ESC Summer School Cardiovascular Sciences June 18, 2013

PROTEOMICS IN VASCULAR BIOLOGY

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- 1. Basics of proteomic mass spectrometry
- 2. Quantitative proteomics
- 3. New developments; Case studies

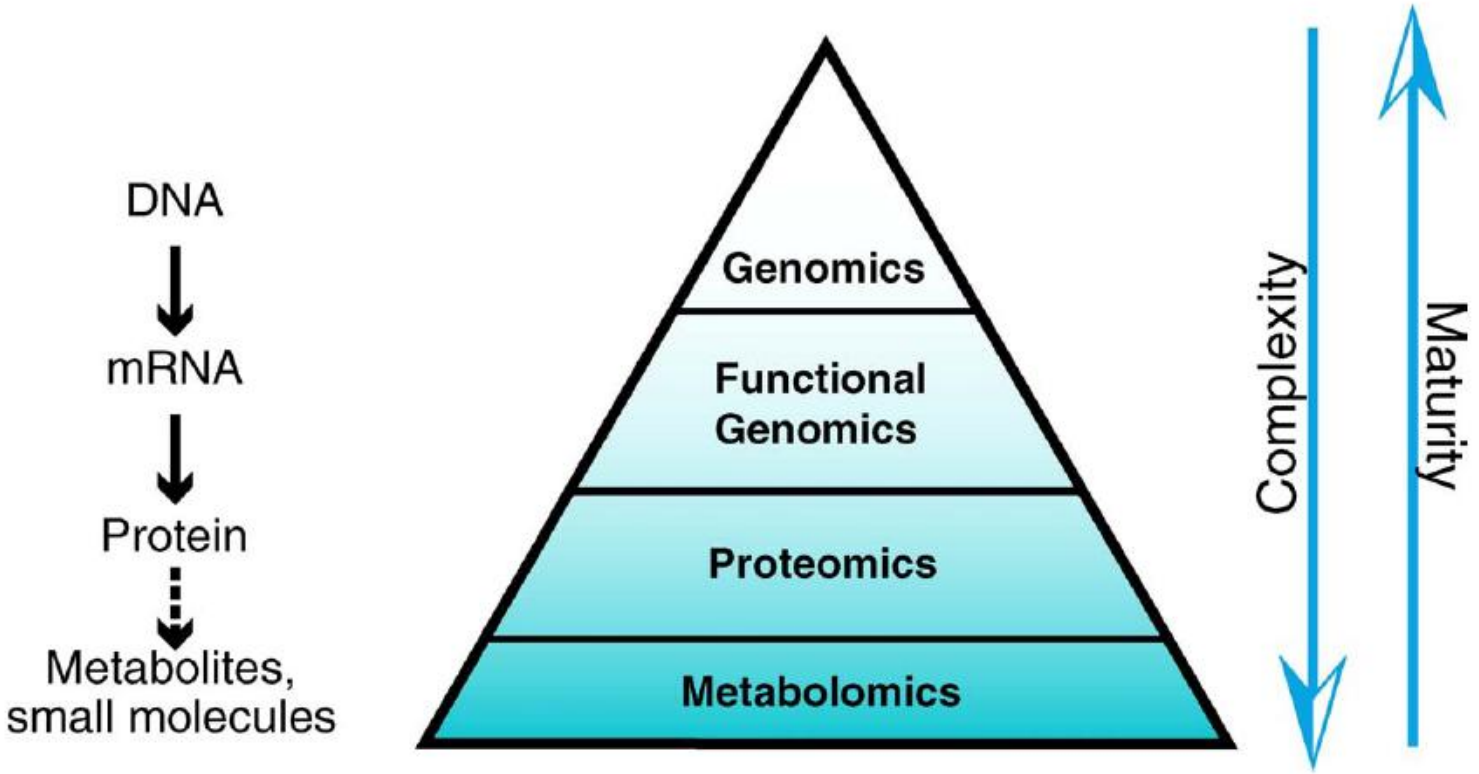
http://genomebiology.com/2001/2/11/comment/2008.1

Opinion

What does a worm want with 20,000 genes?

Jonathan Hodgkin

Moving from genomics to proteomics

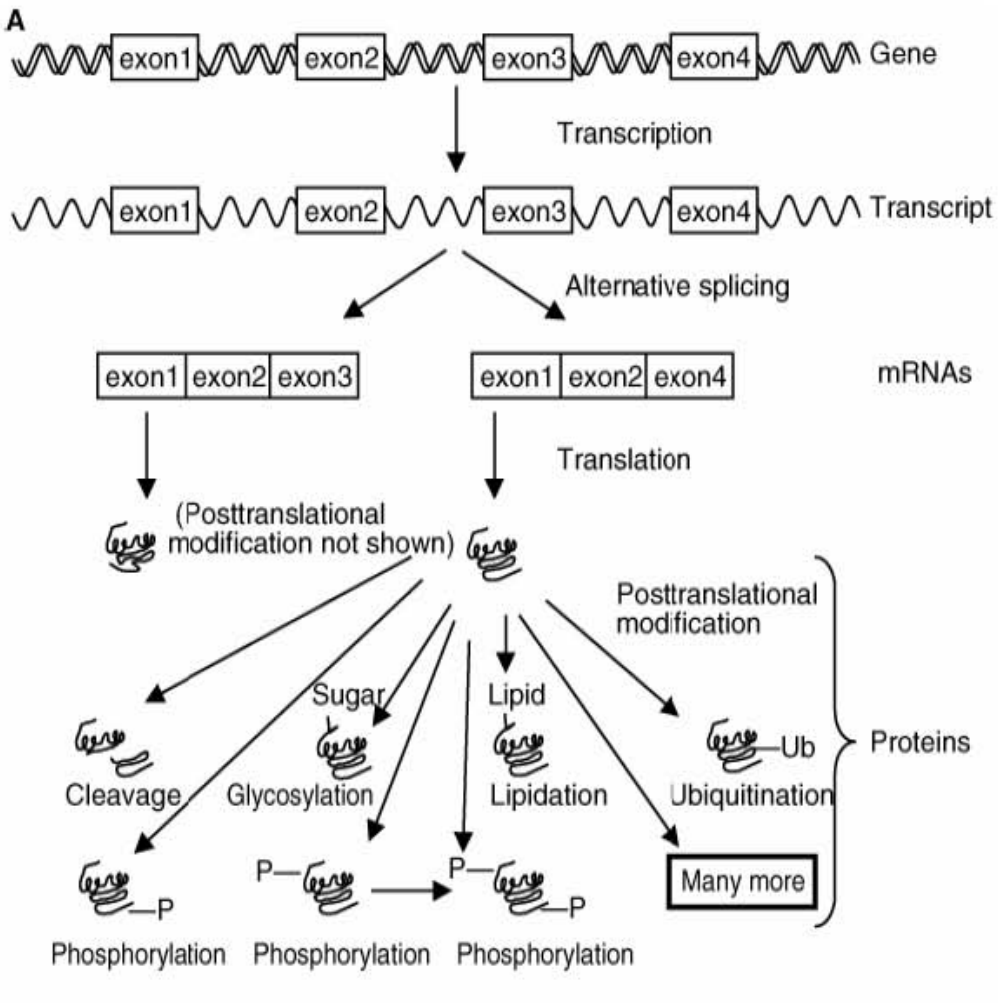


de Hoog CL, Mann M Annu Rev Genomics Hum Genet. 2004;5:267-93

One Genome – Two Proteomes

Inachis io

Complexity of the proteome



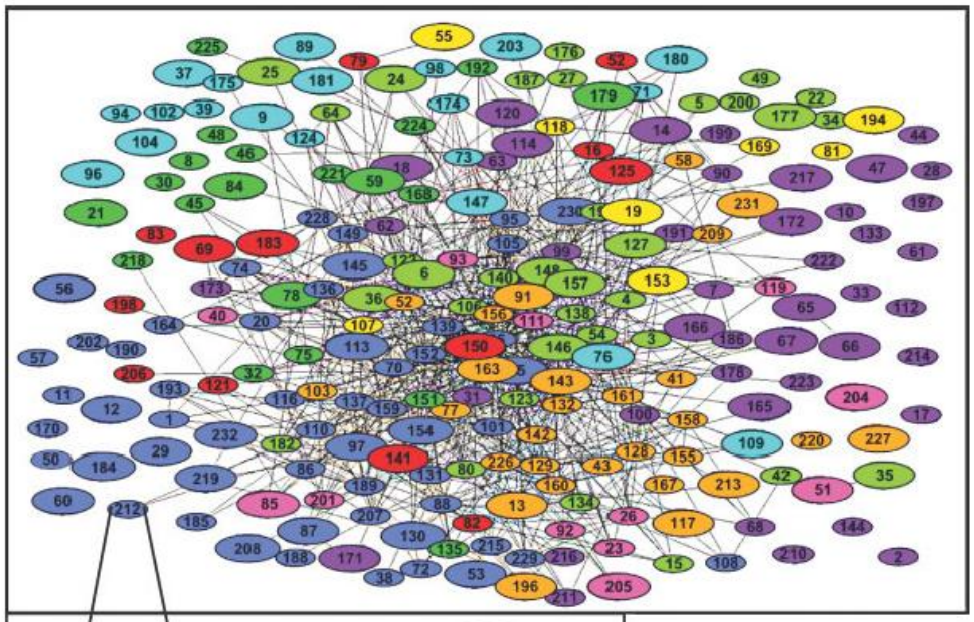
What can happen

What might happen

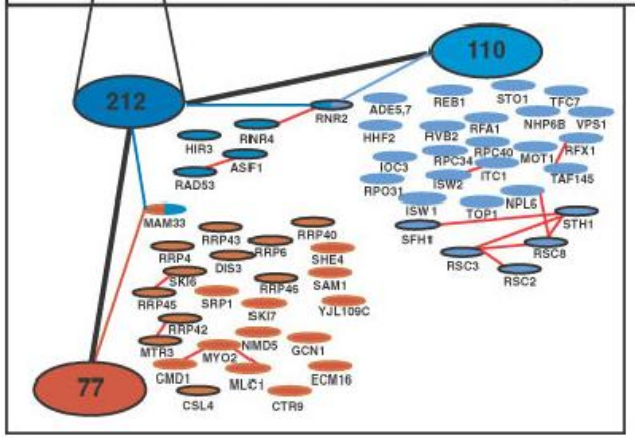
What is likely going to happen

What is happening

The protein complex network in *S. cerevisiae*



- 16830 proteins identified
- 1140 distinct gene product
- 25 % ORF's
- 232 complexes



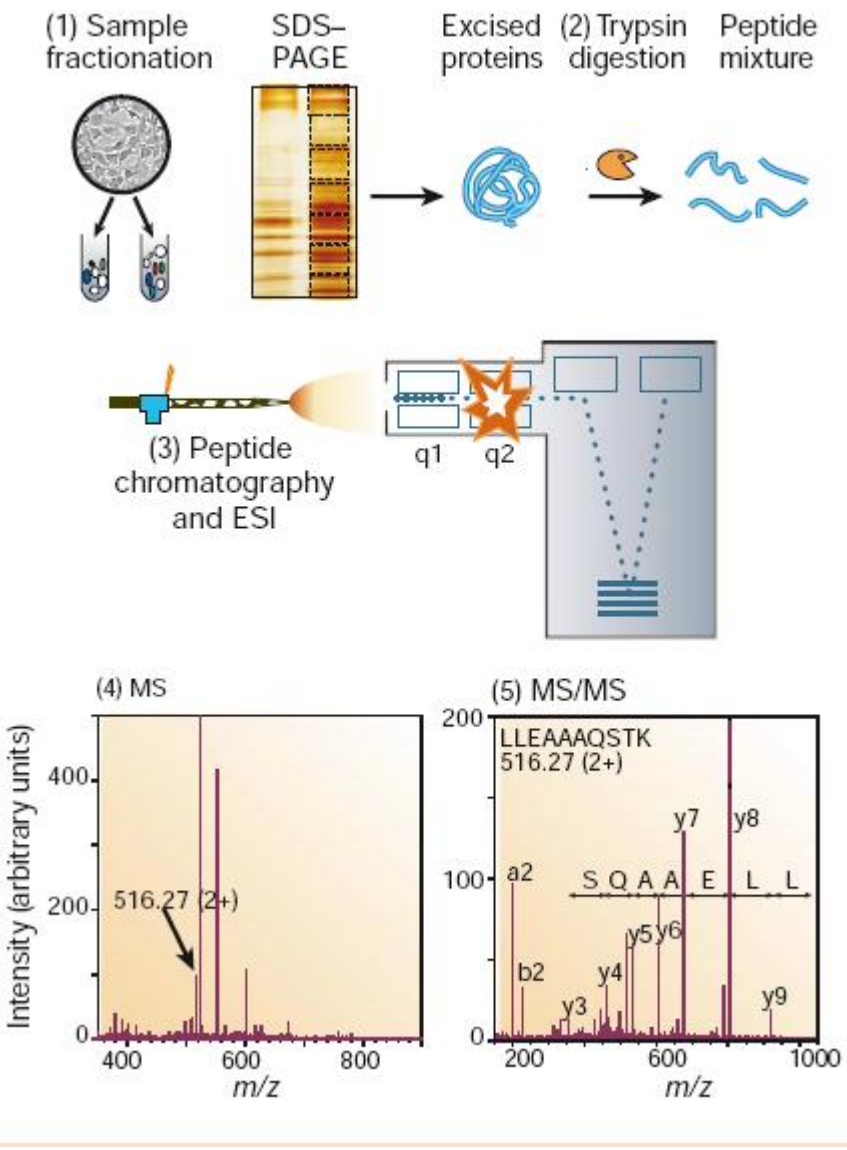
- Many complexes share components
- Complexes in related processes have the same color
- Map provides information for generation of hypothesis leading to further experimental investigations

Protein mass spectrometry develops in various stages

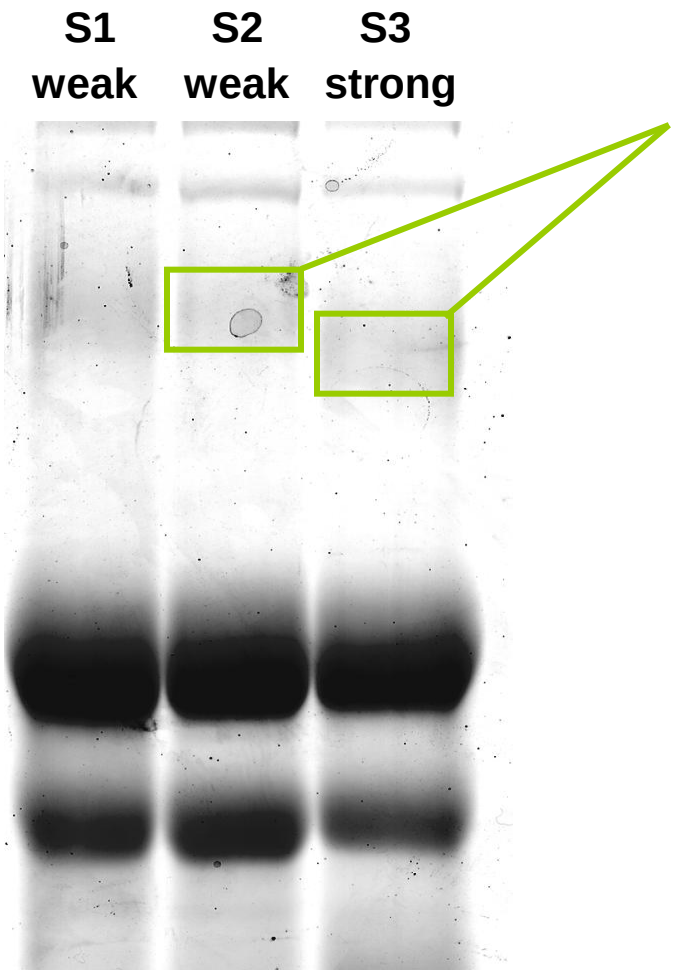
- What is the primary structure and the identity of the protein?
- What is the higher order structure of the protein, which structural domains can be discerned
- What is the subunit composition of functional protein complexes
- What is the topology of a protein and protein complexes
- Which functional domains are present and what is the function of proteins and assemblies of proteins
- How is the function of a protein regulated by post translational modifications
- How is the function of a protein regulated by interaction with other proteins

- 1. Basics of proteomic mass spectrometry
- 2. Quantitative proteomics
- 3. New developments; . Case studies

The basics of MS



Identification



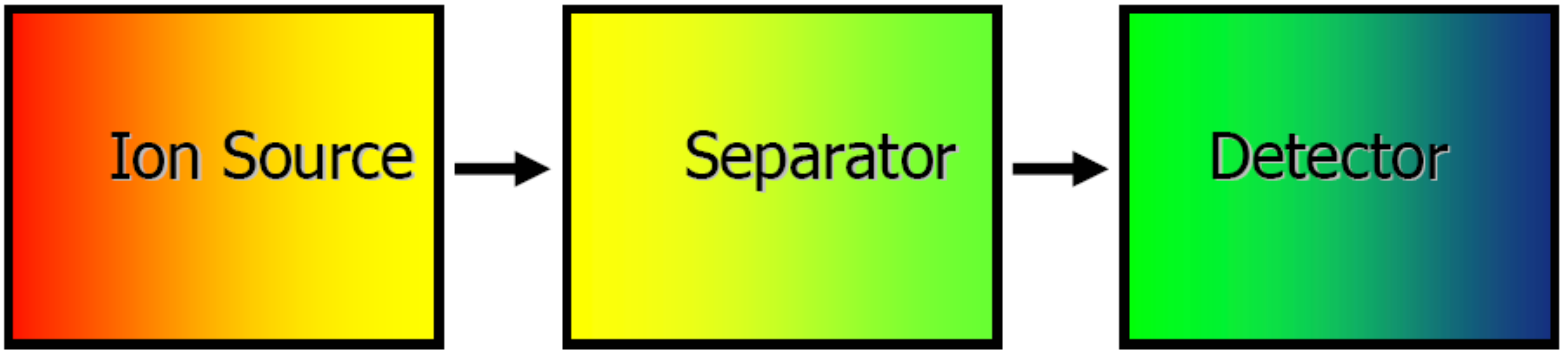
Coomassie SDS-PAGE

What is this protein ‘band’???

