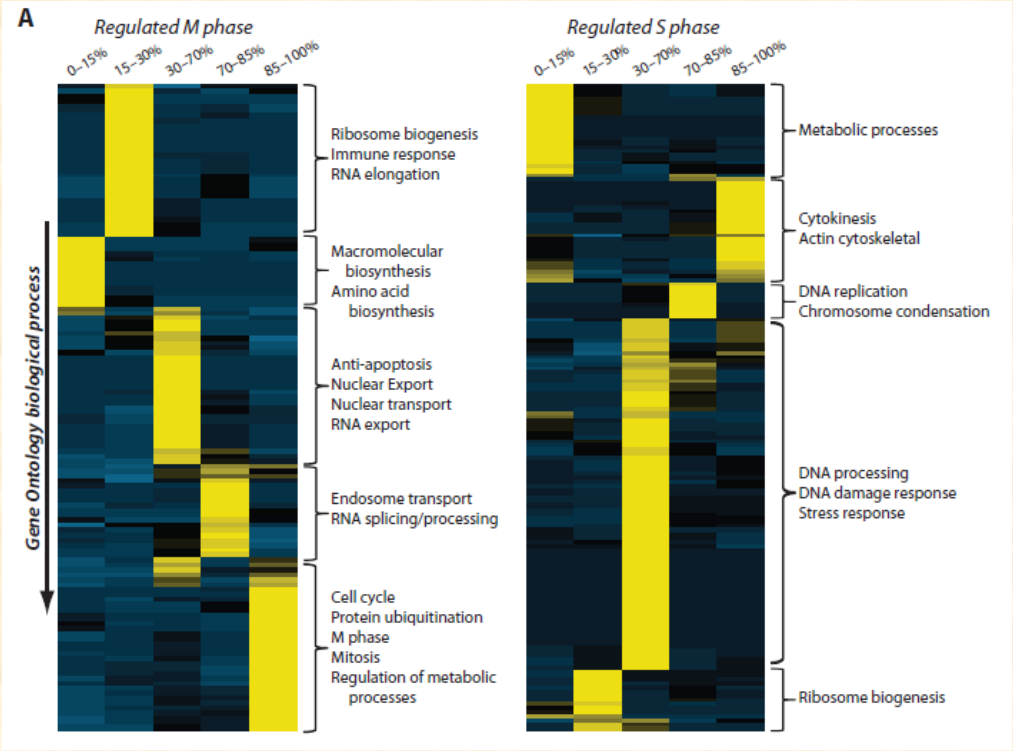
Huang et al., J. Proteomics, 106, 125 (2014)

Phosphoproteome analysis

Olsen J.V. et al., Sci. Signal., 3 (104) ra3 (2010)

- quantified 6027 proteins
- quantified 20,443 unique phosphorylation sites

- **HELA cells**
- **SILAC labeling**
- **TiO_2 enrichment**
- **LC-MS/MS (Orbitrap)**



The panels show the phenotypic phosphoproteome comparison organized by GO biological process for mitotic (left) and S phase (right) cells. Proteins involved in metabolic processes have high-occupancy phosphorylation sites during mitosis, but low-occupancy sites during S phase (color scale: yellow, high overrepresentation; dark blue, high underrepresentation).

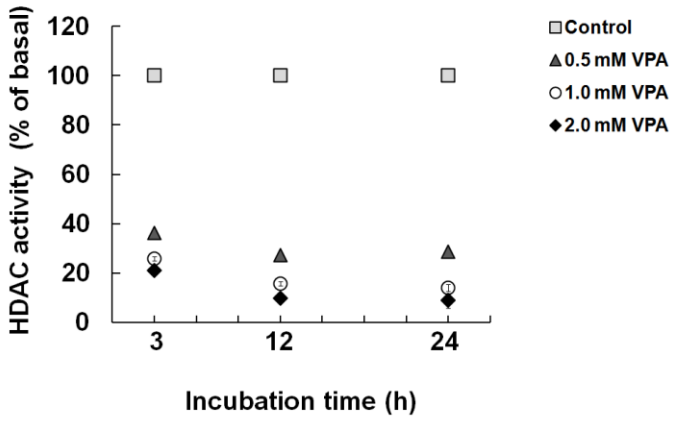
Characterization of effect of histone deacetylase inhibitors

- to **establish a set of methods**

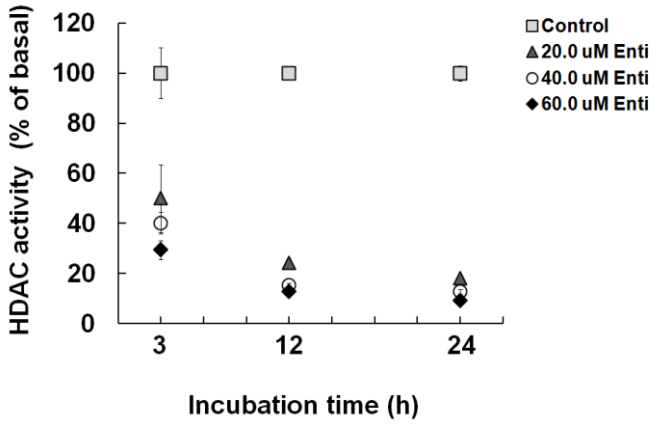
- HDAC Fluorimetric Cellular Activity Assay Kit
- MALDI-MS of N-terminal part of histones (after Glu-C digestion)
- AUT-AU 2-D GE combined with LC-MS/MS analysis

Total HDAC inhibiton effect

Valproic acid



Entinostat



B-CLL MEC-1 cells

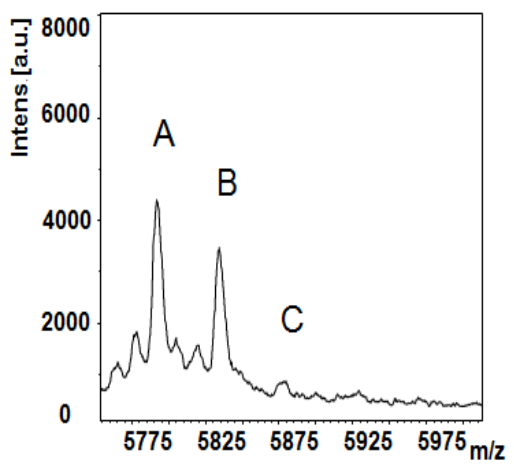
Činčárová et al, Mol. Biosyst. (2012)

Characterization of effect of histone deacetylase inhibitors

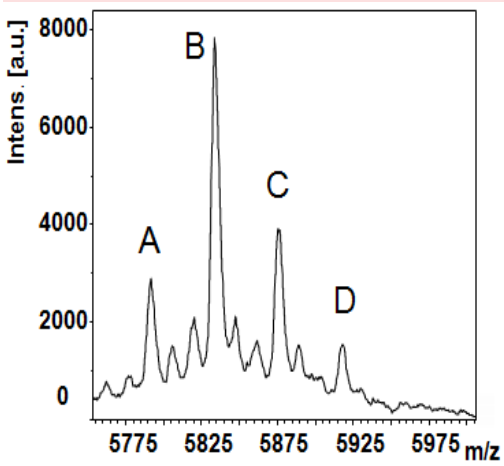
MALDI-MS of Histone H4 acetylated forms

N-terminal fragment (1-53 AA)

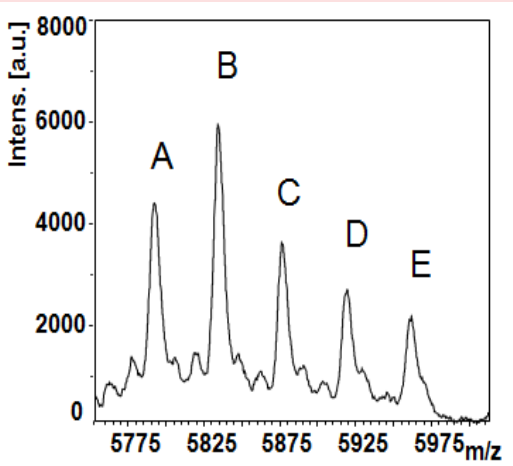
control



Valproic acid



Entinostat



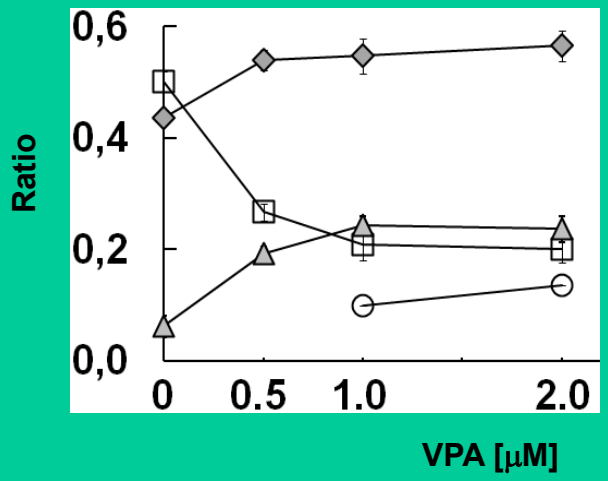
A → E 0 → 4 acetylations

Characterization of effect of histone deacetylase inhibitors

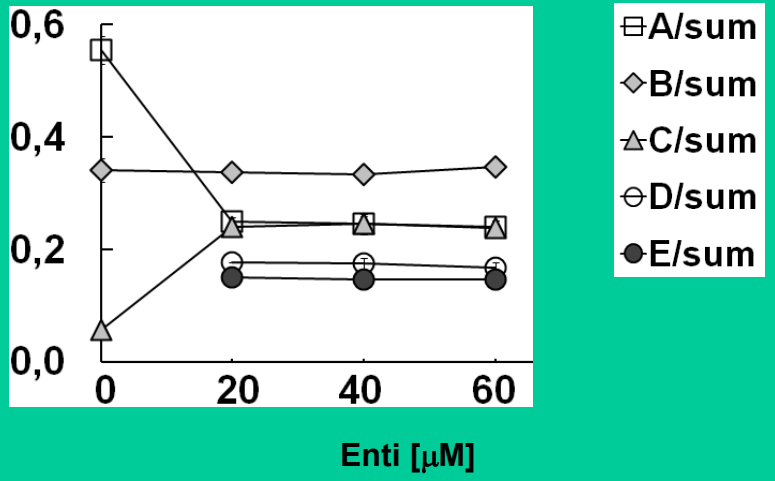
Changes of particular H4 acetylated forms *vs* inhibitor concentration

(24h treatment)

Valproic acid



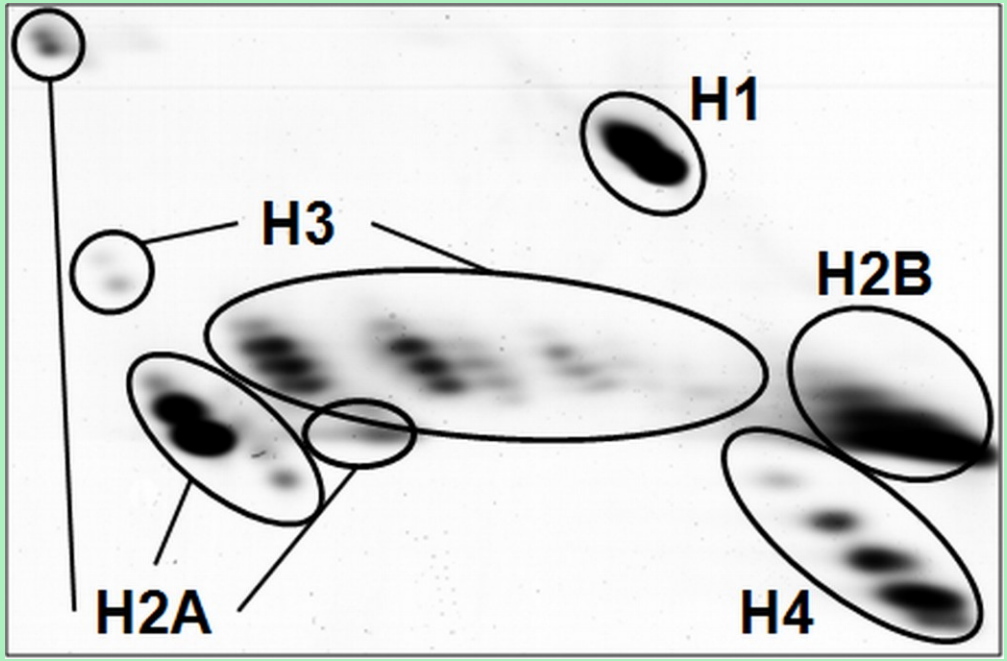
Entinostat



A → E 0 → 4 acetylations

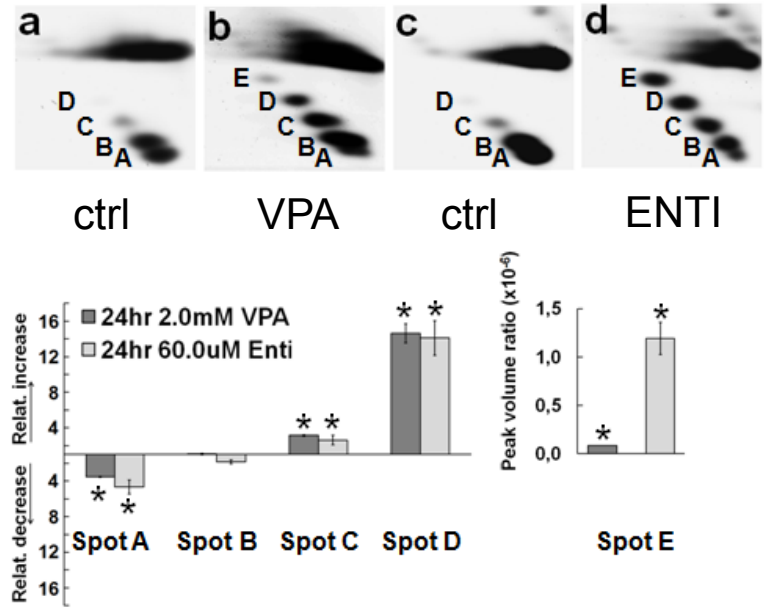
Characterization of effect of histone deacetylase inhibitors

AUT-AU 2-D GE of histone extract



Characterization of effect of histone deacetylase inhibitors

AUT-AU 2-D GE of histone extract



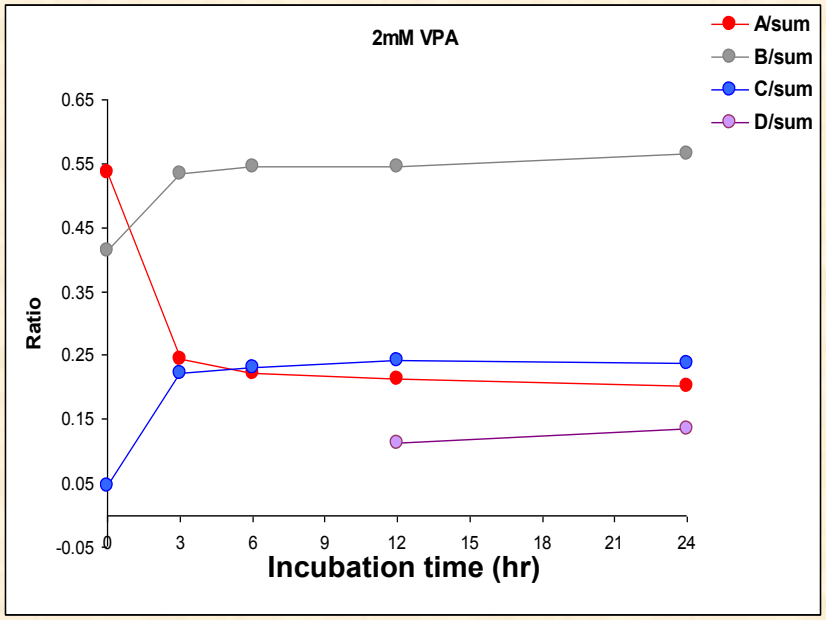
Spot	Relative change VPA Entinostat	Peptide observed
A	-3.53 ± 0.06 -3.60 ± 0.08	GKGGKGLGKGGAK[17]
B	1.06 ± 0.09 -0.80 ± 0.02	GKGGKGLGKGGA**acK**[17]
C	3.14 ± 0.10 1.60 ± 0.50	GKGGKGLG**acK**GGA**acK**[17] GKGG**acK**GLGKGGA**acK**[17] G**acK**GGKGLGKGGA**acK**[17]
D	14.65 ± 1.07 13.10 ± 1.90	GKGG**acK**GLG**acK**GGA**acK**[17] G**acK**GG**acK**GLGKGGA**acK**[17] G**acK**GG**acK**GLGKGGA**acK**[17]
E	> 100 > 100	G**acK**GG**acK**GLG**acK**GGA**acK**[17]

A → E 0 → 4 acetylations

Characterization of effect of histone deacetylase inhibitors

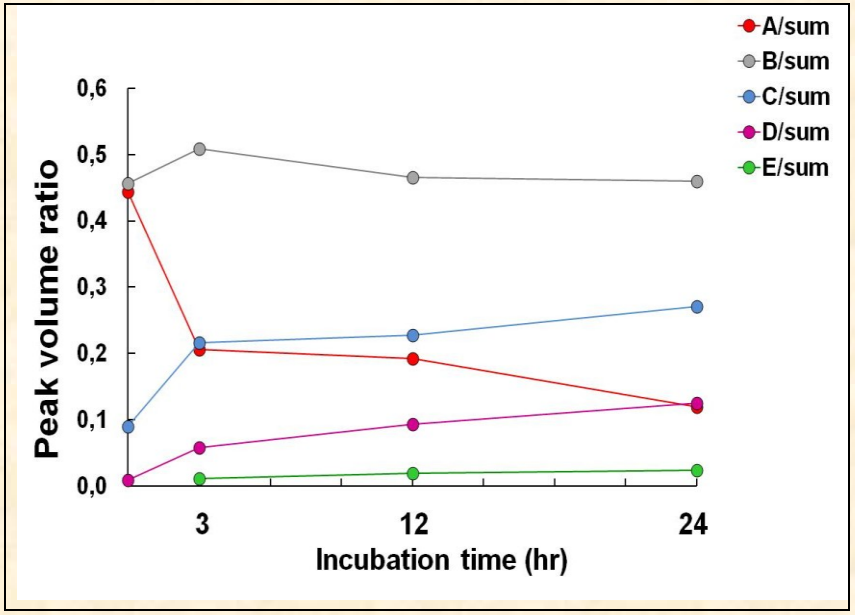
Changes of histone H4 acetylated forms in dependence on incubation time (**2 mM VPA**)