How would you solve this question?

Principle of Biomolecular Mass Spectrometry

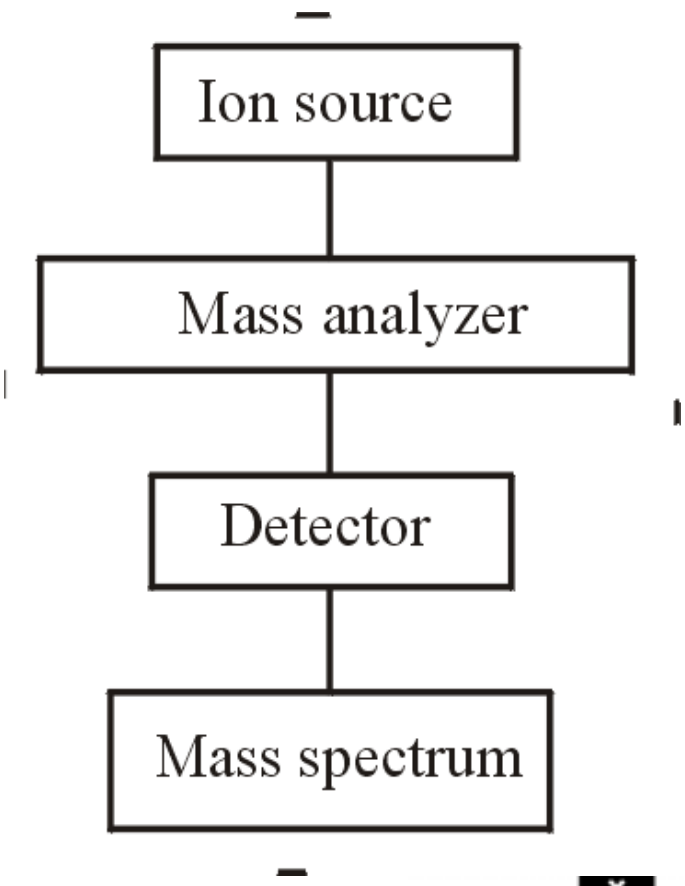
- transfer biomolecule into the gas phase
- ionize biomolecule
- apply electromagnetic fields
- analyse ion movements
- determine m/z of the biomolecule and the number of ions



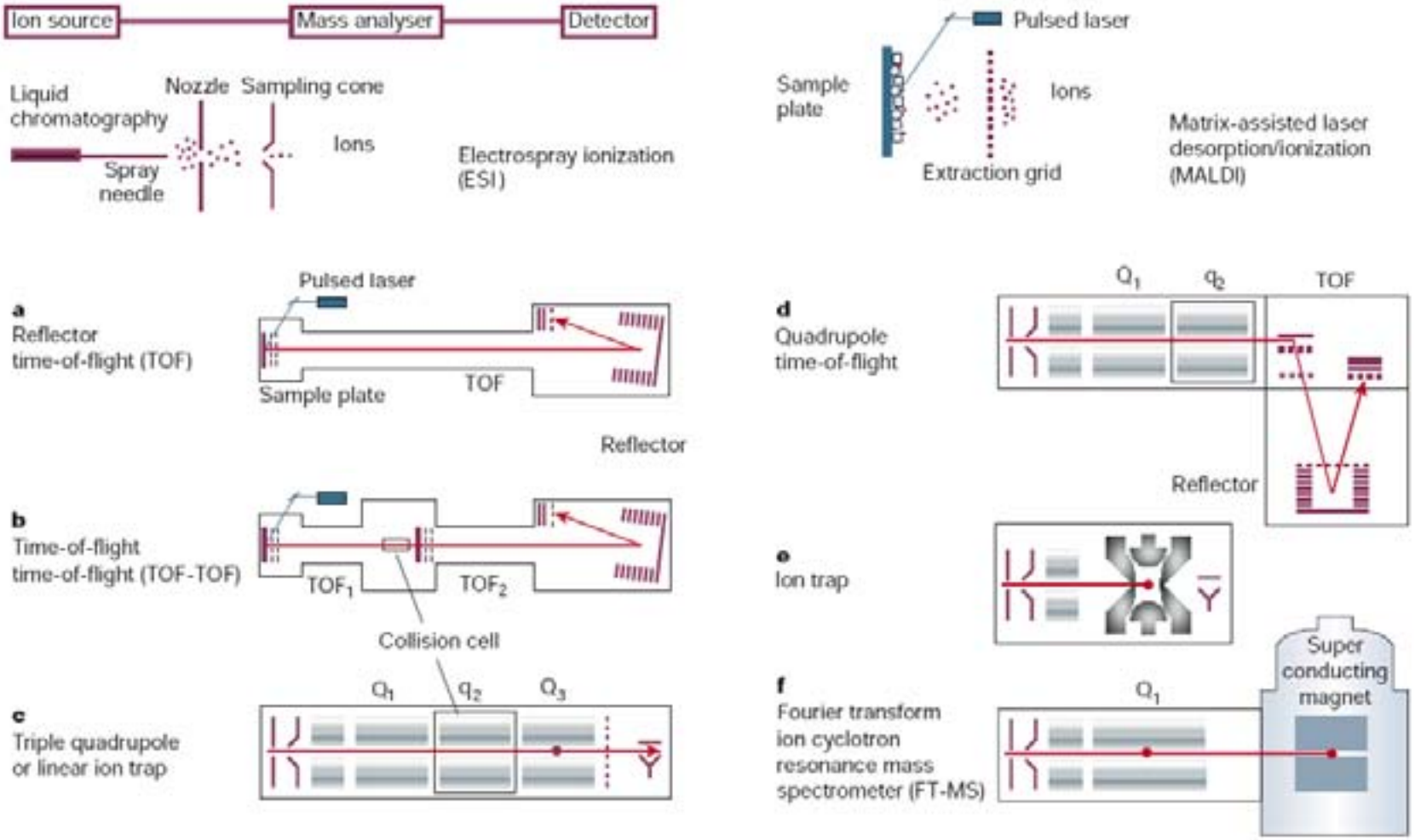
What is a mass spectrometer?

A mass spectrometer is an instrument wherein

- Ions are generated
- Ions are separated as a function of mass over charge ration m/z
- Ions are detected
- The mass over charge ratio and the amount of ions are determined



Many types of Mass spectrometers

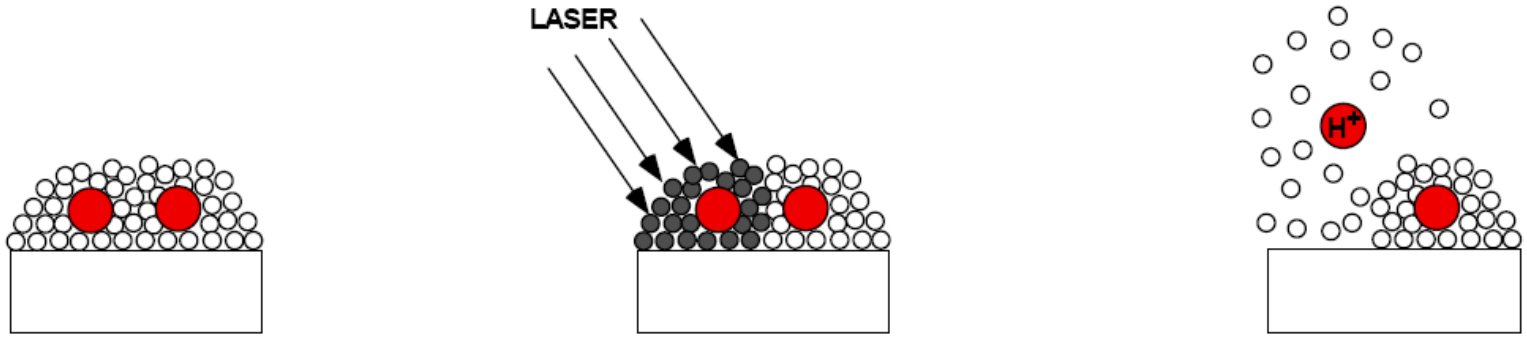


Matrix-Assisted Laser Desorption Ionization (MALDI)

Sample embedded in light-absorbing matrix

LASER-excitation of matrix molecules

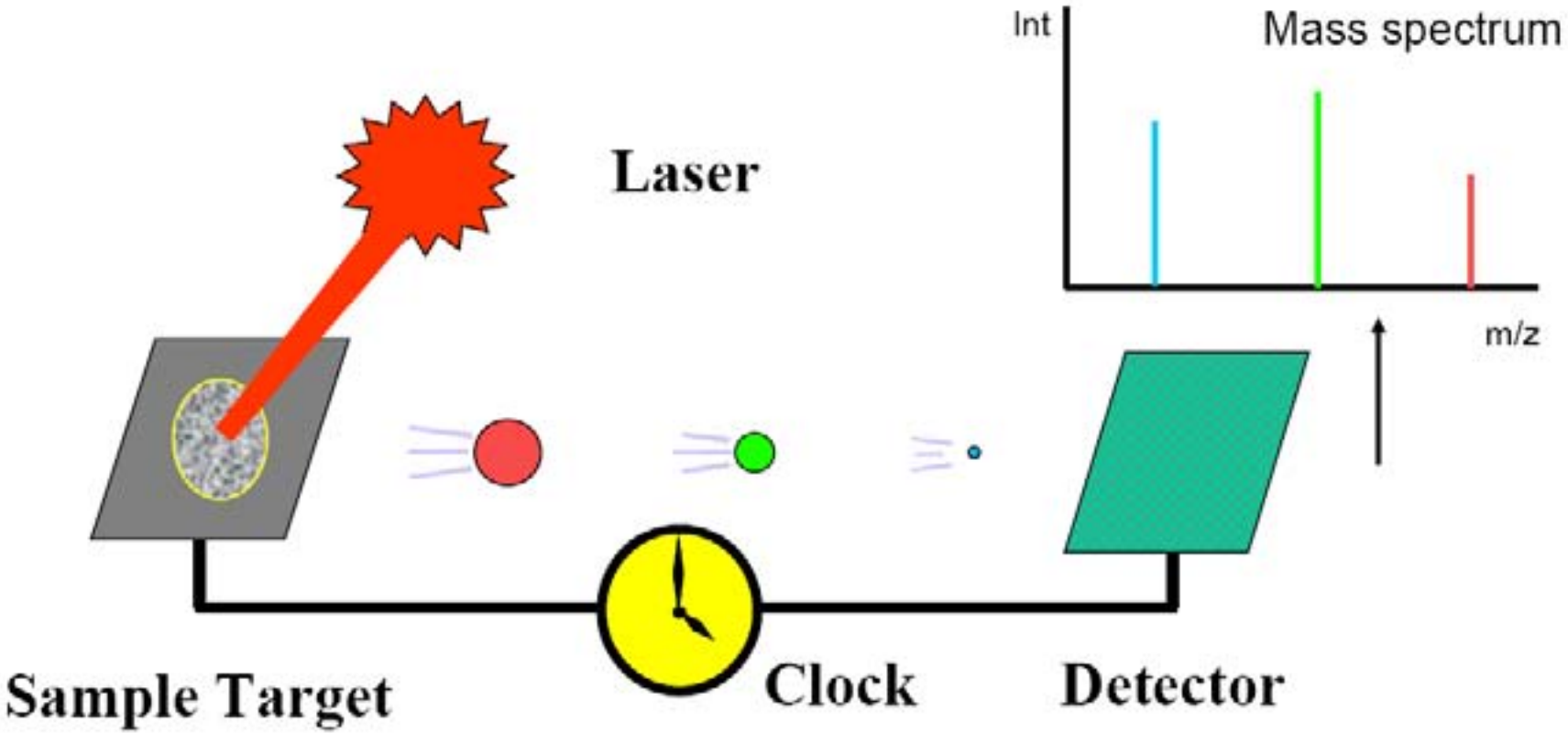
Sample desorption and protonation



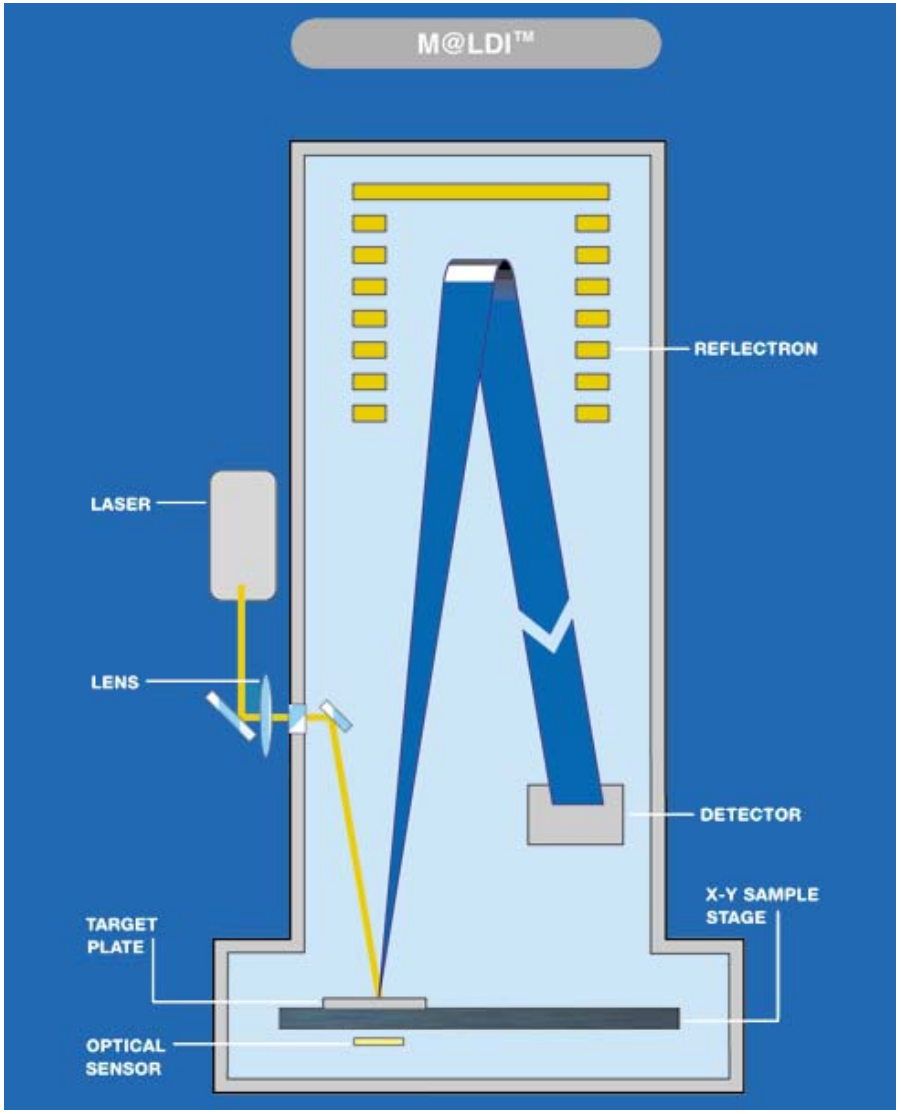
Matrix for Proteins: sinapinic acid, dihydroxybenzoic acid etc.
Matrix for Peptides: 4-hydroxy-α-cyanocinnamic acid, DHB

it co-crystallizes, absorbs laser energy, evaporates and acts as acid

Principle MALDI-TOF-MS



Time-of-Flight → Molecular Mass



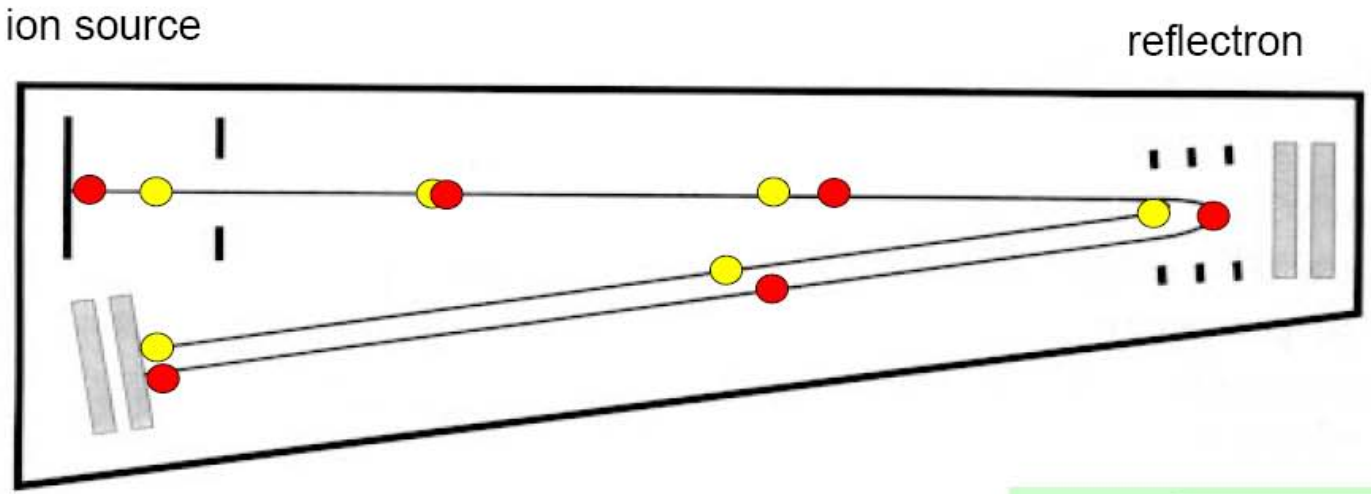
MALDI-TOF MS

- Matrix-Assisted Laser Desorption Ionization Time Of Flight ms.
- sample in UV absorbing matrix hit by UV laser pulse.
- peptideH^+ desorbed, accelerated, enters field-free flight tube
- flight time = $A.m^{1/2}$
- reflectron increases mass accuracy