MALDI-MS



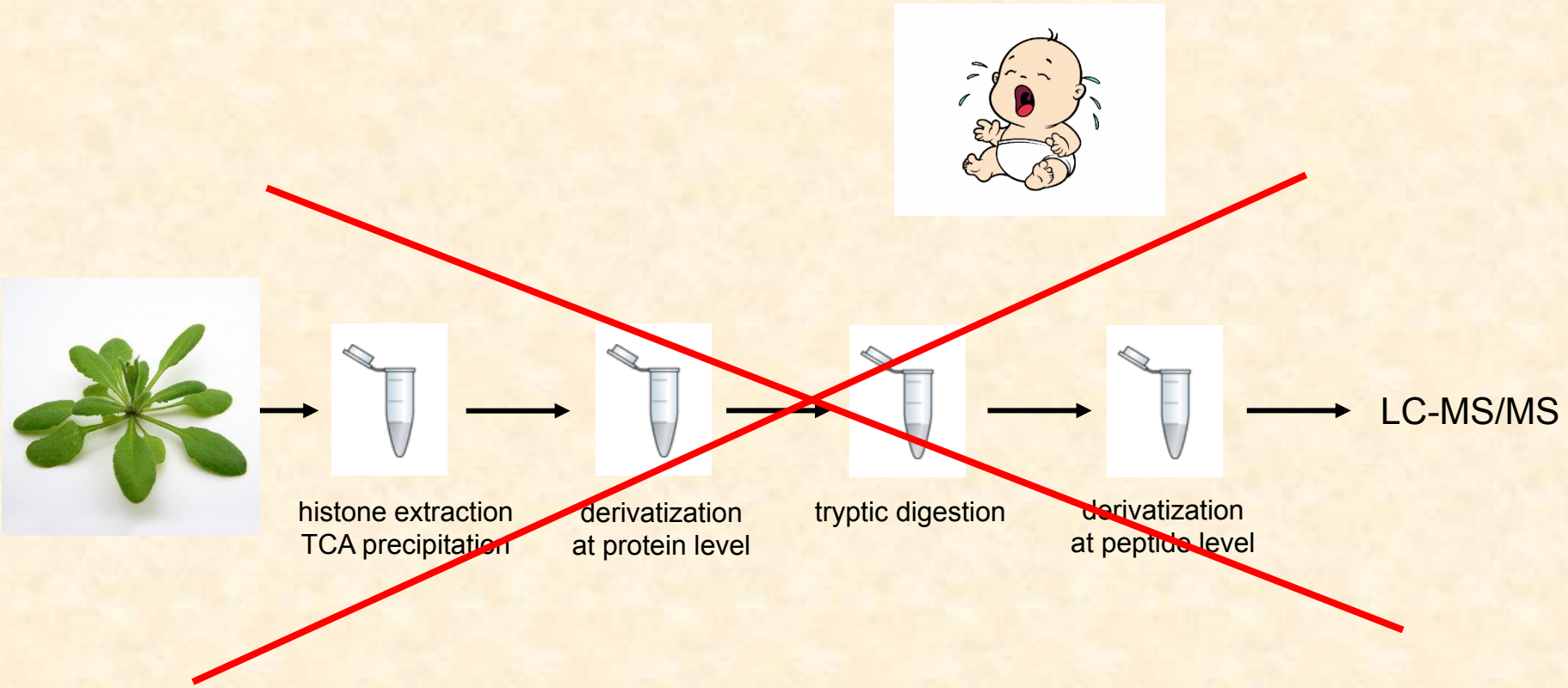
peak intensity

2-D AUT-AU GE



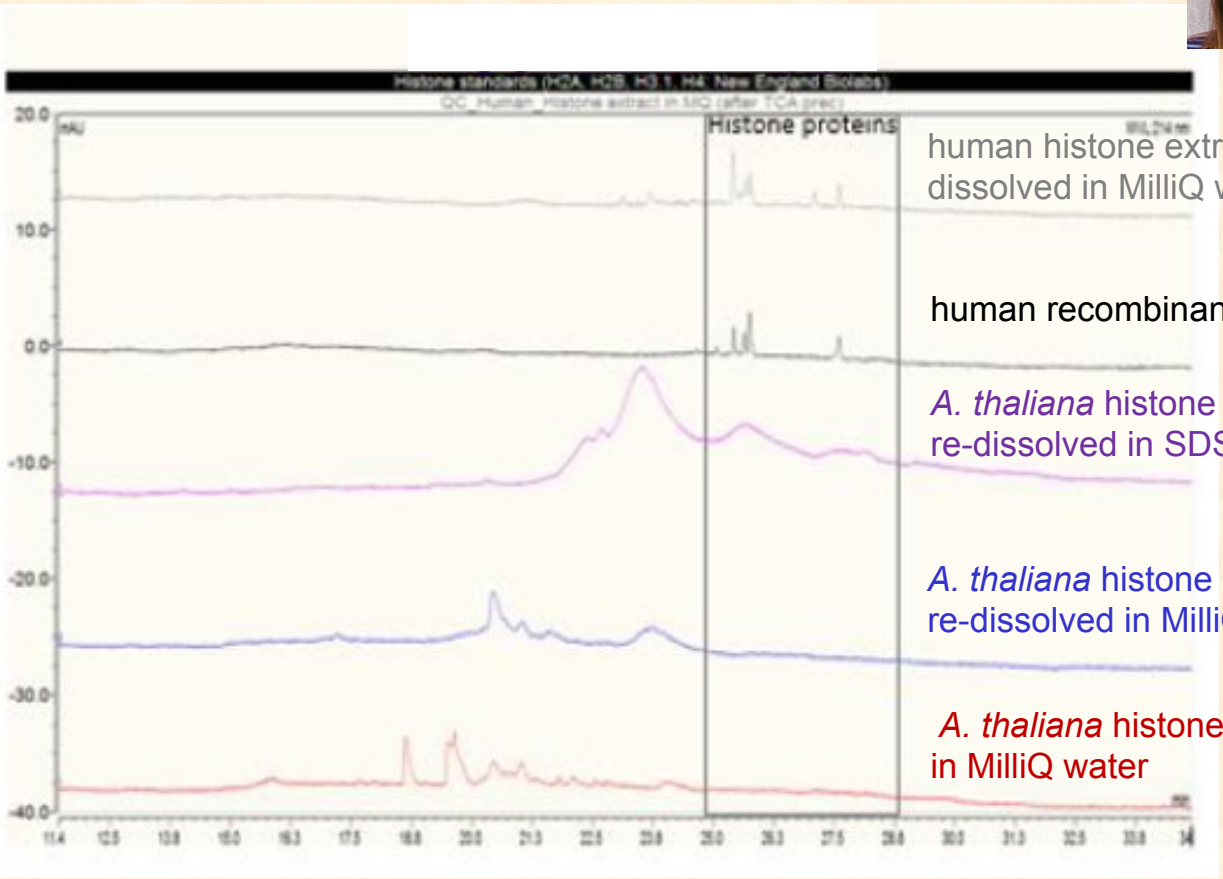
spot intensity

Characterization of Post-Translational Modifications of Histones



e.g. in Sidoli S. et al., J. Vis. Exp. 111:e54112 (2016).

Filter-Aided Sample Preparation Procedure for Mass Spectrometric Analysis of Plant Histones



human histone extract after TCA precipitation re-dissolved in MilliQ water

human recombinant histone standards

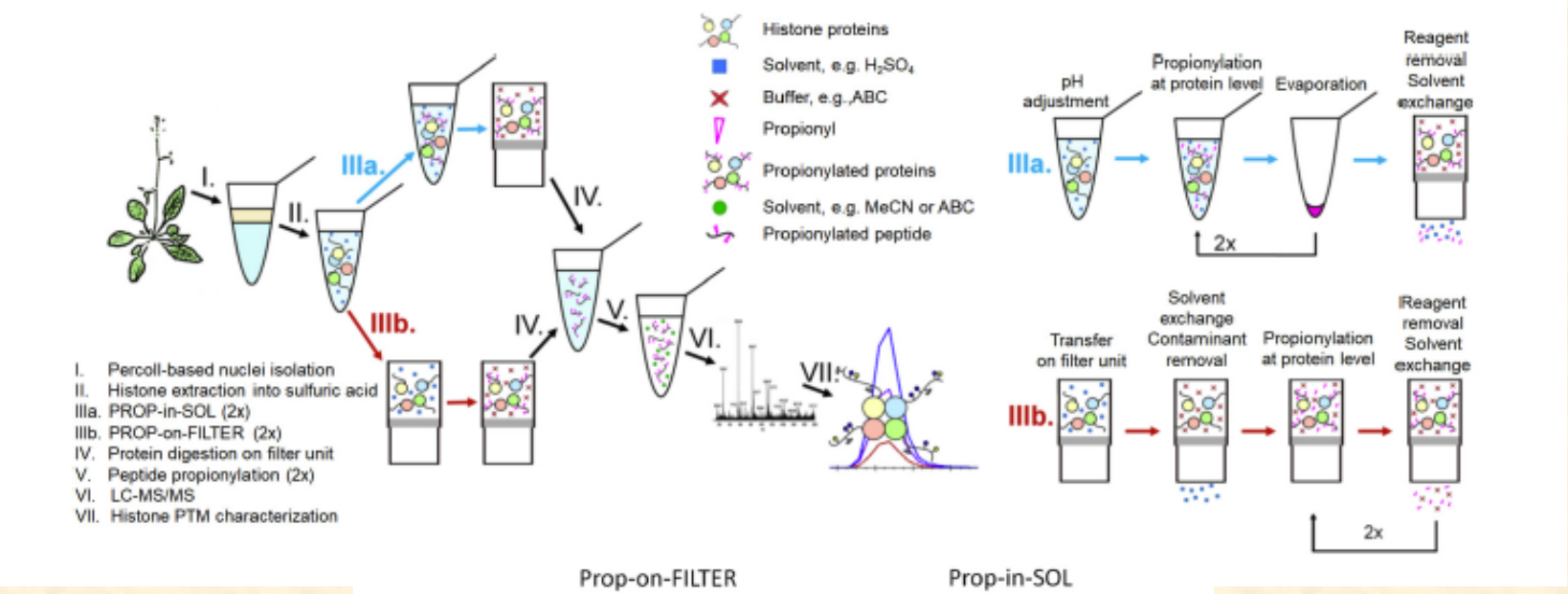
A. thaliana histone extract after TCA precipitation re-dissolved in SDS

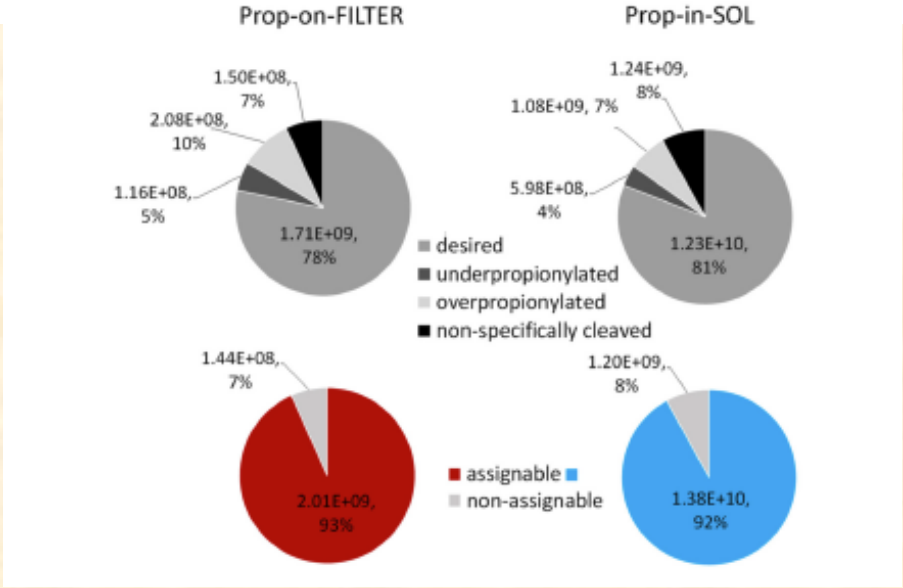
A. thaliana histone extract after TCA precipitation re-dissolved in MilliQ water

A. thaliana histone extract in sulfuric acid diluted in MilliQ water

Ledvinova D.. et al, Front. Plant Sci., ***9****, article 1373 (2018)*

Filter-Aided Sample Preparation Procedure for Mass Spectrometric Analysis of Plant Histones





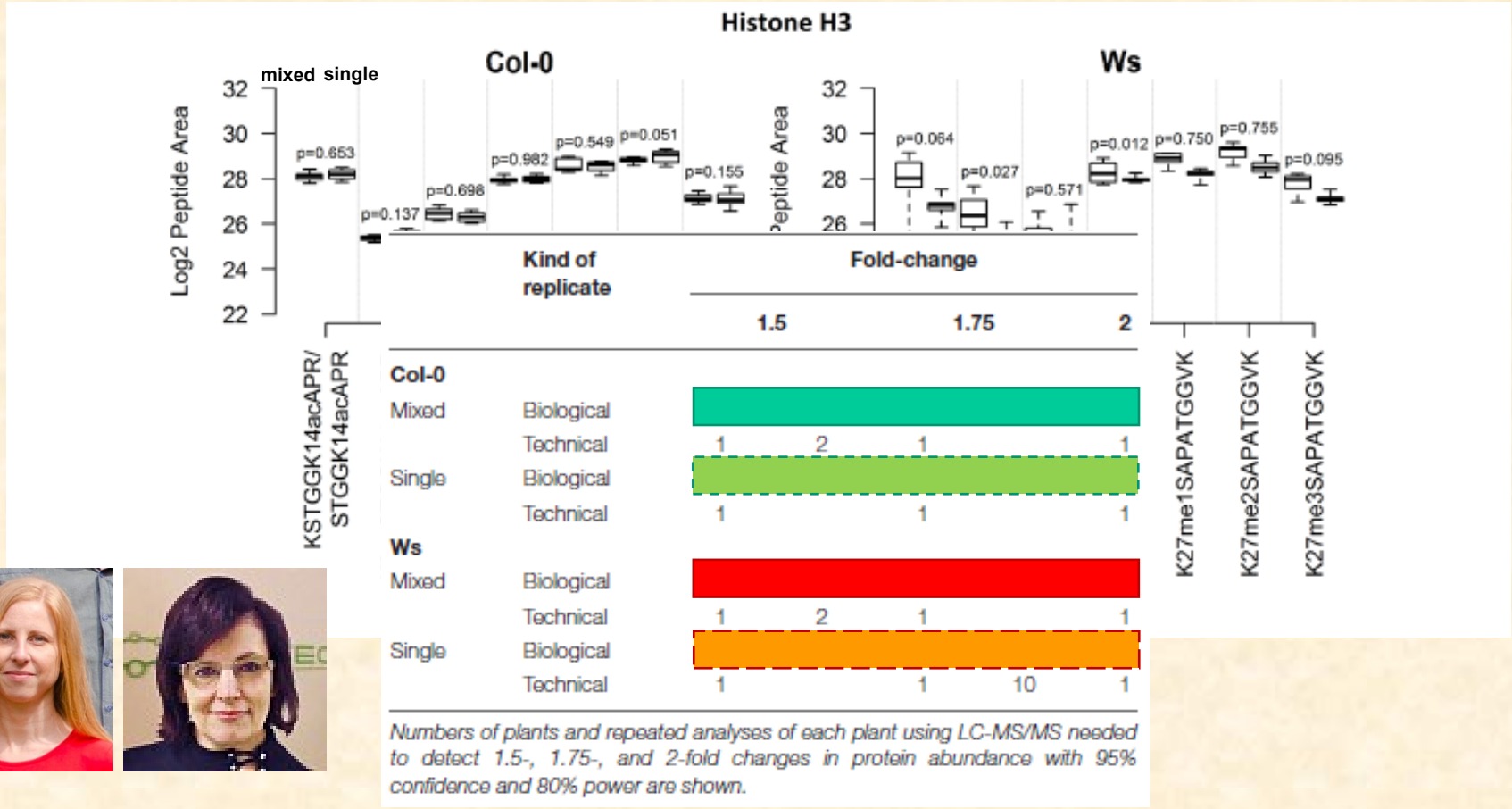
*Ledvinova D.. et al, Front. Plant Sci., **9**, article 1373 (2018)*

Inter-individual variations of Histone Modification Patterns

C7250

cooperation with Prof. Fajkus group, CEITEC-MU

two *Arabidopsis thaliana* ecotypes **Columbia 0** (Col-0) and **Wassilewskija** (Ws) grown from seeds collected from a **single parent plant** (Single) and a **set of parent plants** (Mixed).



*Brabencova S. et al, Front. Plant Sci., **8**, article 2084 (2017)*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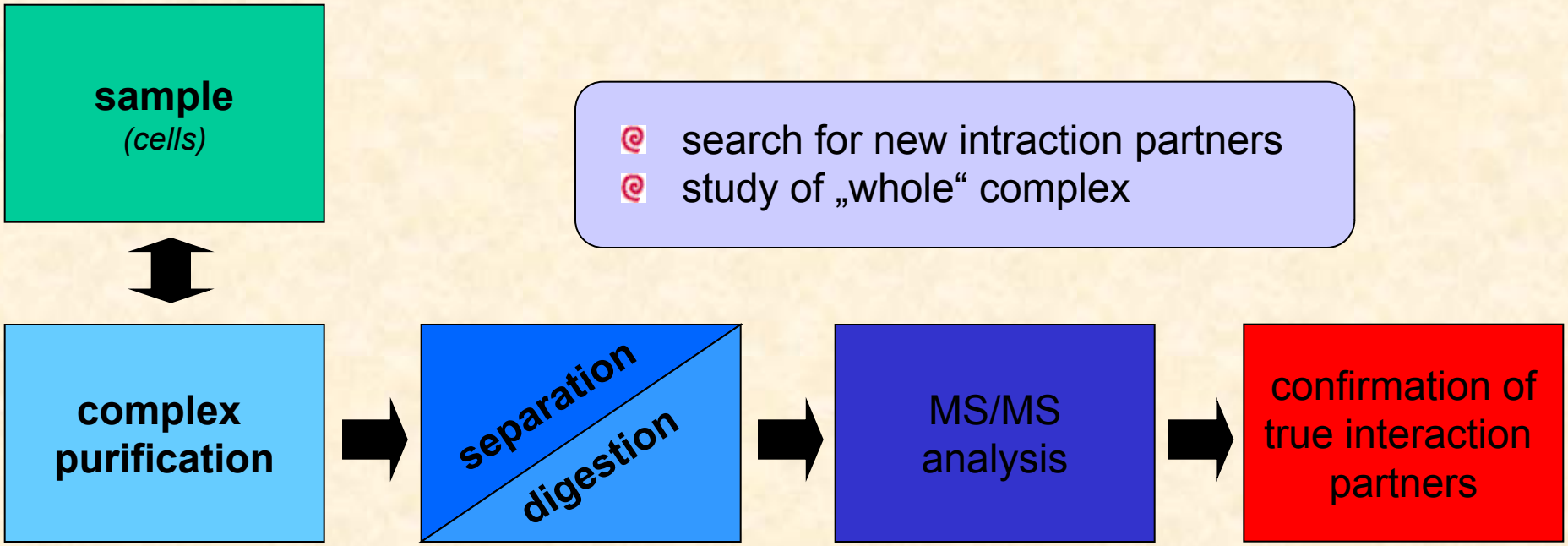
Charakterization of protein complexes
functional proteomics