Identical ions with slightly different kinetic energies are focused by the reflectron

- ions with a relatively high kinetic energy travel deeper into the reflectron than identical ions with a relatively low kinetic energy
- therefore, the faster "high energy ions" reside for longer times in the reflectron then the slower "low energy ions"
- as a result, identical ions with slightly different kinetic energy reach the detector at the same time, under properly choosen conditions

MALDI-TOF-MS Resolution: Ion trajectories in a reflectron time-of-flight mass spectrometer, where U is the kinetic energy and dU the difference in kinetic energy of two ions with mass m



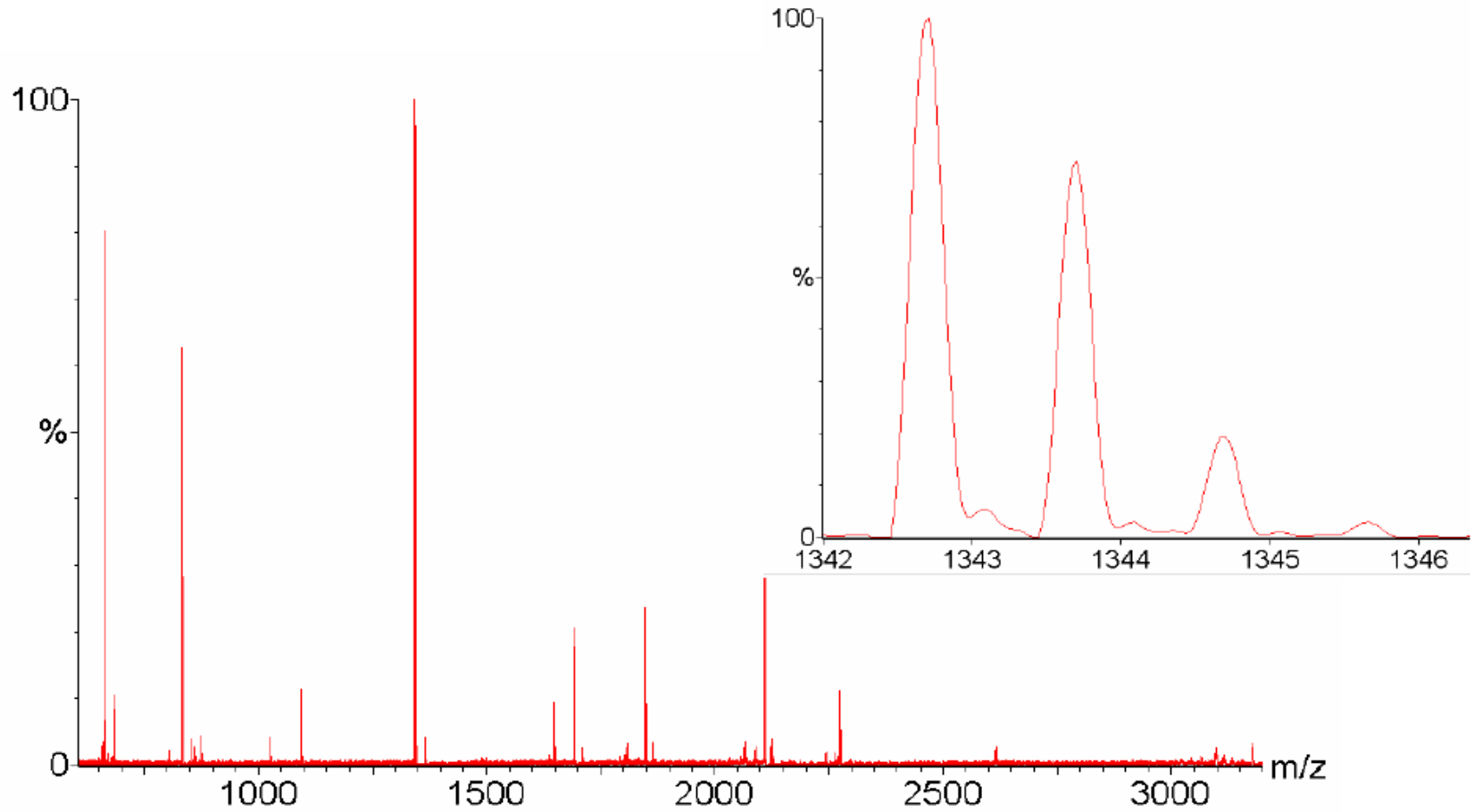
$$t_r = 2\frac{\sqrt{2m(U+dU)}}{zeE_r}$$

where t_r is the flight time in the reflectron field E_r

● U ● U+dU

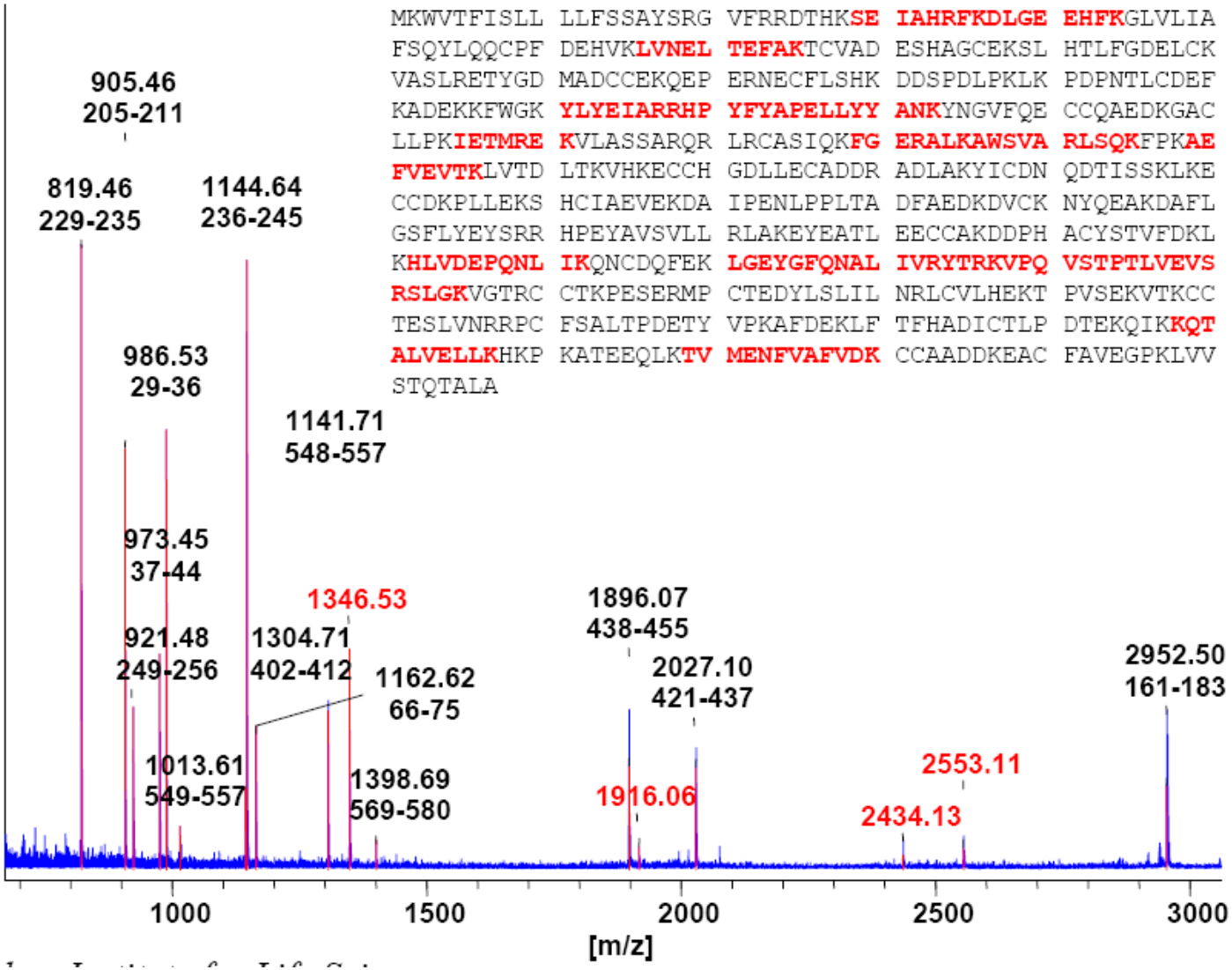
UNIVERSITEIT VAN AMSTERDAM

Mass spectrum of tryptic digest of aldolase obtained by MALDI-TOF MS



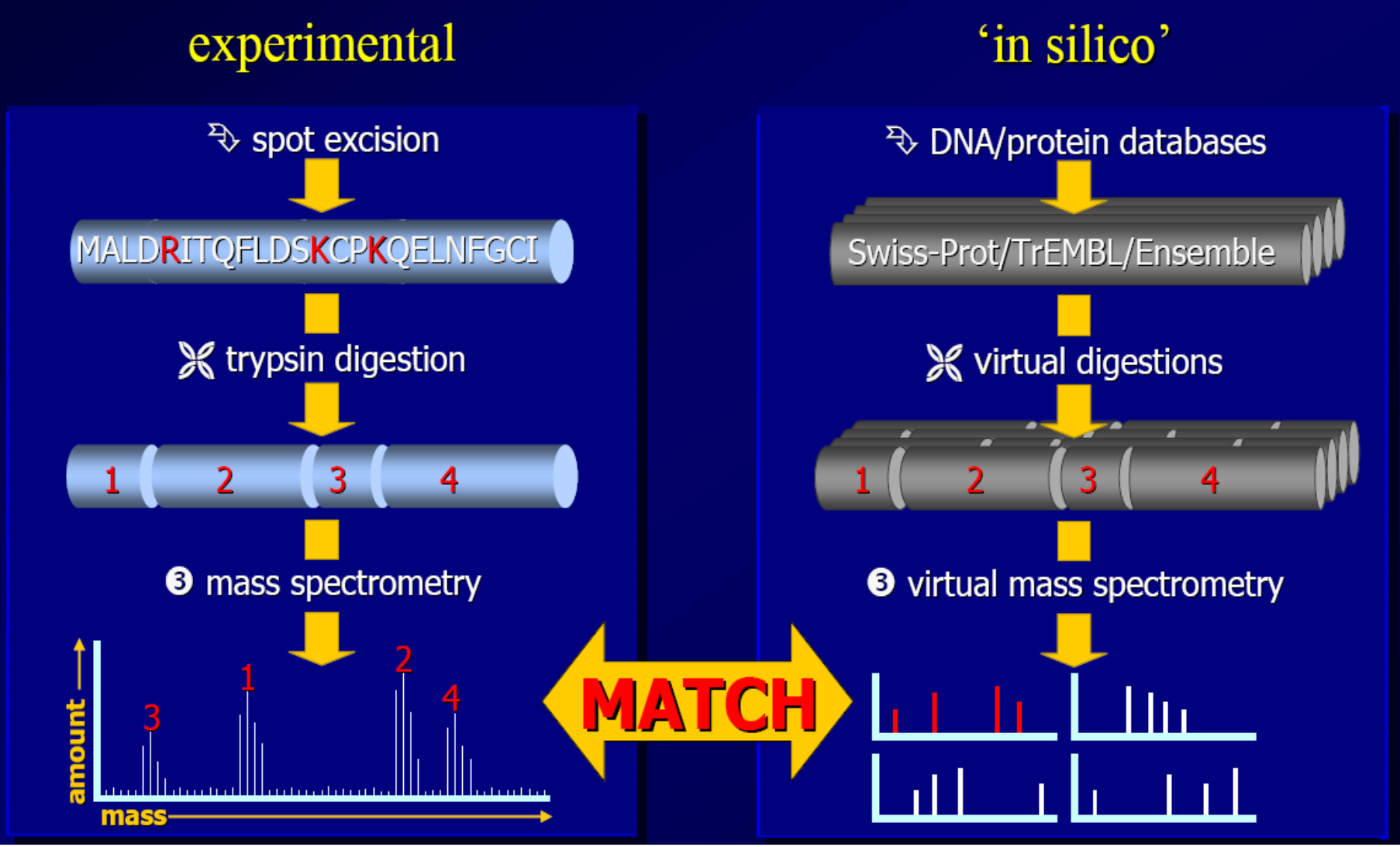
N.B. a mass spectrum like this contains enough information to identify the protein if present in a database

Lys C Digest of Bovine Serum Albumine

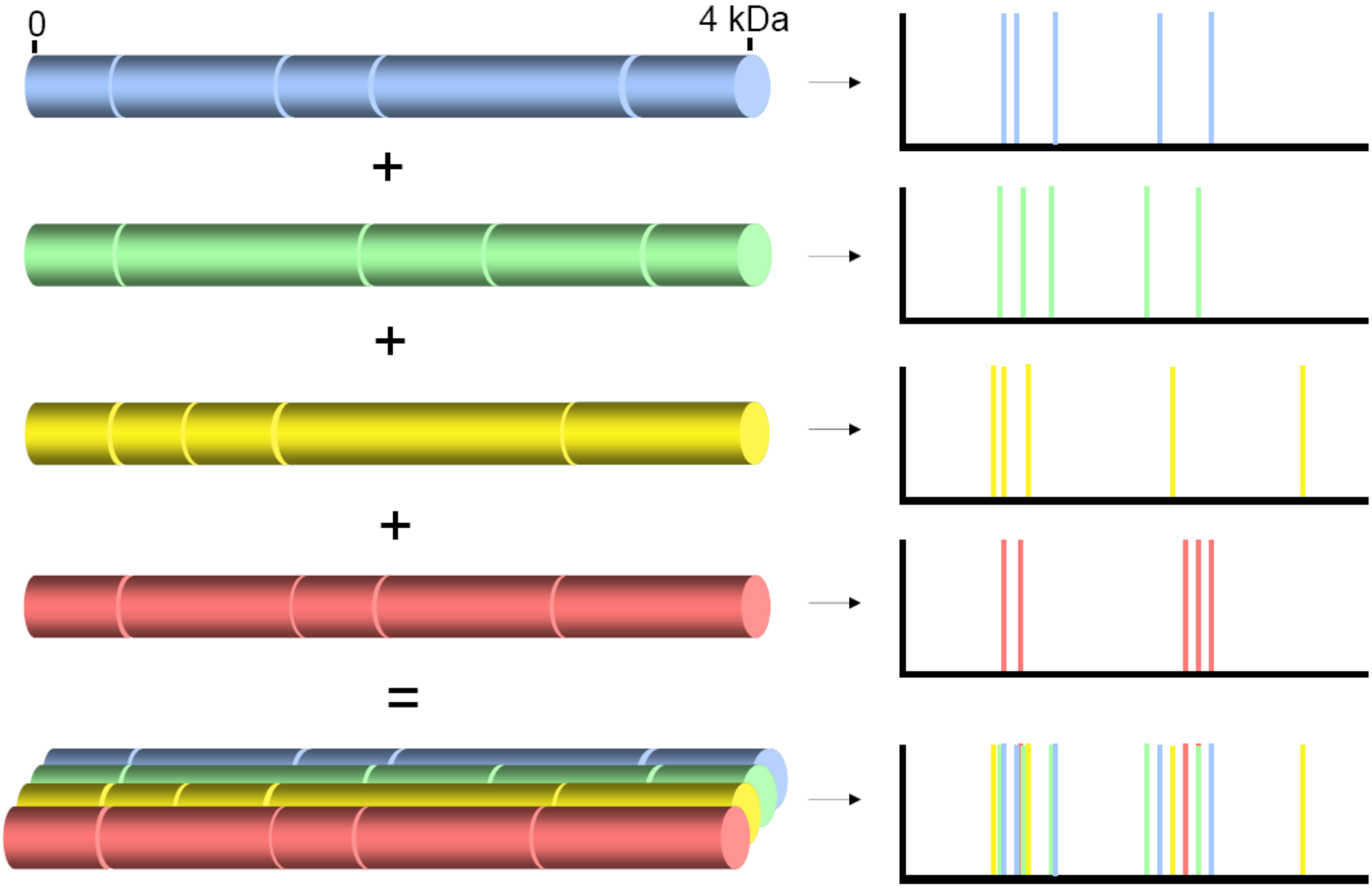


MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
STQTALA

Peptide mass finger printing



Mass Accuracy and PMF: database searching



Suppression effect

- Different peptides have different propensities to ionize
- The ionization of some peptides is suppressed by the presence of other, better ionizable peptides
- So, the signal intensity of a peak in a mass spectrum of a mixture of peptides is not a measure of the concentration of the peptide concerned