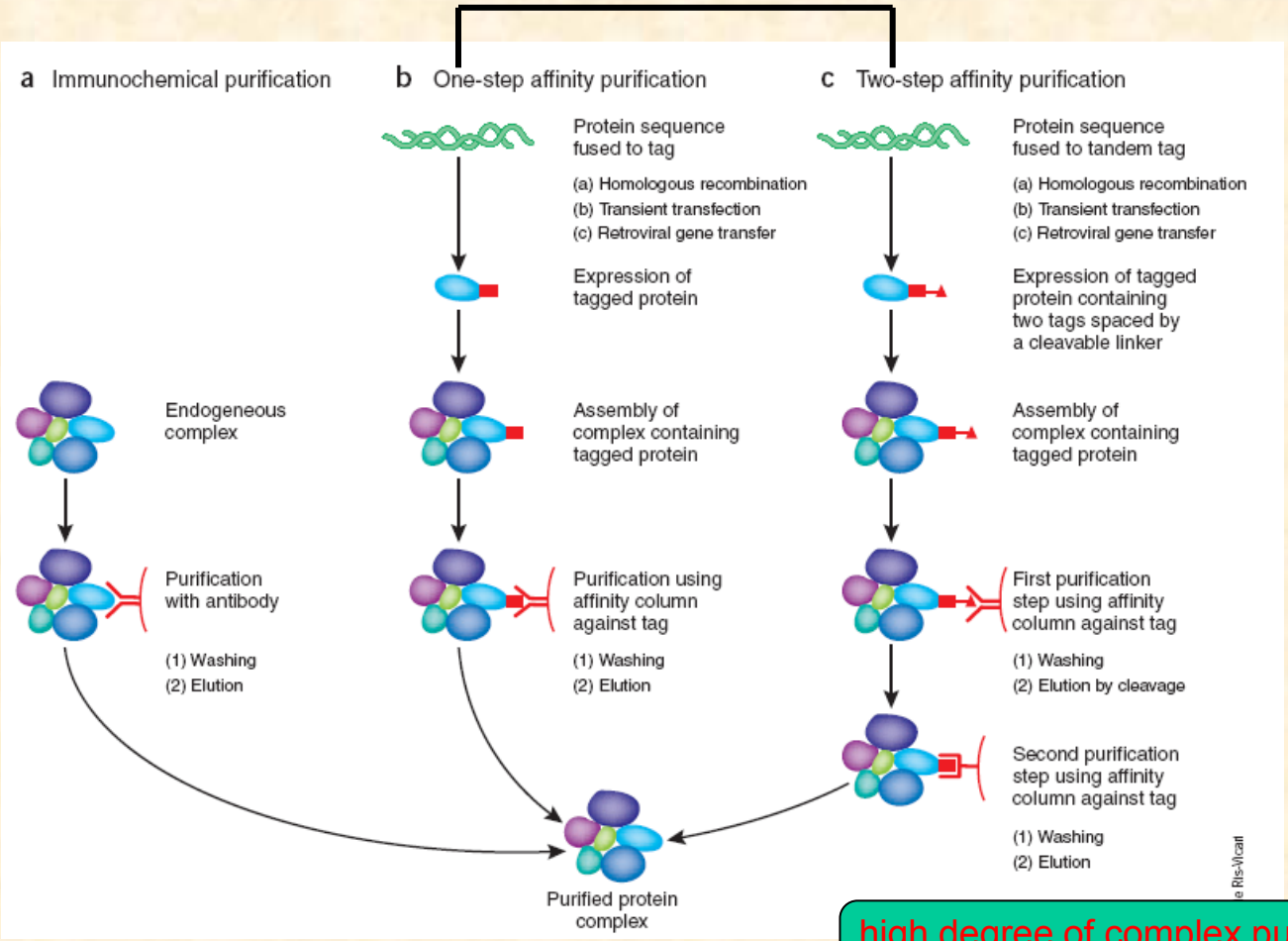
- > 80% proteins is functional only as a part of complex
- ~ 10000 types of interactions

Aloy P., Russell R. B.: Nat. Biotechnol. ***22*** *(10), 1317-1321 (2004)*



Purification of protein complexes

in vivo expression of bait protein with a tag

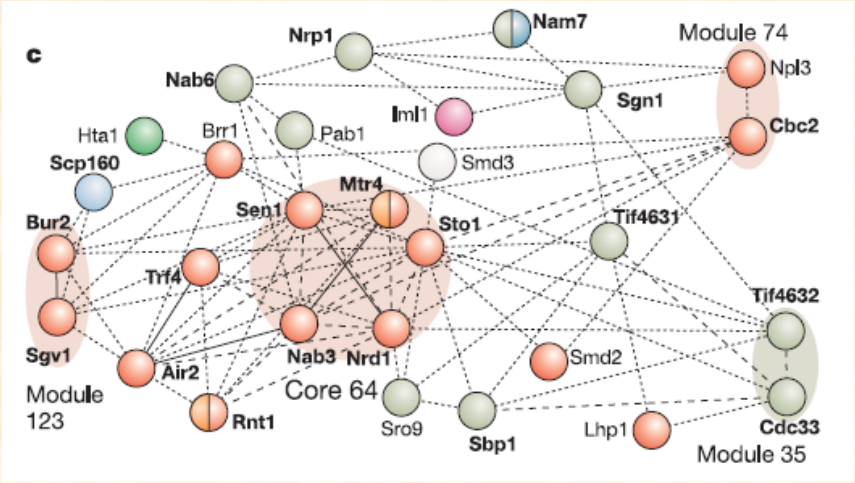


high degree of complex purity
loss of weakly binded interaction partners

*T. Köcher, G. Superti-Furga: Nat. Methods, **4**(10), 807-815 (2007).*

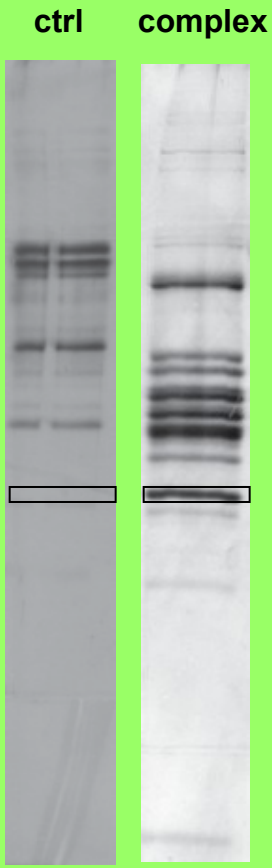
MS capabilities in protein complex analysis

- identification of individual complex members including their PTMs
- confirmation of interaction partners (exclusion of nonspecific interactors)
- determination of complex stoichiometry
- determination of 3D structure of complex (cross-linking)



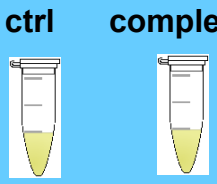
Gavin A.-C. et al.: Nature, **440** (30), 631-636.