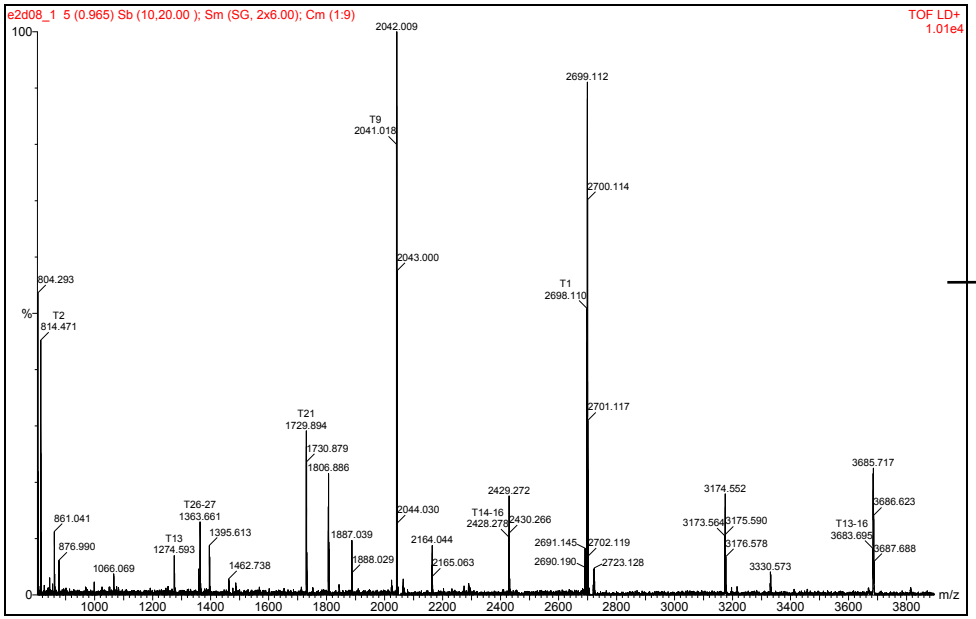
Mass analysis by MALDI-TOF-MS

- Mostly singly charged analyte ions $(M + H)^+$, minor or no fragmentation
- Mass range of analytes: 500-300000 Da
- Resolution: $m/\Delta m$; Δm: "full width at half maximum" (fwhm)
- Resolution with MALDI-TOF-MS ~20.000: no "isotopic resolution" at m/z values > 10.000
- Accurate mass measurements requires internal standards
- Mass accuracy
  - 500-3000 Da: ~ 30 ppm (~ 0.1 Da at 3000)
  - 3-10 kDa: ~ 100 ppm (~ 1 Da at 10000)
  - 10-30 kDa: ~ 300 ppm (~ 10 Da at 30000)
- Peptide mass fingerprinting demands ±0.2 Da mass accuracy
- Sensitivity for peptides: < 10 fmol

Peptide mass fingerprinting

ion

int.



m/z

814.489
861.105
1274.612
1363.655
1395.664
1729.908
1806.013
1806.869
1886.063
2041.015
2042.155
2163.046
2428.265
2690.126
2697.335
2698.127
2699.038
2719.405
2721.204
3172.451
3173.639
3682.893
3683.684

Protein dBase

Peptide dBase

compare

match

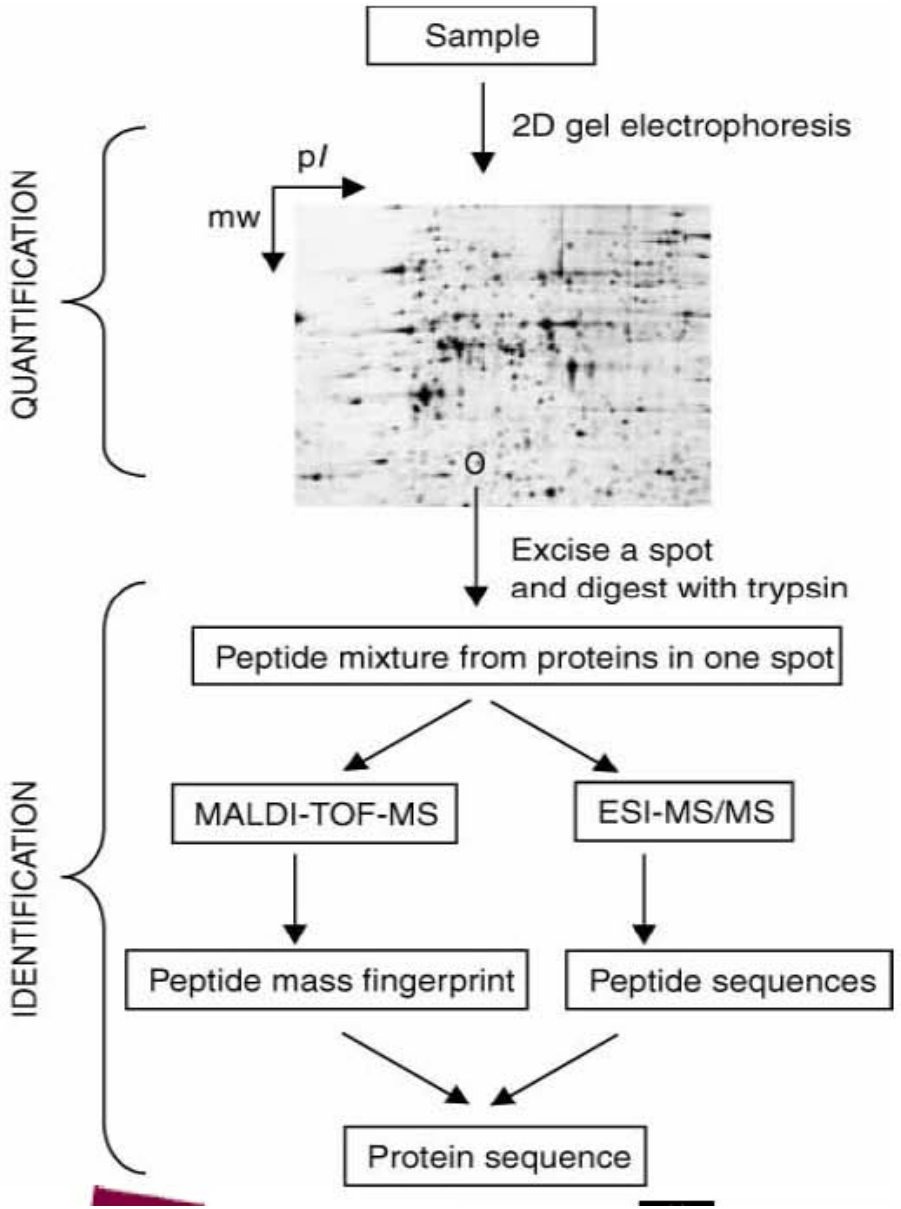
Ionisation efficiency !

Example: HSP26_yeast

Proteome analysis using two-dimensional gel electrophoresis and mass spectrometry (2DE/MS)

"a major goal of proteomics is the global and quantitative measurement of the proteins expressed in cells or tissues"

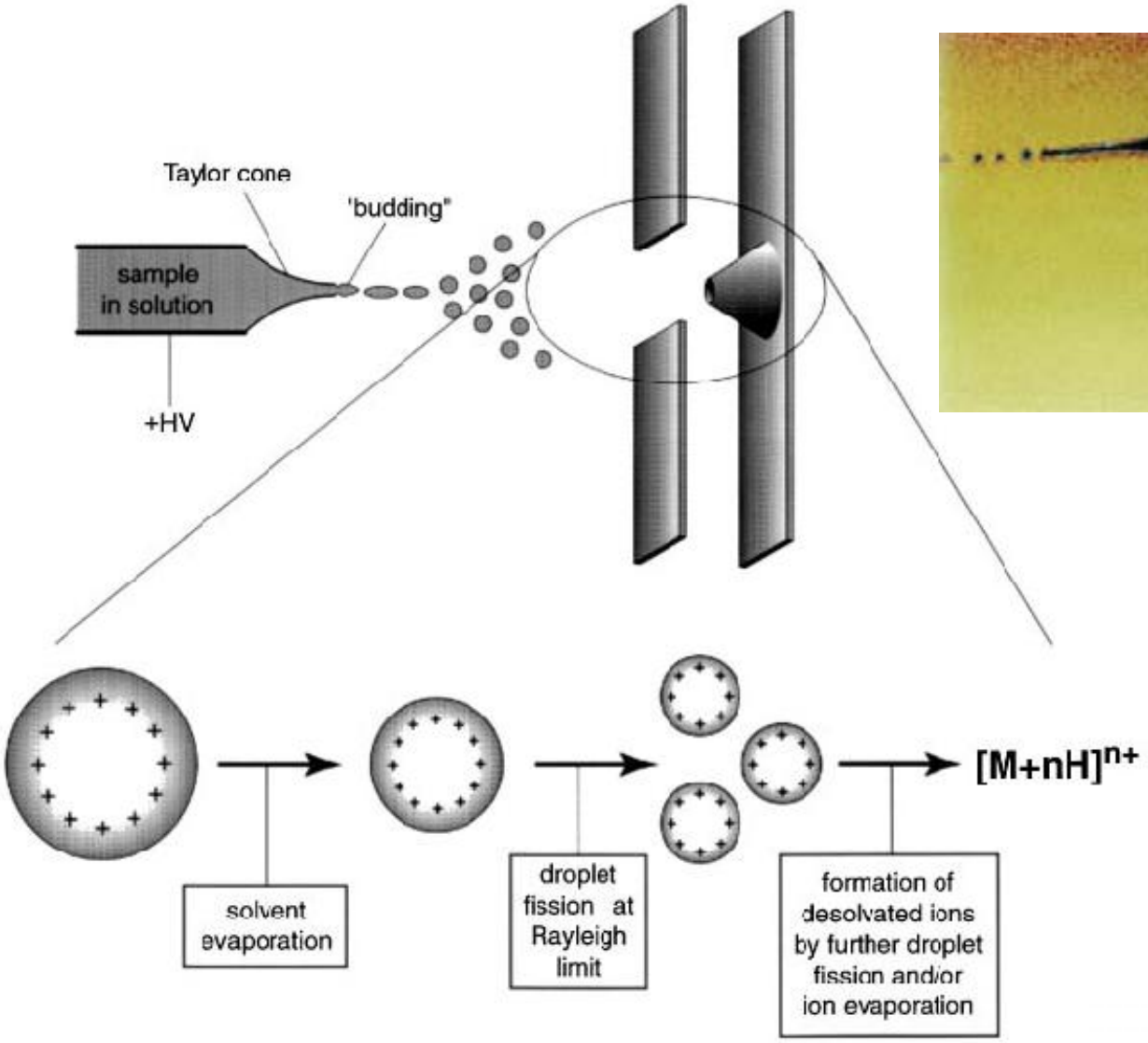
"the qualitative and quantitative comparison of proteomes under different conditions to further unravel biological processes"



Protein sequencing by mass spectrometry

- electrospray ionisation of proteins and peptides
- MSMS principles and instrumentation to fragment gas phase ions
- amino acid sequence determination of peptides

Electrospray ionisation



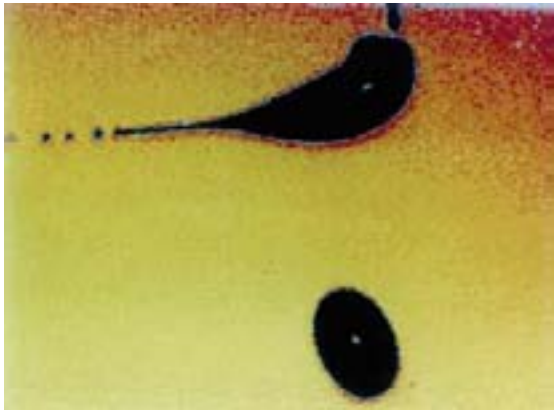
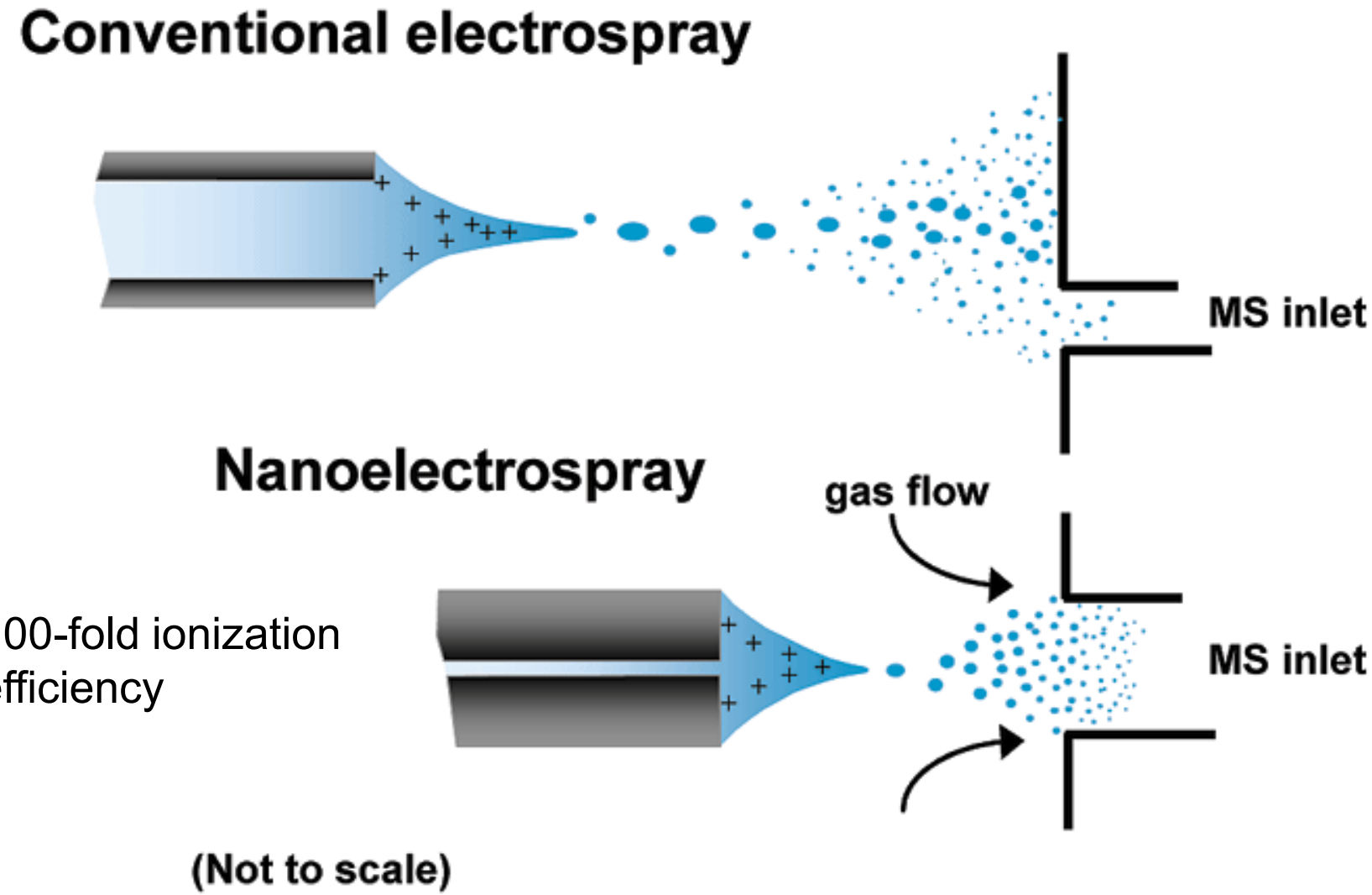


Figure 2. Droplet production in the electrospray interface.

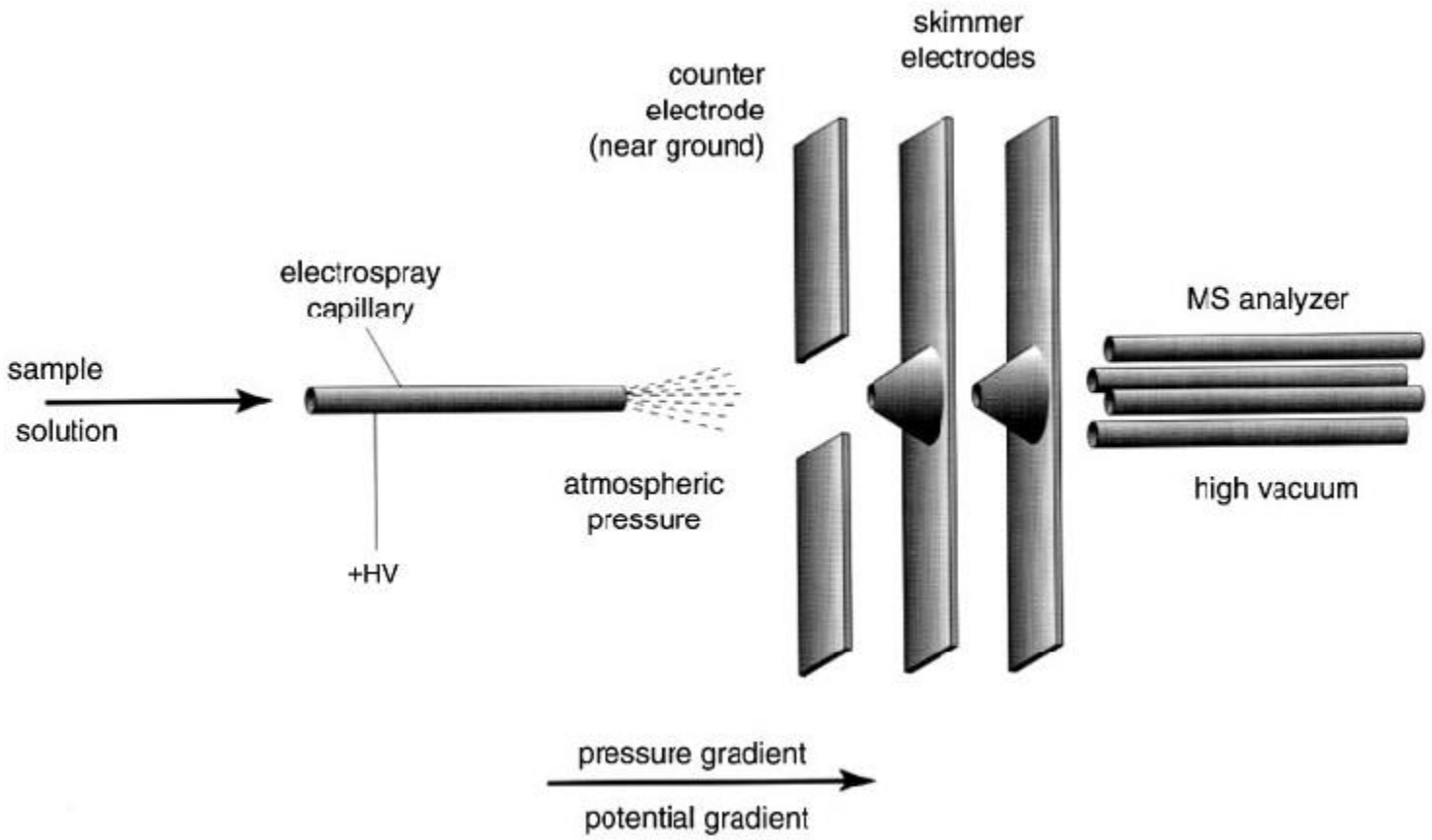
Normal flow rate electrospray (top) vs a lower flow rate electrospray (bottom) that produces smaller droplets



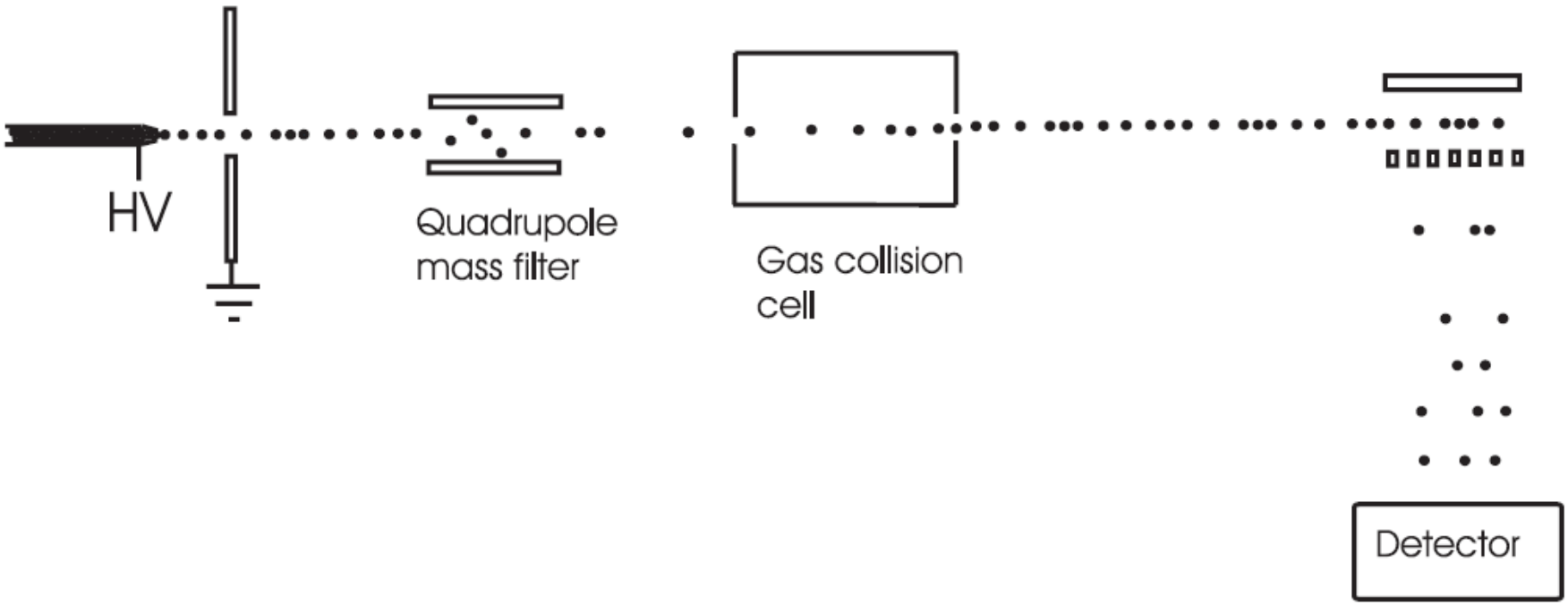
100-fold ionization efficiency

Acc. Chem. Res., **37** (4), 269 -278, 2004

Electrospray ionisation



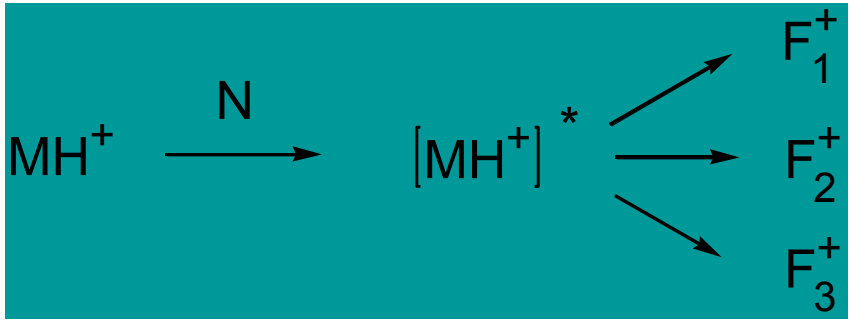
Simplified schematic drawing of an electrospray ionization quadrupole time of flight mass spectrometer (ESI Q-TOF MS) in the MS/MS or MS^2 mode



Collision-induced Dissociation

Collisions between ions with a certain kinetic energy and noble gas atoms or small molecules, such as N_2 and O_2.

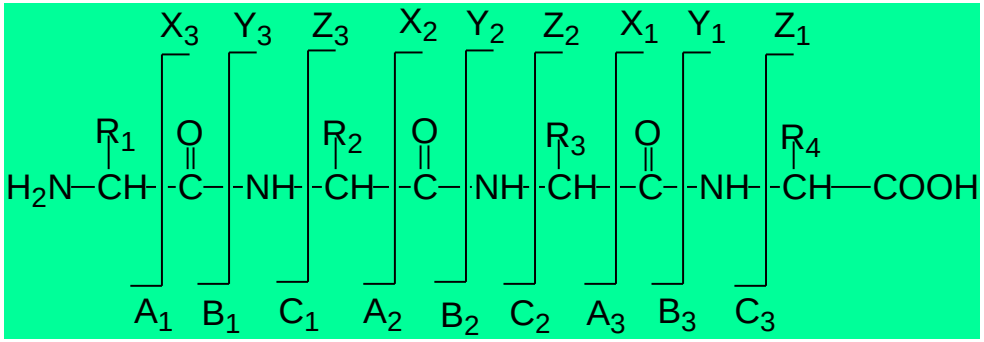
Conversion of kinetic energy into internal energy results in dissociation of the mass selected ion.



Peptide fragment ions

Nomenclature: P. Roepstorff and J. Fohlman, Biomed. Mass Spectrom., 11 (1984) 601); K. Biemann, Biomed. Environ. Mass Spectrom., 16 (1988) 99.

Roepstorff, Fohlman:

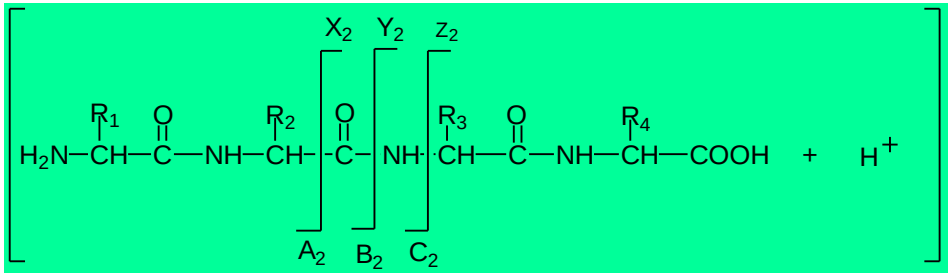


Biemann:

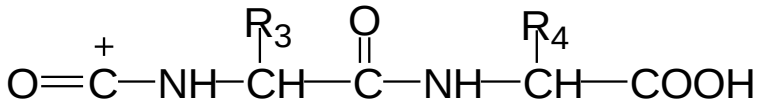
non-capital letters : a_n, b_n, c_n, x_n, y_n, z_n

y_{n+2} instead of Y_2"

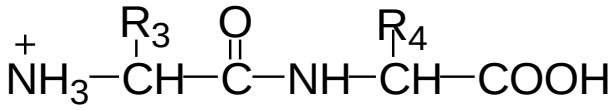
Assumed structures of ions formed by "backbone" fragmentation



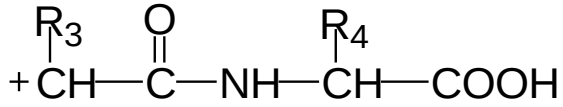
X_2 / x_2 ions:



Y_2"/ y_{2+2} ions:



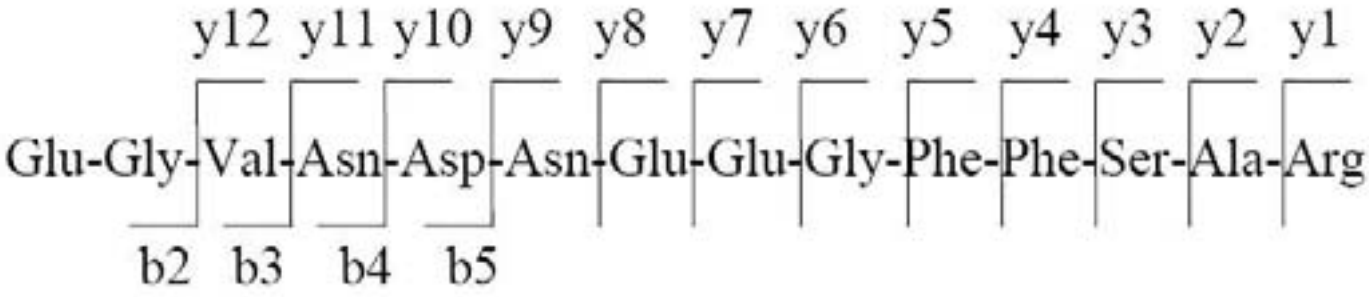
Z_2 / z_2:

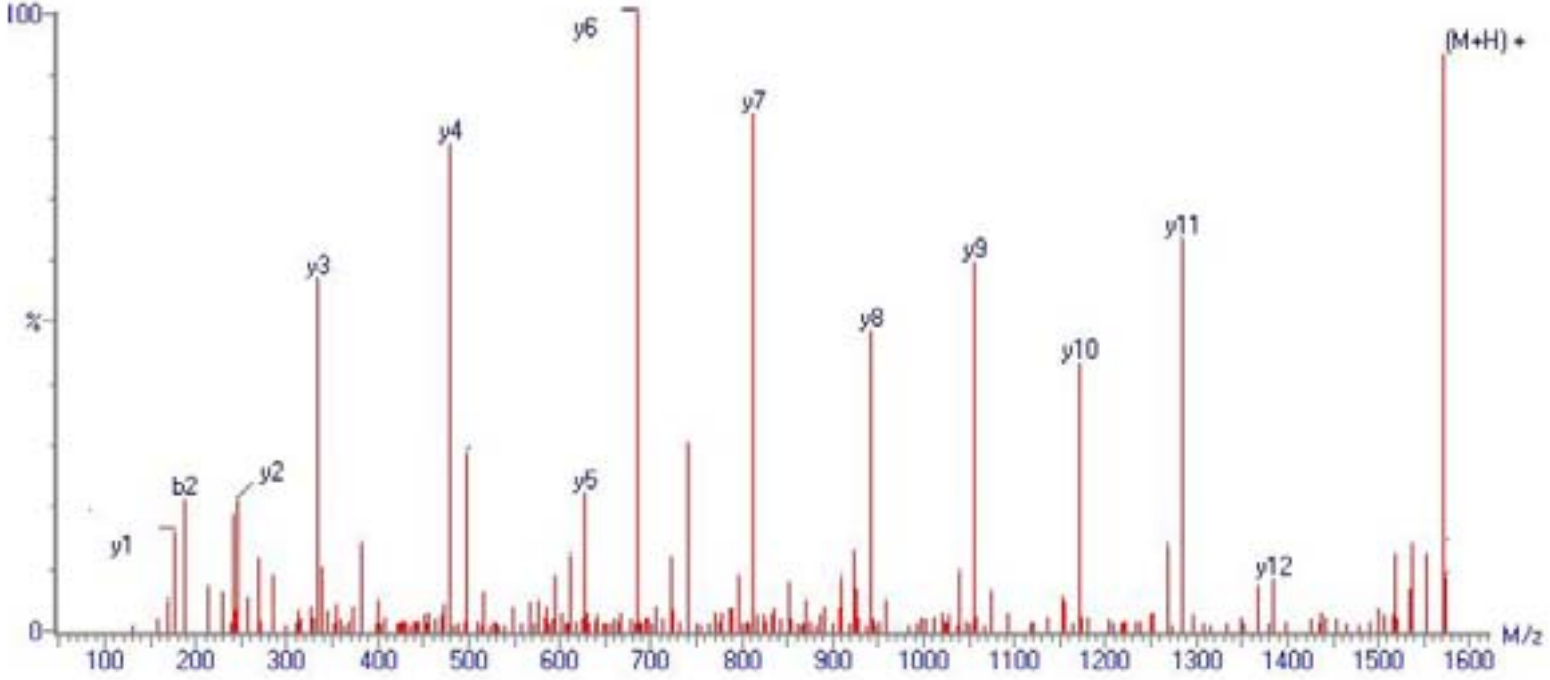


Other peptide fragment ions: a_{n+1}, z_{n+1}, d_n, v_n and w_n ions

(d_n, v_n and w_n ions formed by "sidechain" fragmentation)