Identification of individual interaction partners



digestion in gel

LC-MSMS



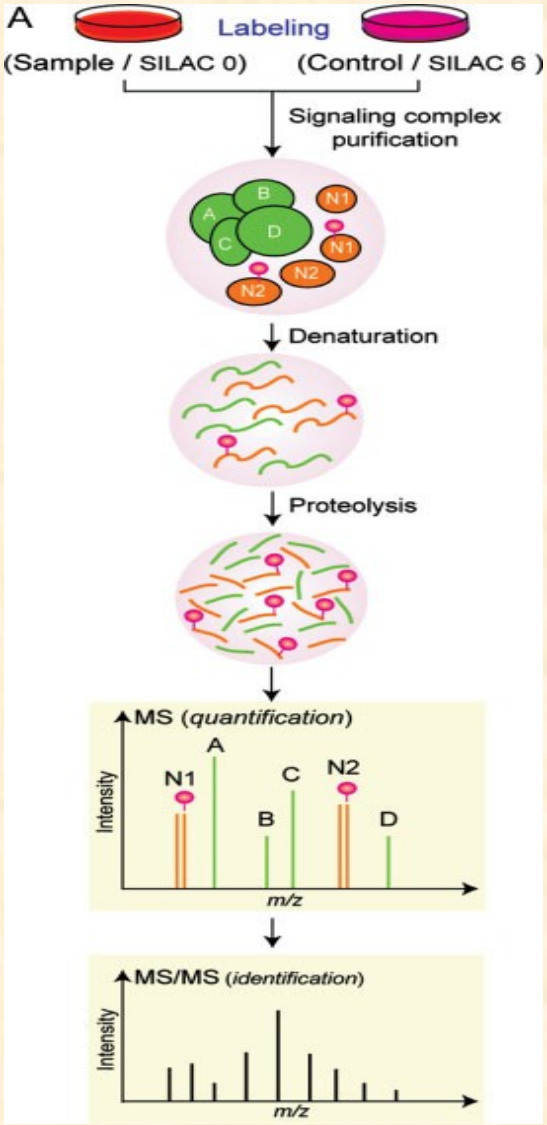
sample prep

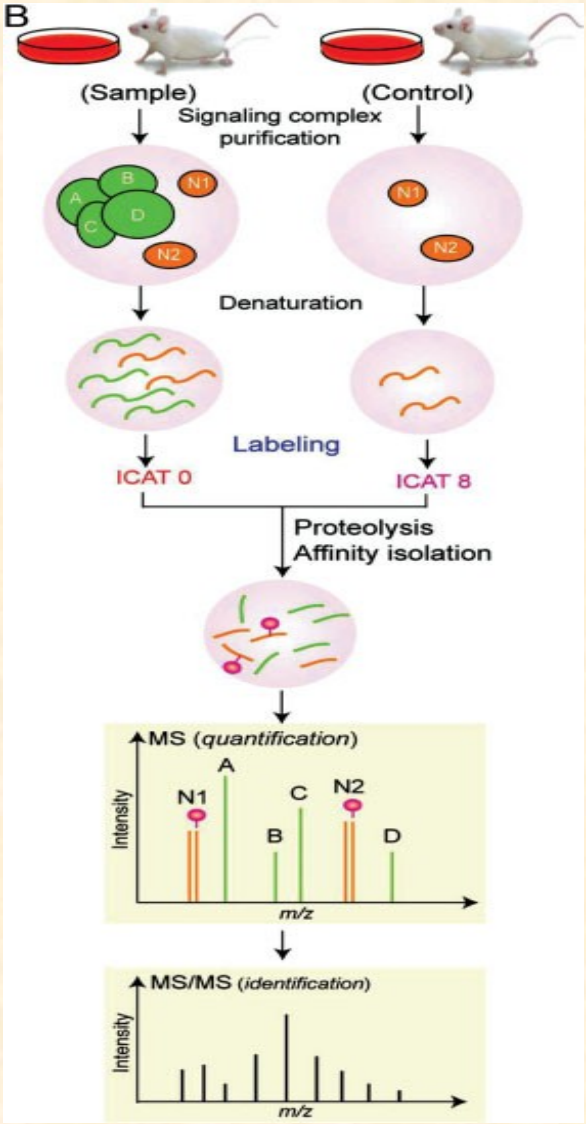
digestion in solution

LC-MSMS

possibility to label samples by tags for relative quantification and to pool the samples for further processing

Confirmation of true interaction partners





non-specific interaction 1:1

W. Yang *et al., Proteomics* 2008, *8,* 832–851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Austrian
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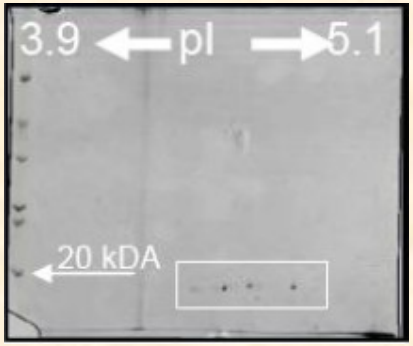
Sequence confirmation and determination of OBP protein isoforms