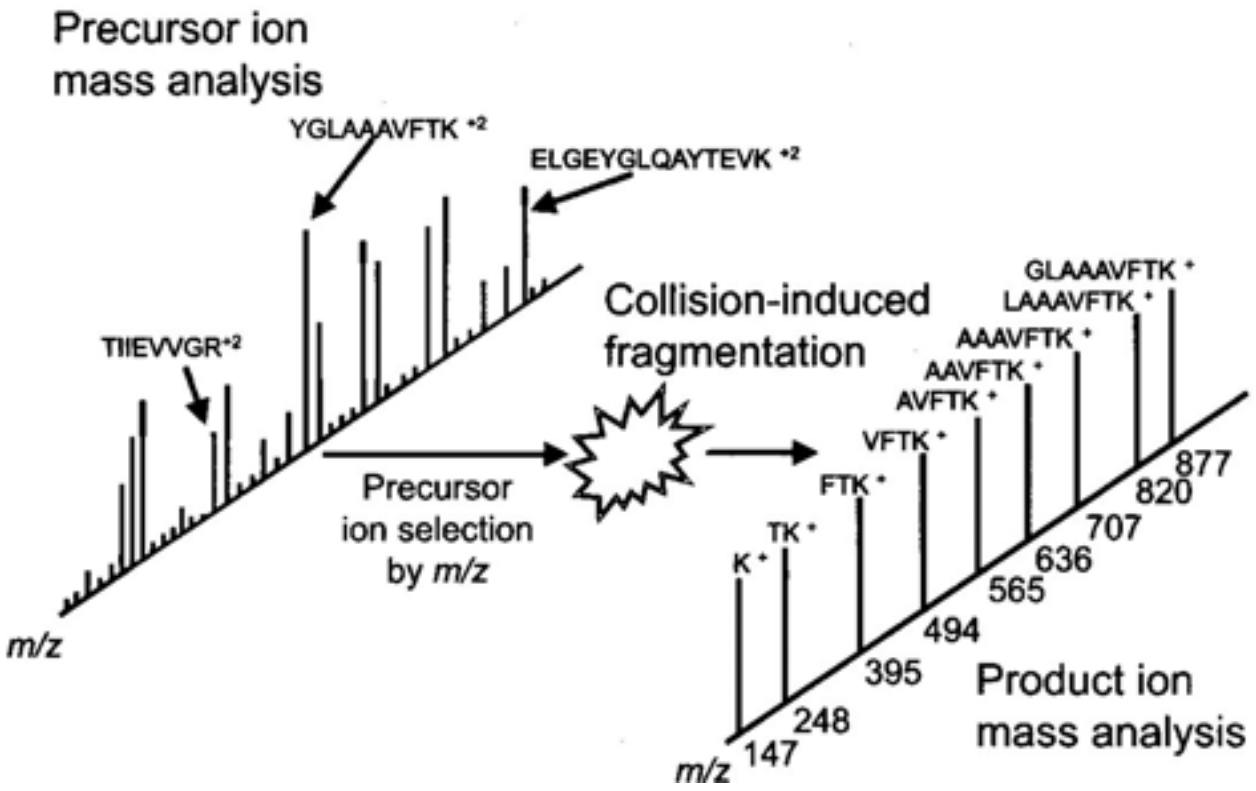
MS/MS spectrum of the $[M + 2H]^{2+}$ ion of GluFib



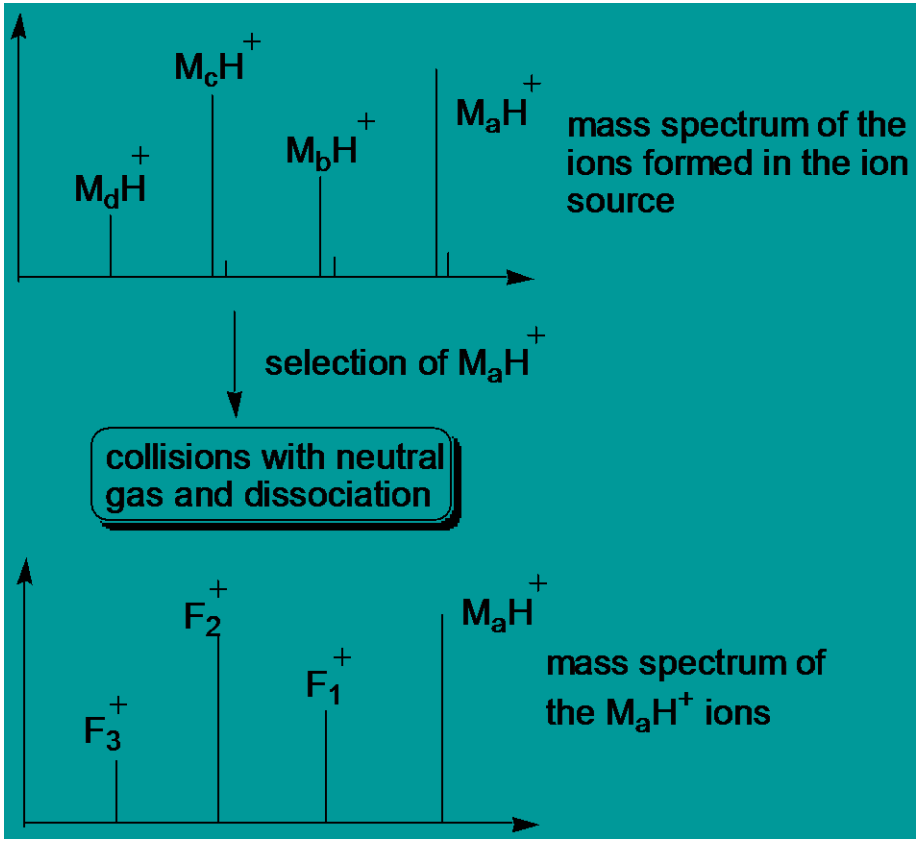


Cleavage sites of trypsin in profilin

MS-MS spectra of protonated peptides



Tandem mass spectrometry

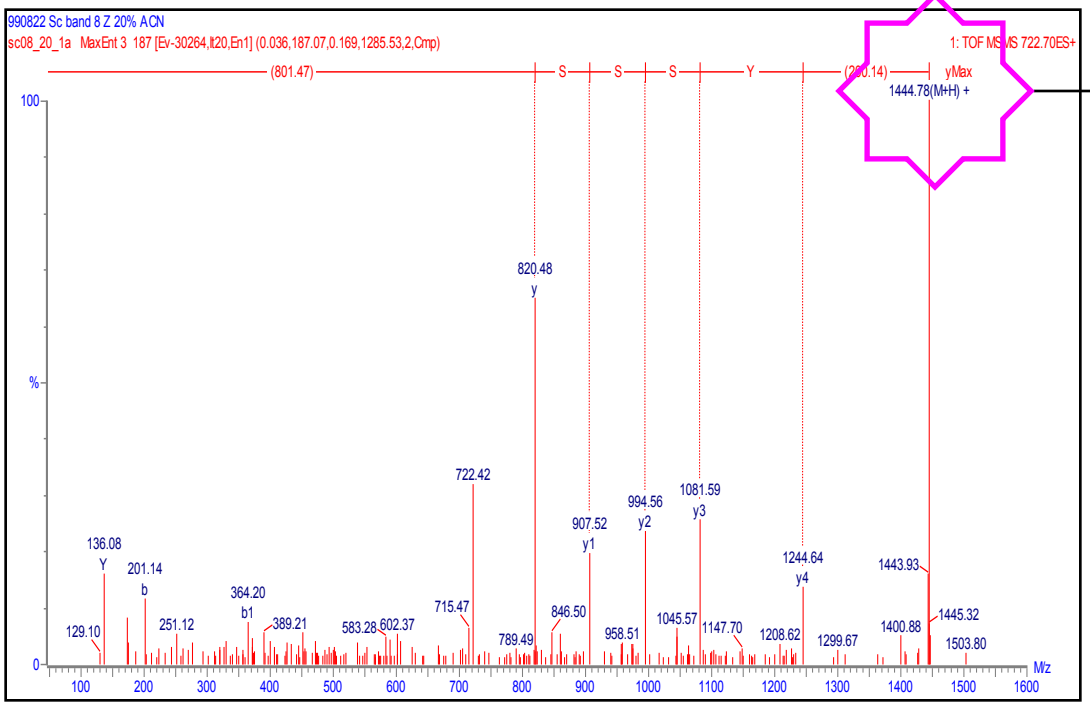


Recording of mass spectra of ions selected according to their m/z ratio.

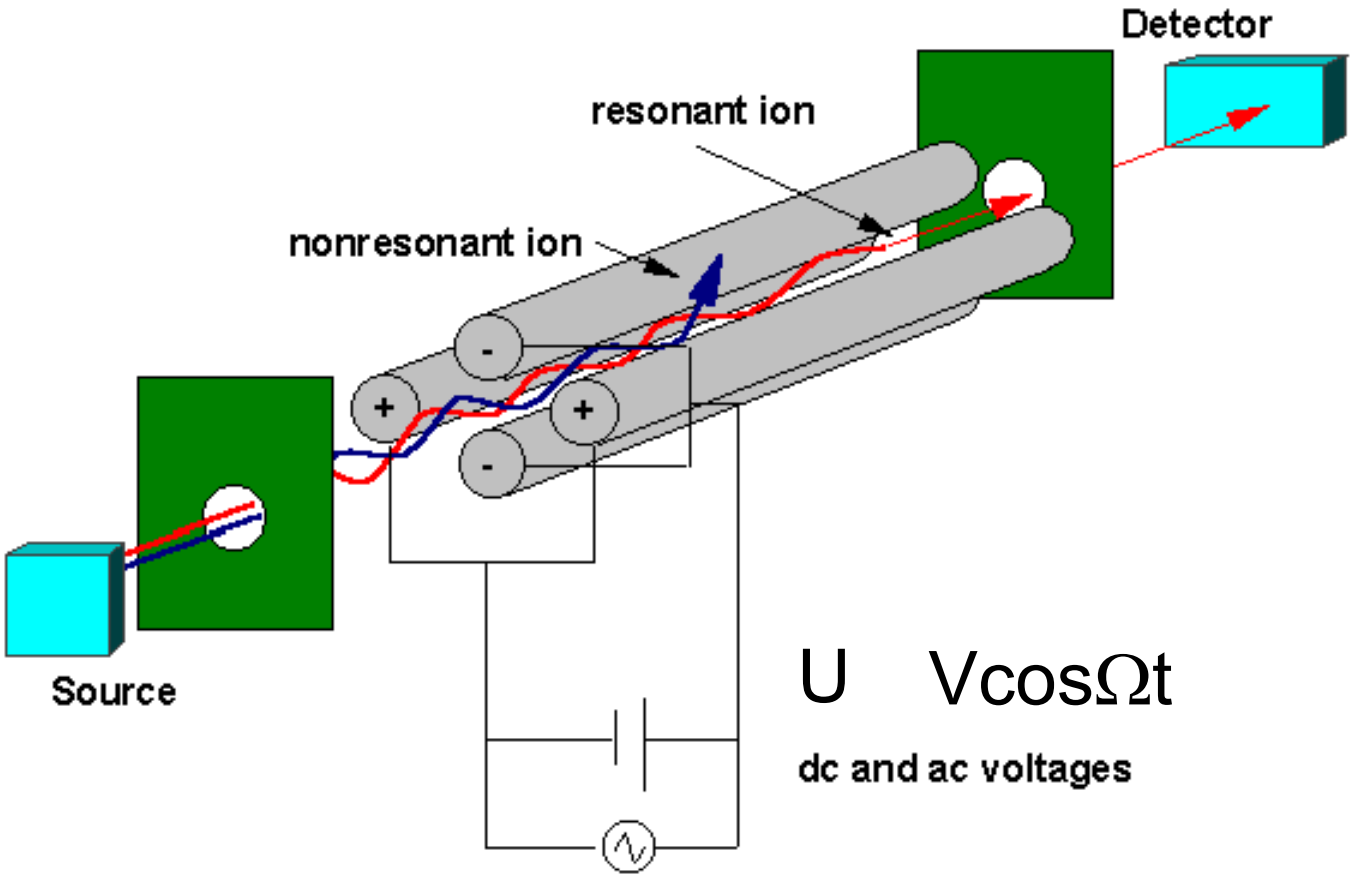
MSMS: classical sequence TAG

Format: 409,76-----$T_1A_2G_3$-------528,13

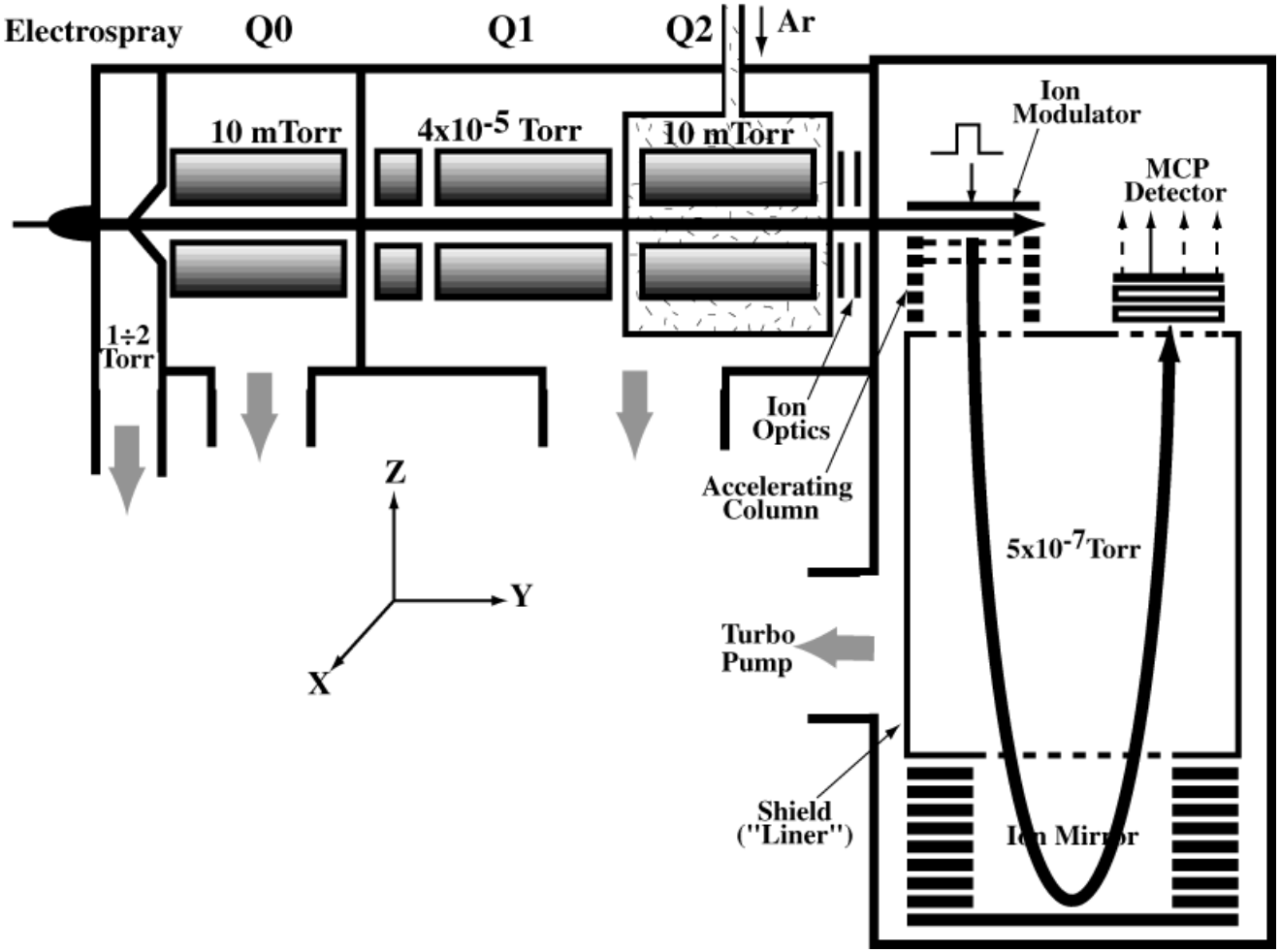
mass1 internal sequence mass2



Quadrupole analyser



Electrospray ionisation tandem mass spectrometry



Swammerdam Institute for Life Sciences

- 1. Basics of proteomic mass spectrometry
- 2. Quantitative proteomics
- 3. New developments; Case studies

Quantitative proteomics

How much do you see?

Suppression effect

- Different peptides have different propensities to ionize
- The ionization of some peptides is suppressed by the presence of other, better ionizable peptides
- So, the signal intensity of a peak in a mass spectrum of a mixture of peptides is not a measure of the concentration of the peptide concerned

Proteomic comparison

- Are differences ‘real’ (gel to gel and stainingvariations) ?

Possible solutions: pre purification, more gels, DIGE (Diff. gelelectrophoresis, ‘cydyes’: increases sensitivity)

- Local stresses per gel differ....

Solution: DIGE (again…..)