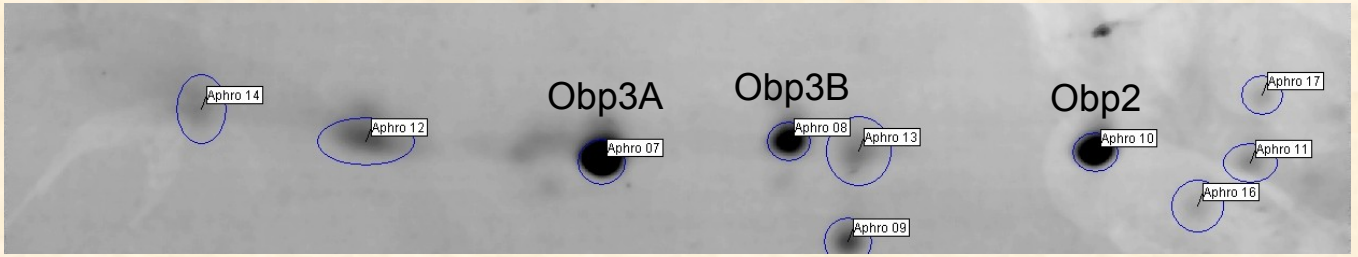
de novo sequencing

Myodes glareolus

- saliva
- 2D gel electrophoresis
- MS/MS of selected spots

- unknown genome
- no antibodies

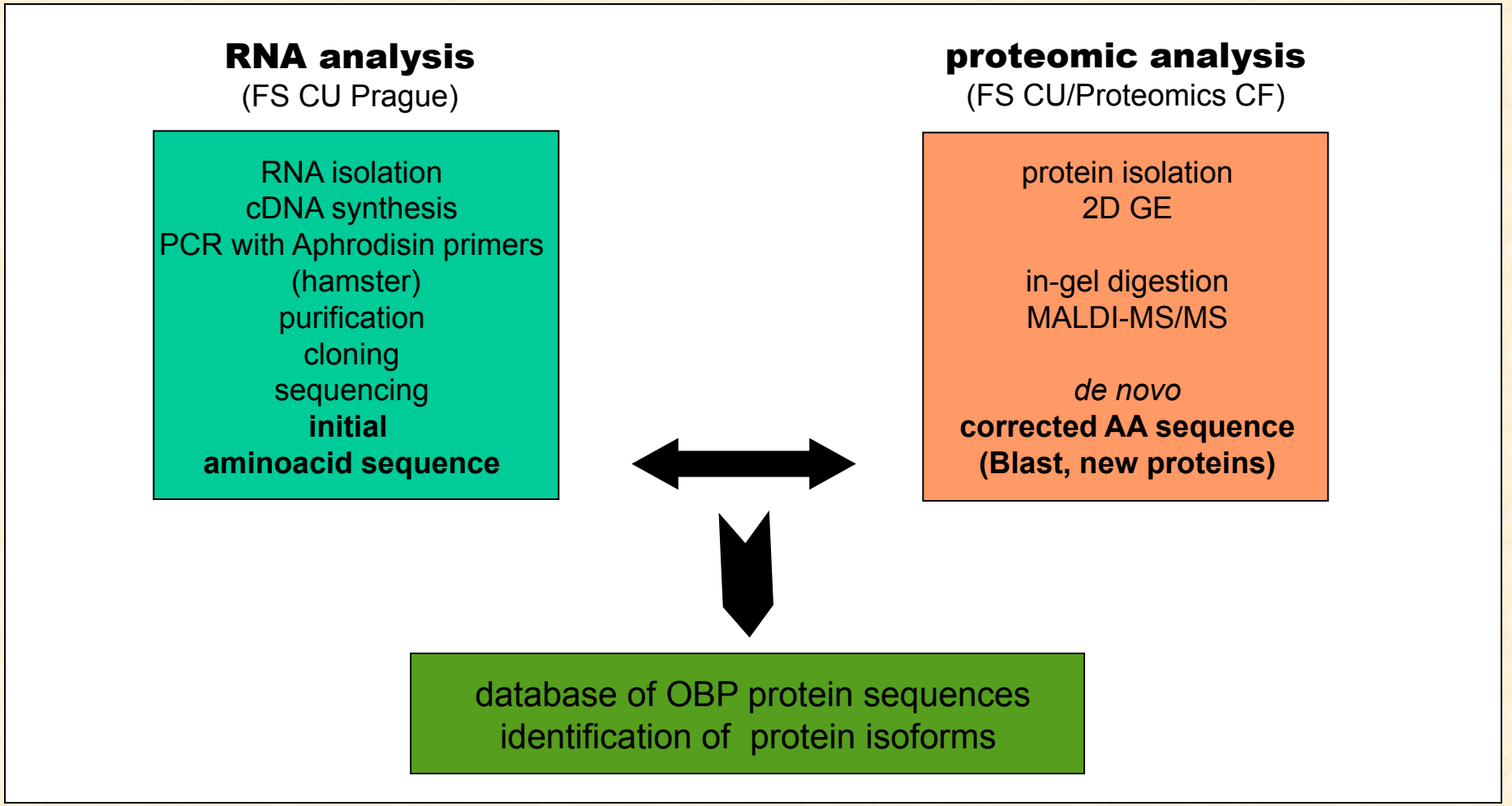




cooperation with prof. Stopka FS CU, Prague

*Stopková R., Zdráhal Z., Ryba Š. et al, BMC Genomics 2010, **11**:45*

Sequence confirmation and determination of OBP protein isoforms



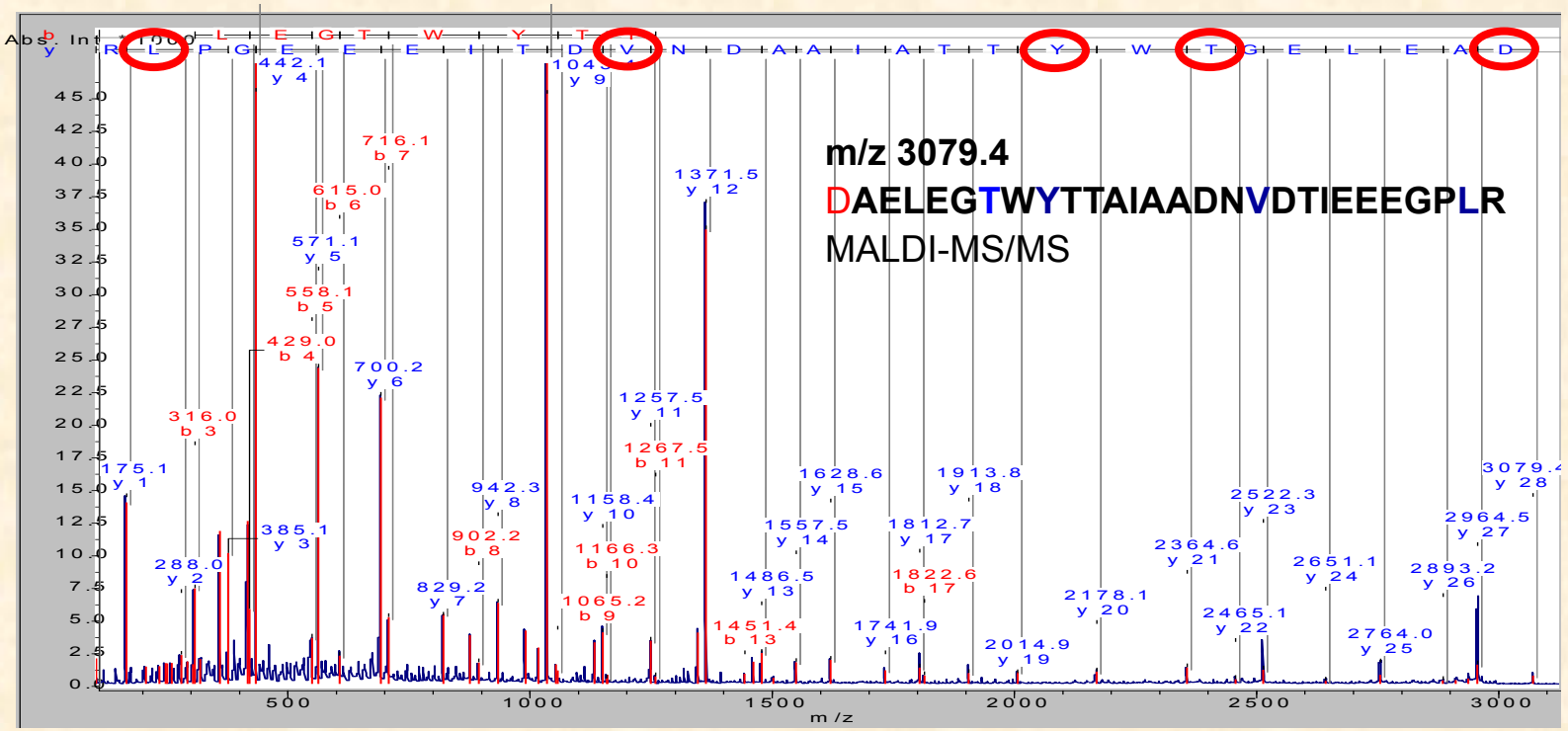
Sequence confirmation and determination of OBP protein isoforms

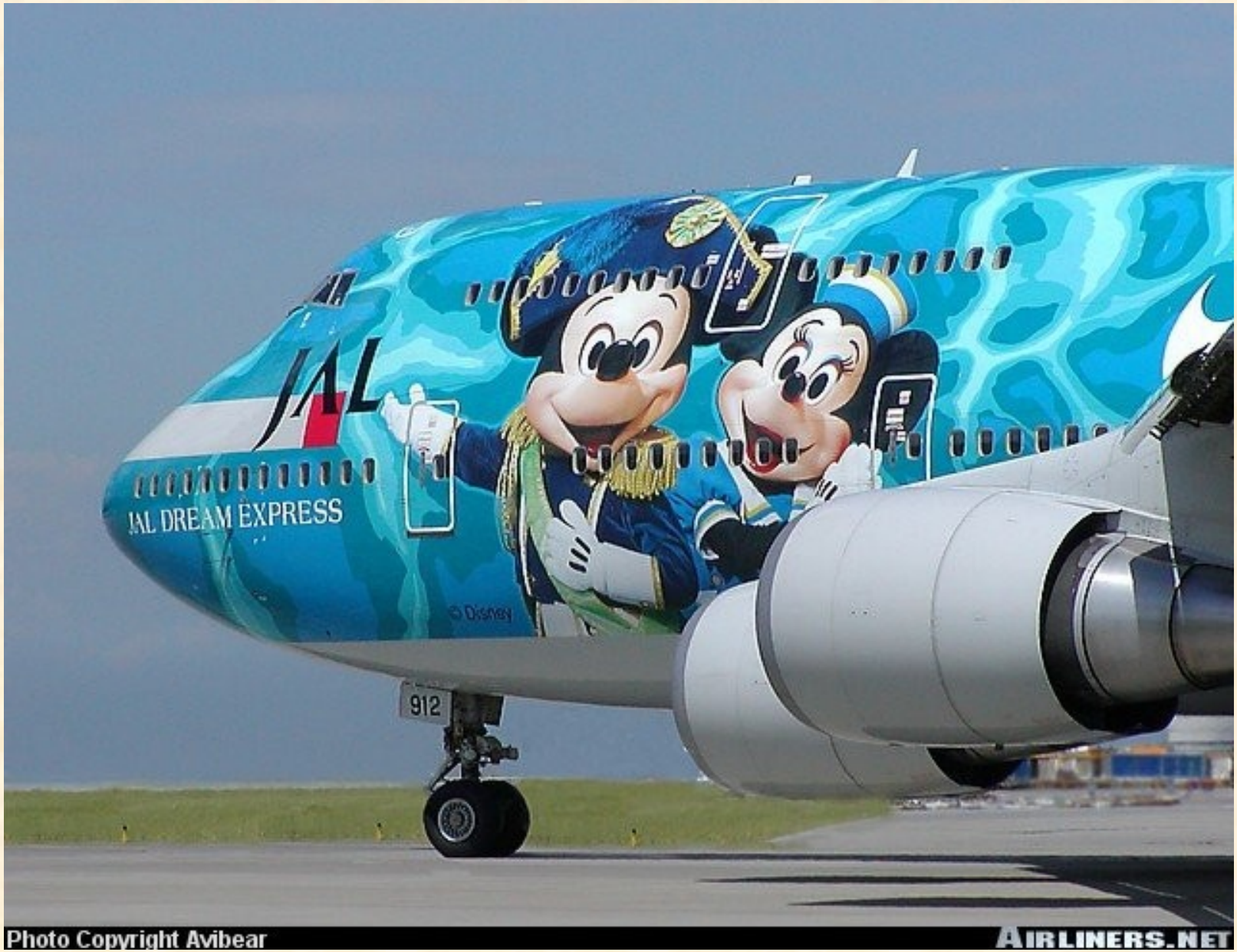
MALDI-MS/MS a LC-MS/MS
manual spectra interpretation

original sequence - QAELEGKWVTTAIAADNIDTIEEEGPMR (OBP3)

DAELEGTWYTTAIAADNVDTIEEEGPLR

HAELEGTWYTTAIAADNVDTIEEEGPLR

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JAL DREAM EXPRESS
912
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MS Imaging

MALDI-MS imaging

samples:

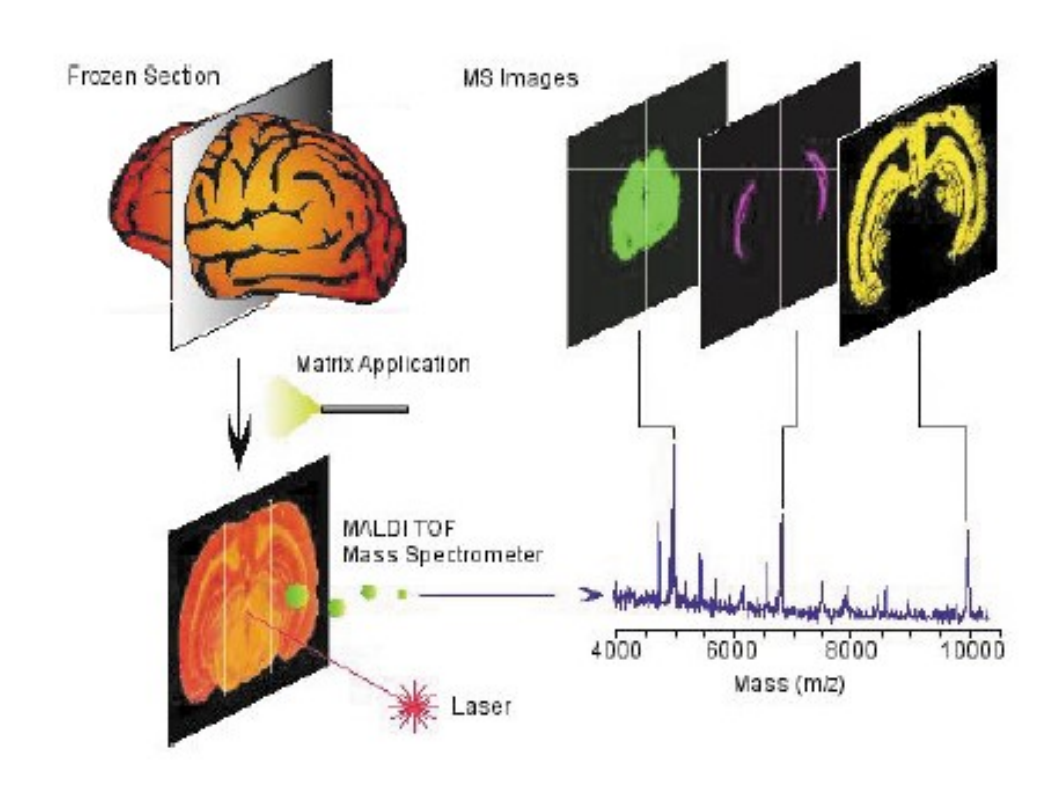
- fresh frozen tissue sections
- individual cells or clusters of cells isolated by **laser-capture microdissection** or **contact blotting** of a tissue on a membrane target.

MS analysis

- scanning of sample area point by point
- image corresponds to planar distribution of individual *m/z*

distribution of peptides (proteins, lipids,...)

MALDI-MS imaging



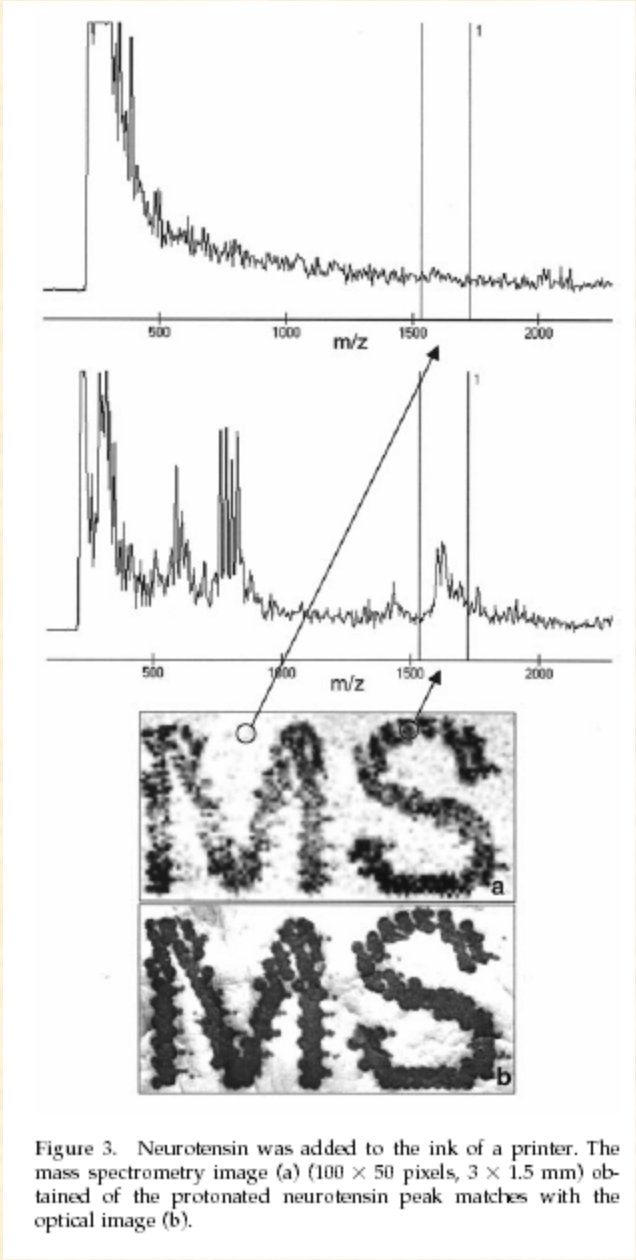


Figure 3. Neurotensin was added to the ink of a printer. The mass spectrometry image (a) (100 × 50 pixels, 3 × 1.5 mm) obtained of the protonated neurotensin peak matches with the optical image (b).

M. Stoeckli, T.B. Farmer, R.M. Caprioli: *J Am Soc Mass Spectrom*, **10**, 67–71 (1999)

MALDI-MS imaging

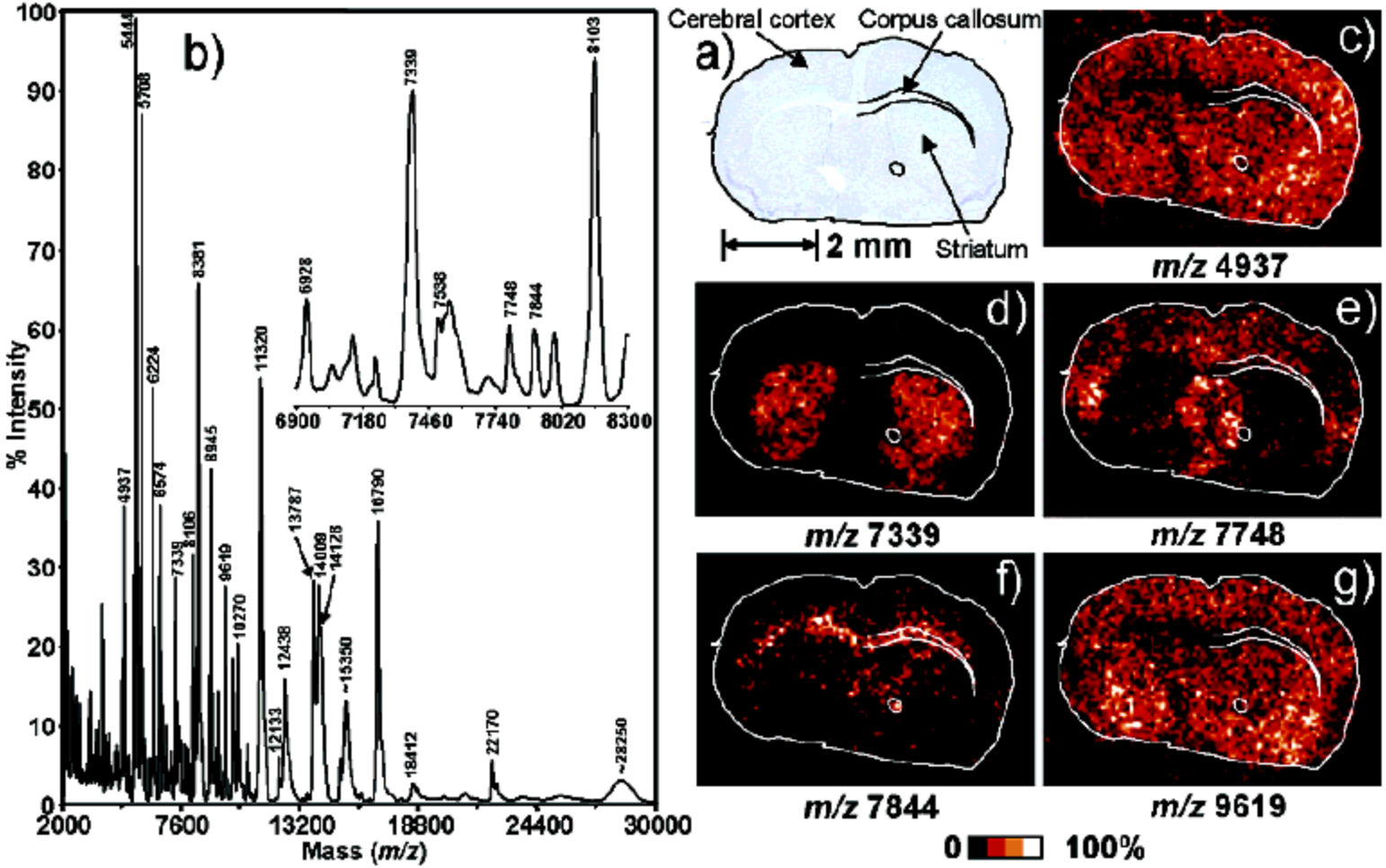
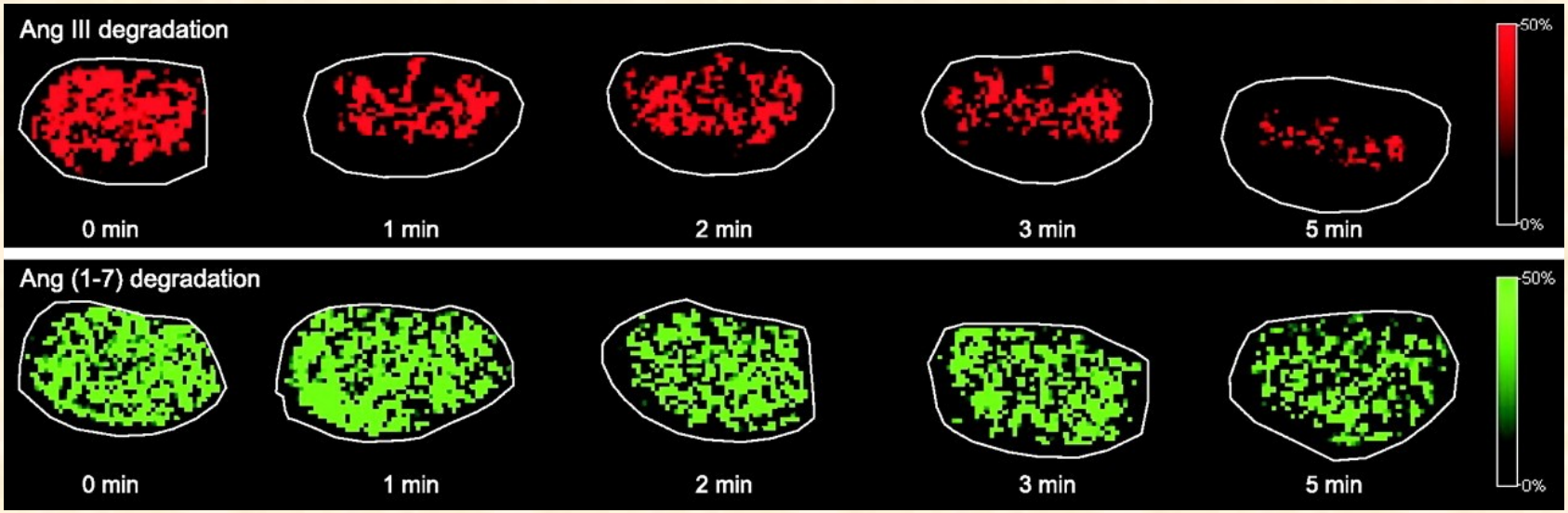


Figure 8. IMS analysis of a 12-μm coronal mouse brain section. (a) Photomicrograph of a Cresyl Violet-stained section showing different anatomic brain substructures. (b) MALDI-MS protein profile obtained after homogeneous matrix deposition averaging all of the individual spectra acquired from the section. (c–g) Ion density maps obtained at different *m/z* values with an imaging resolution of 100 μm. The ion density maps are depicted as pseudocolor images with white representing the highest protein concentration and black the lowest.

Chaurand *et al*:*Anal. Chem.***2004,** 76,1145-1155

Degradation of Ang III and Ang-(1–7) in mouse kidney sections.



Grobe N. et al, Am. J. Physiol. Endocrinol. Metab., 302 (8), E1016 (2012)

The end