A method for quantitative proteomics should …

- Be reproducible
- Be reliable
- Be sensitive
- Cover many proteins
- Have a large dynamic range

ABSOLUTE vs relative

- Absolute :
  - Concentration
  - Copy number / cell
- Relative:
  - up – down regulation
  - comparison of states

Methodology

- Gel based
  - Staining + Image analysis
  - Prederivatized proteins
- Without gels
  - Isotope free vs isotope labeling
  - Multi-dimensional LC
  - Affinity separations

Gel based methods

1. Stain (CBB/Ag/Sypro)
2. Image analysis
3. Repeat for reproducibility

Alternatively:

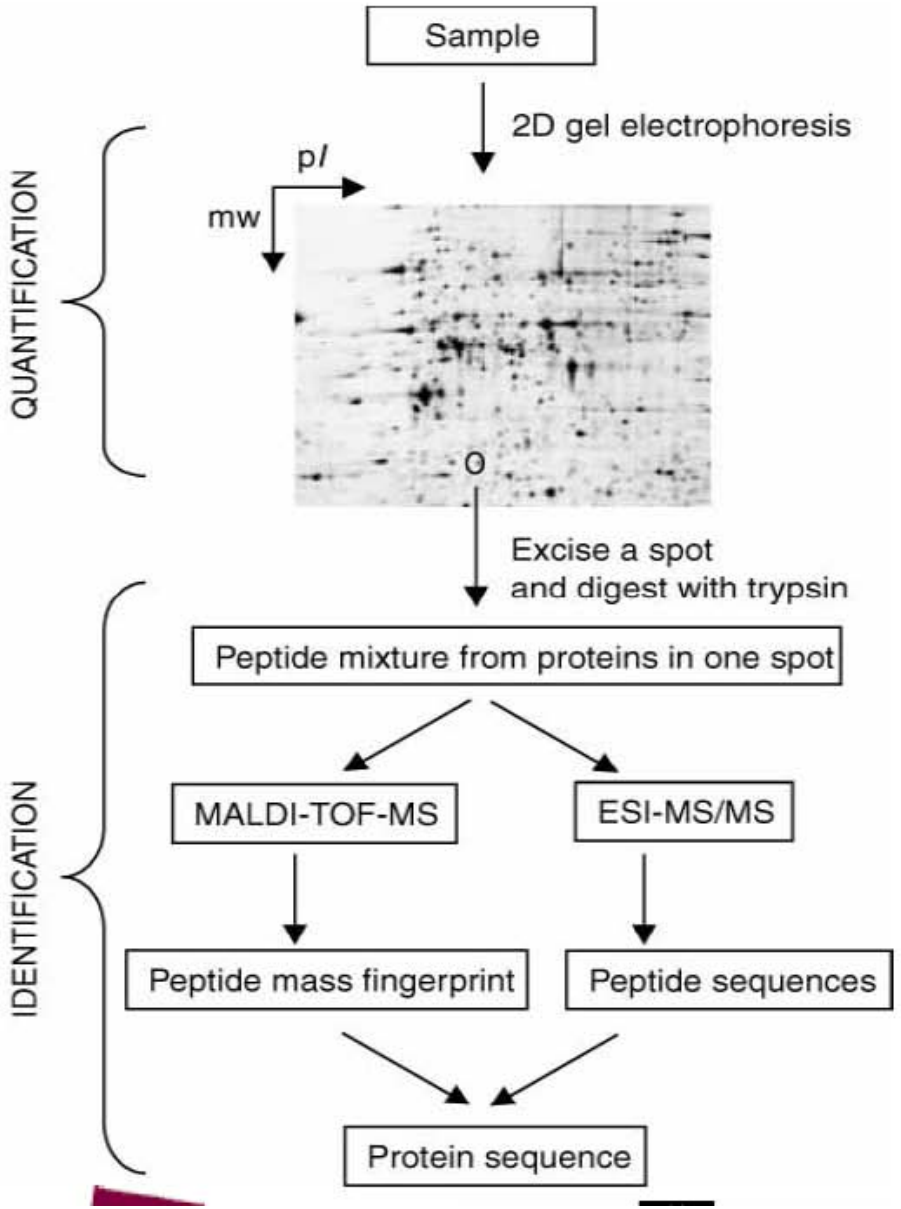
Fluorescent derivatization prior

to 2D-GE

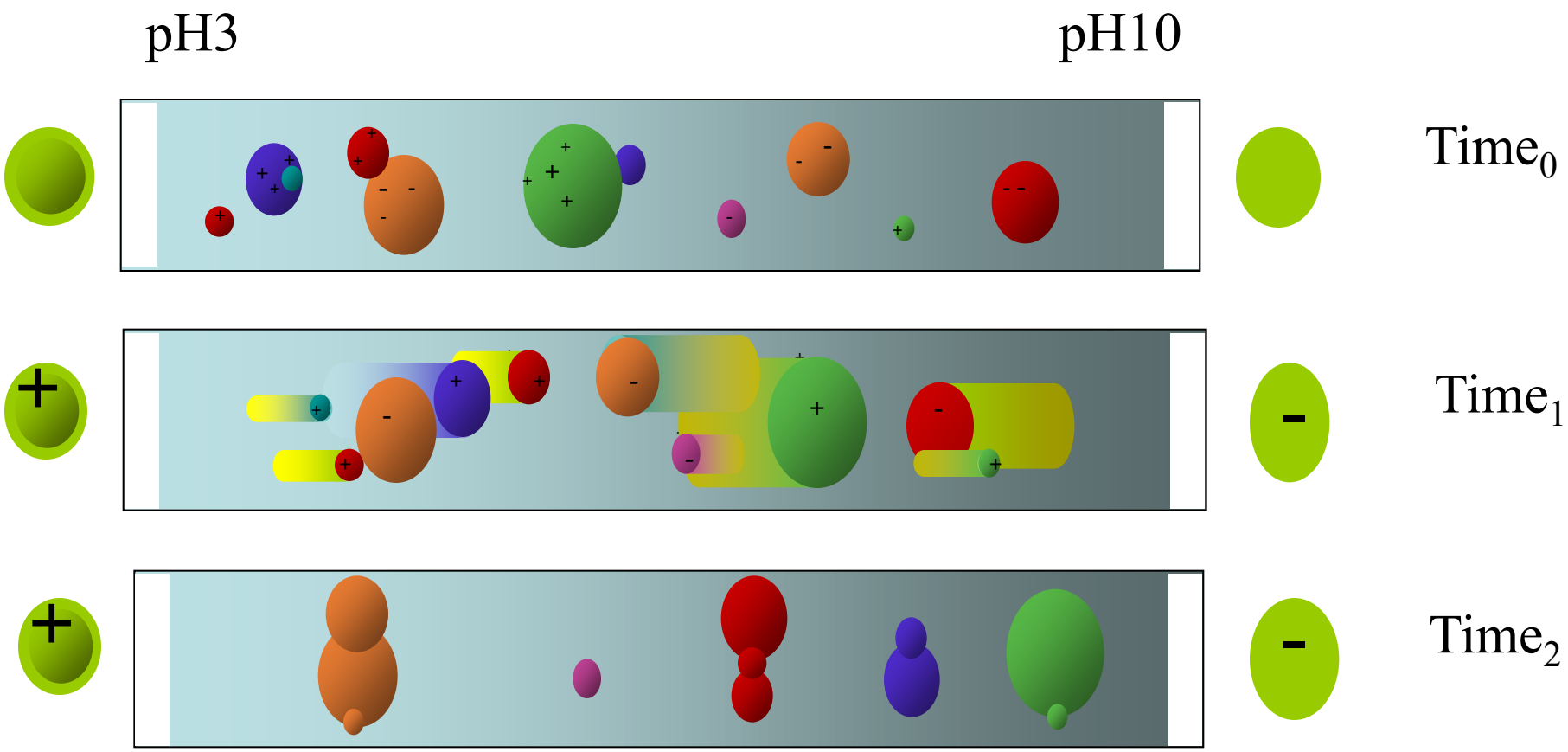
Proteome analysis using two-dimensional gel electrophoresis and mass spectrometry (2DE/MS)

"a major goal of proteomics is the global and quantitative measurement of the proteins expressed in cells or tissues"

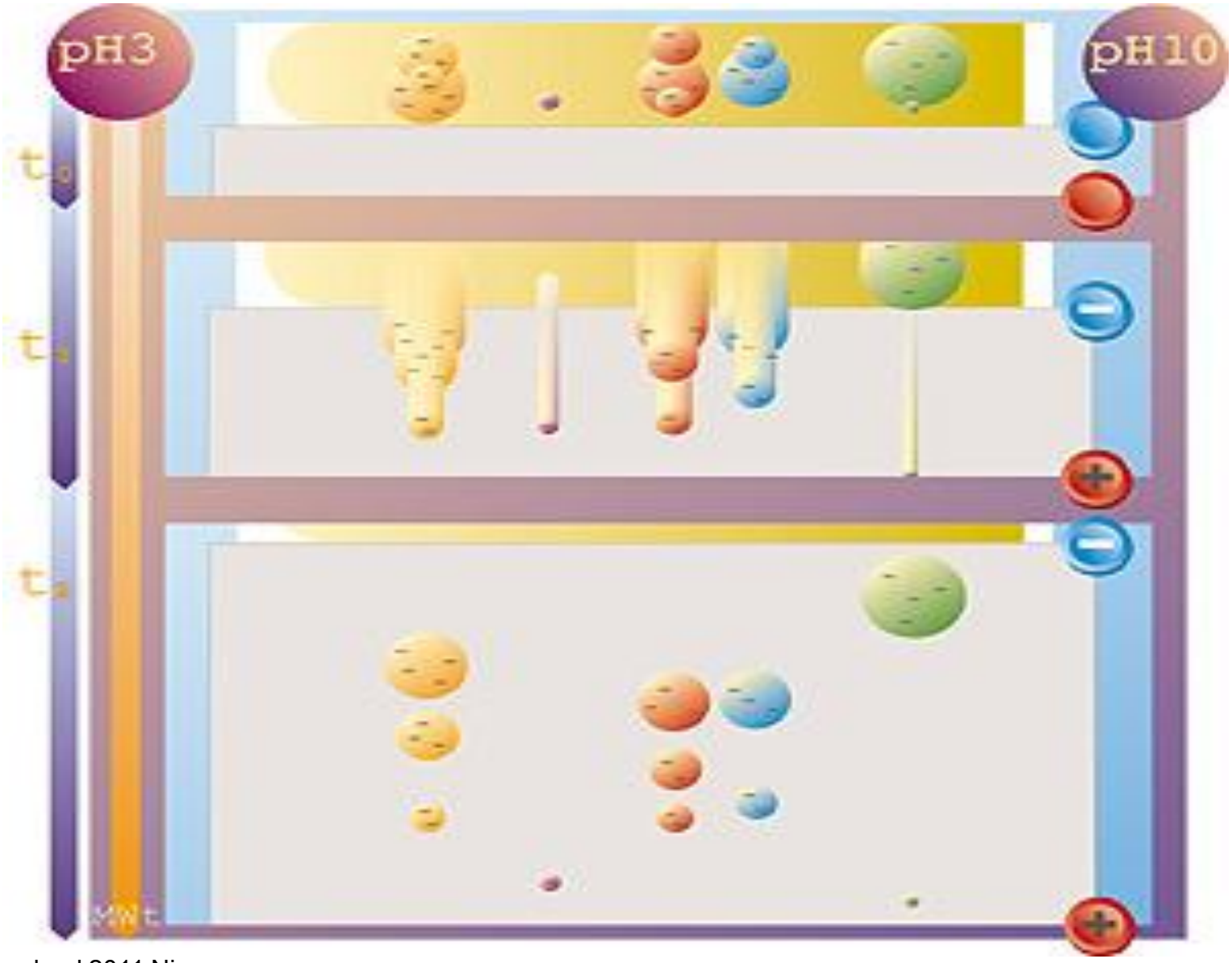
"the qualitative and quantitative comparison of proteomes under different conditions to further unravel biological processes"



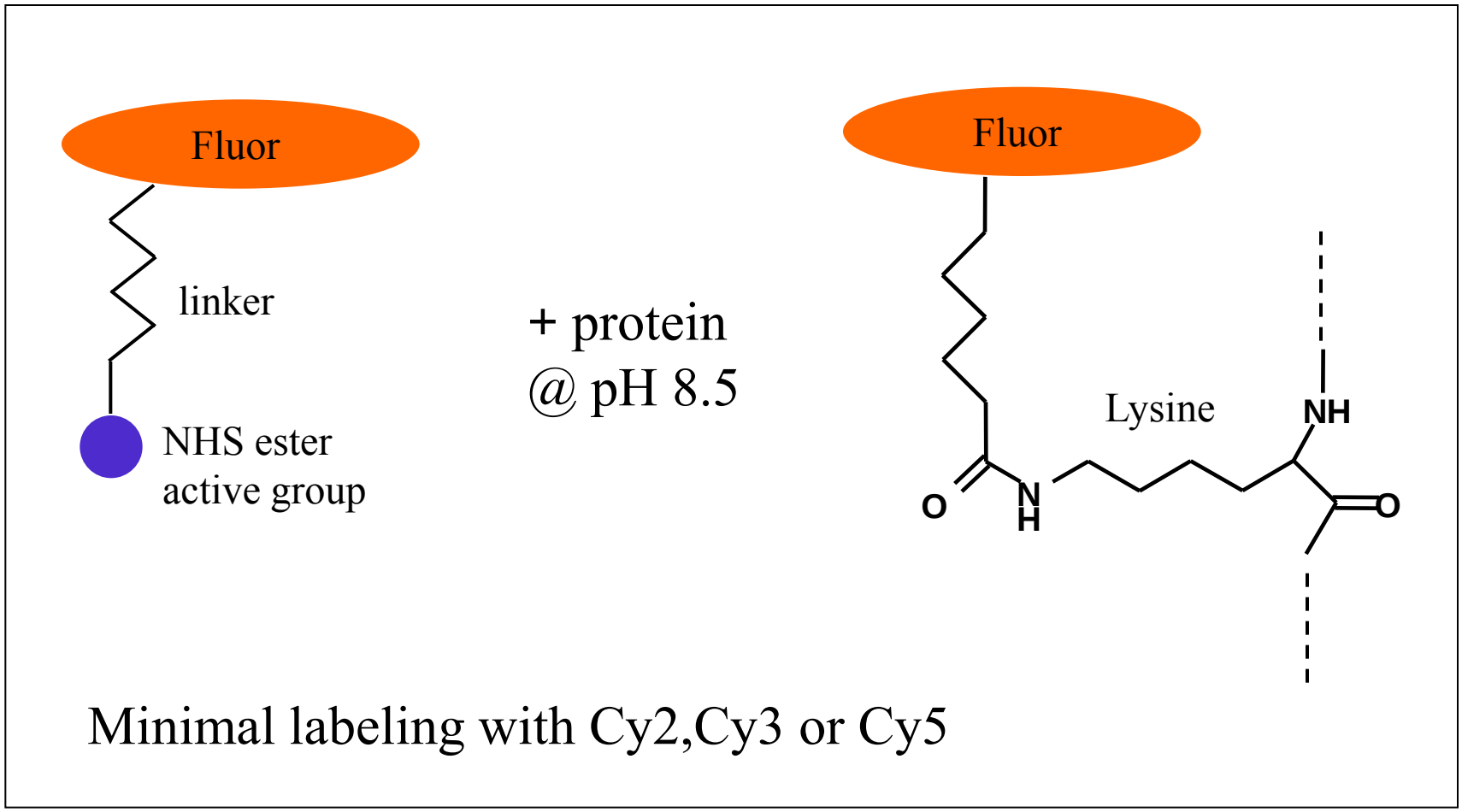
1st Dimension: Iso Electric Focussing



2nd Dimension: SDS Page



CyDye DIGE fluor Structure



Minimal labeling with Cy2,Cy3 or Cy5

Sensitivity: 125 pg/protein, linear response over 10^5 range.
Compare: Coomassie >100 ng and Silver >10 ng.

DIGE: Difference Gel Electrophoresis

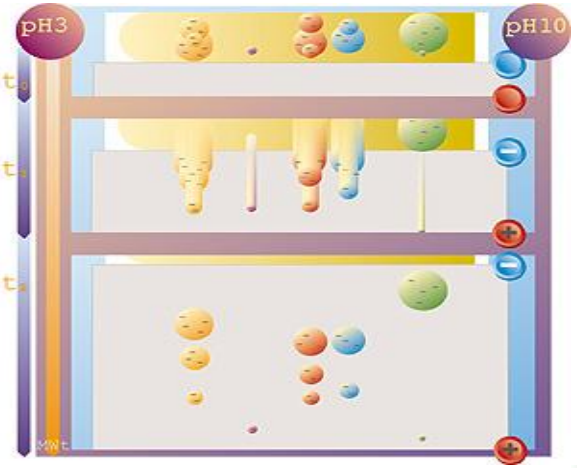
Protein extract 1

Label with fluor 1

Mix labeled extracts

Protein extract 2

Label with fluor 2



Separate by 2-D PAGE

Excitation wavelength 1

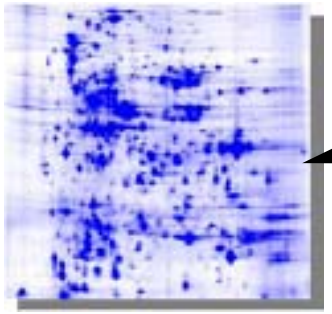
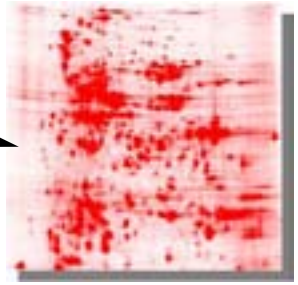


Image gel



Excitation wavelength 2

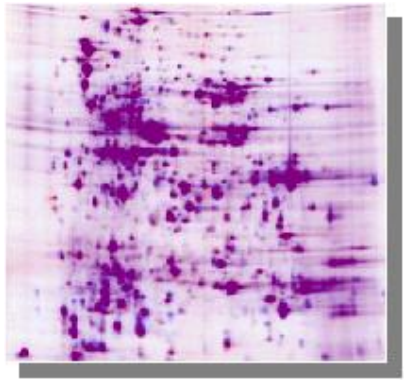
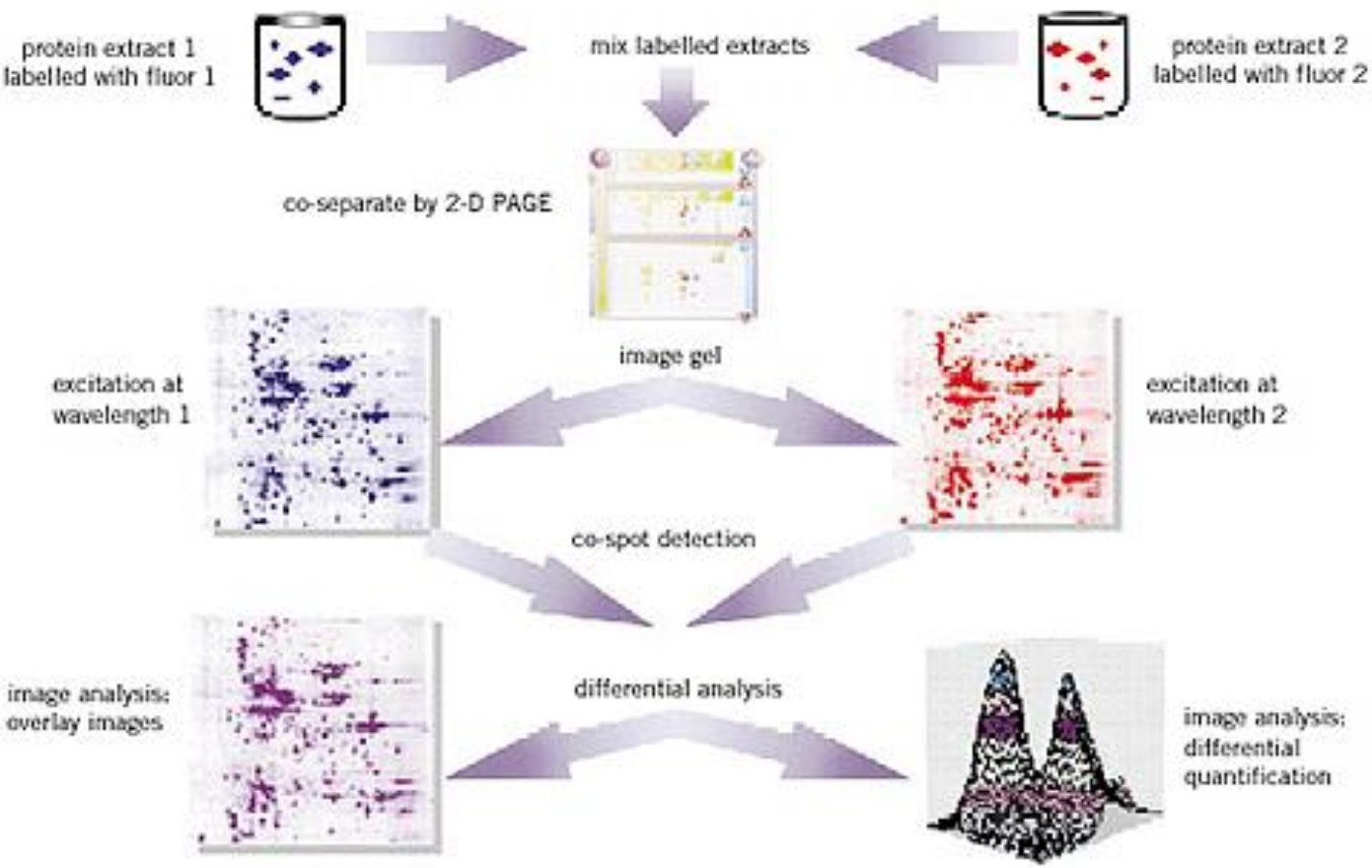


Image analysis: overlay images

Analysis of difference

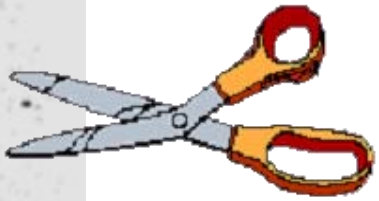
Image analysis: data quantitation

DeCyder procedure

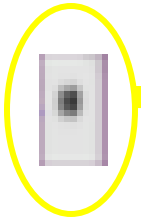


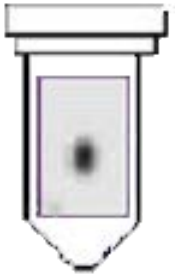
Sample

2D Page

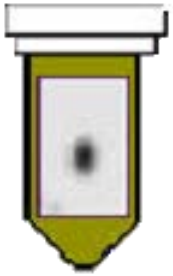


Cut





Isolate



Digest

Peptides

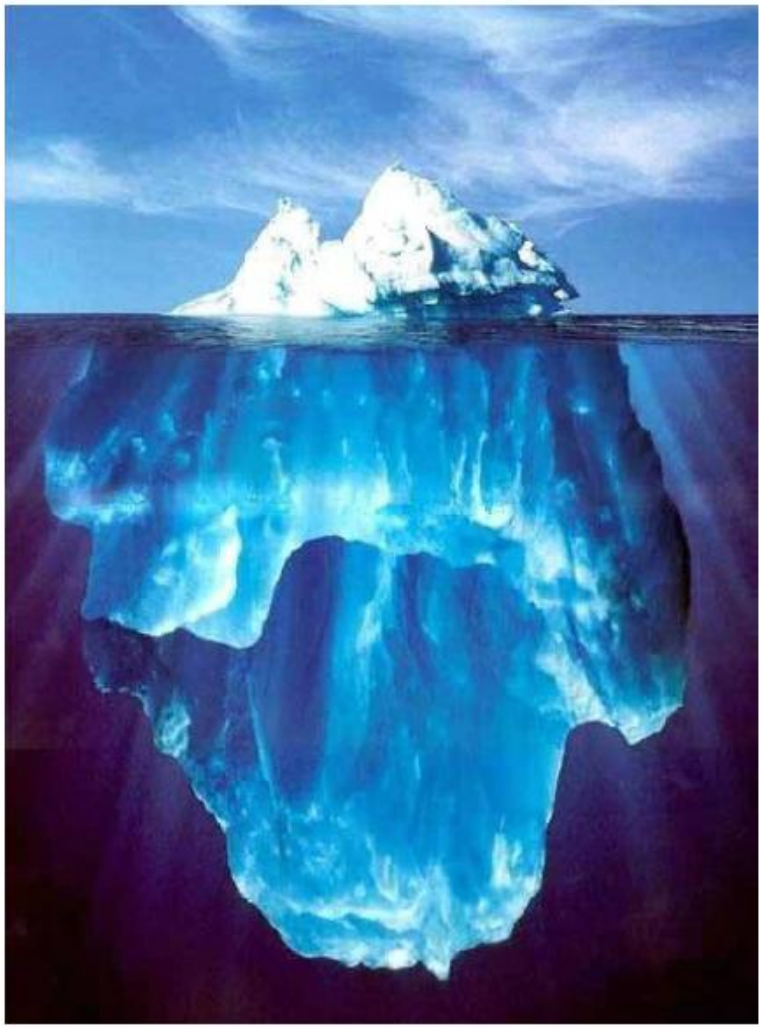
Gel based methods

Dynamic range	Stain: 10^3 (?) Fluor: ???
Reproducibility	Stains: worse than 2 Fluor: up to 1.3 (ideal)
Separating power	Thousands of SPOTS (not equal to proteins)
Bias	Soluble, abundant

Limitations of 2-D gel electrophoresis

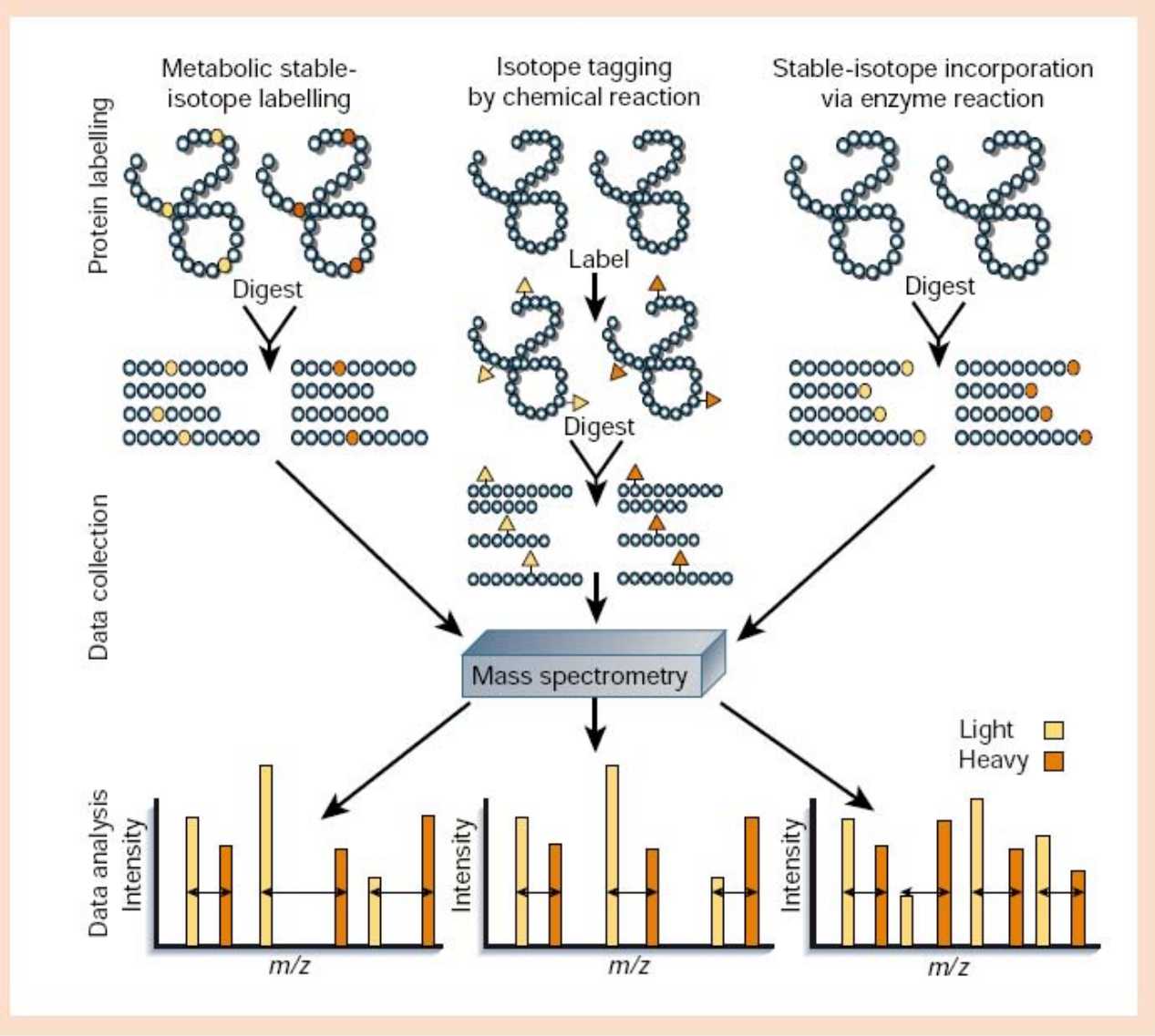
- large proteins (> 100 kDa) are underrepresented
- hydrophobic membrane proteins (30% of entire proteome) tend to aggregate during the first dimension and do not enter the second dimension gel
- highly basic and acid proteins are problematic
- relatively low dynamic range $10^3 - 10^4$ of 2-D gels prevents detection of less abundant proteins

Limitations of 2-D gel electrophoresis



- relatively low dynamic range 10^3 – 10^4 of 2-D gels prevents detection of less abundant proteins

Isotpe labelling



Isotope labels or isotope free

Mass spectrometry is not quantitative

- Ionisation propensity
- Co-suppression
- Co-enhancing

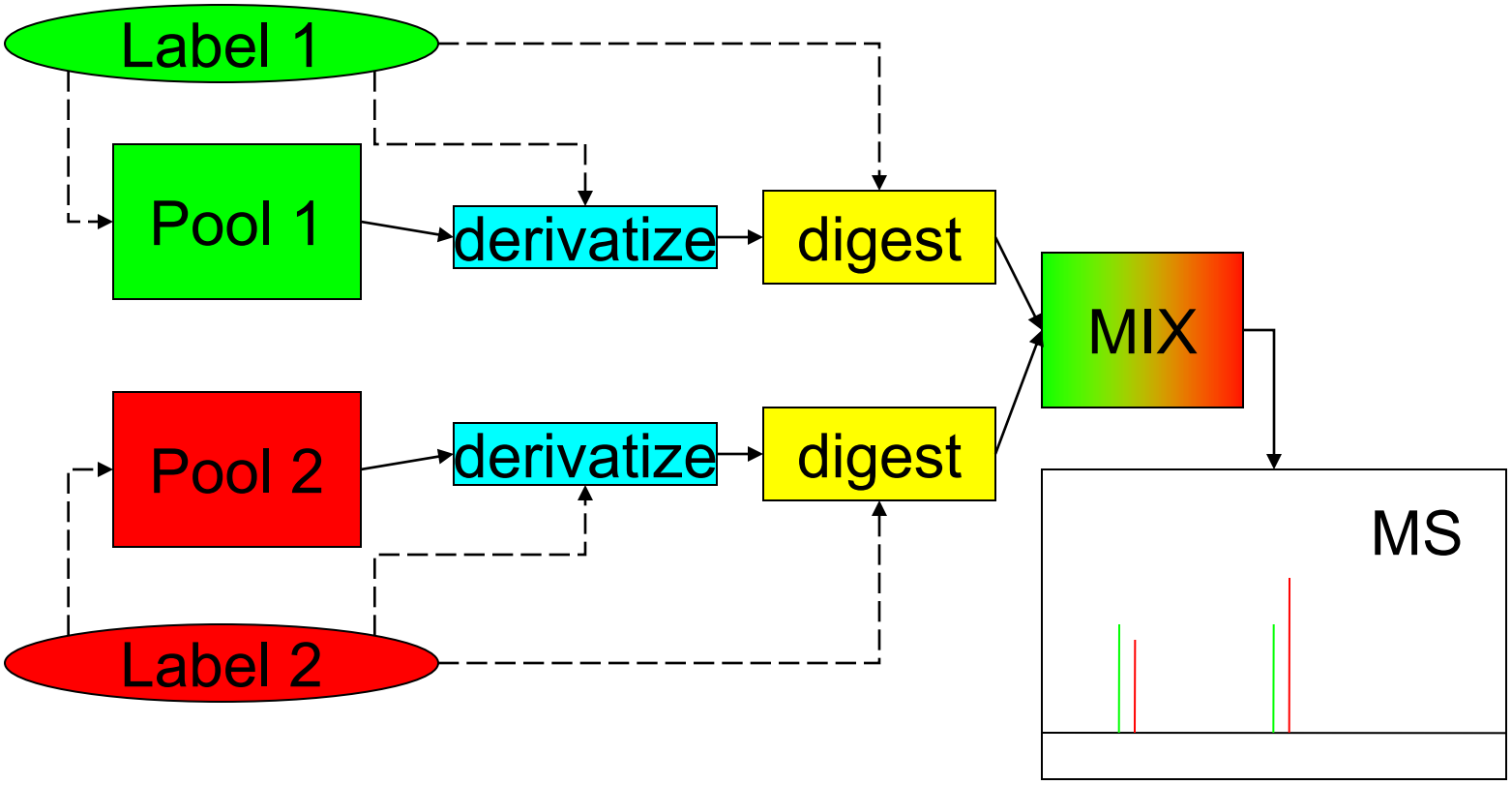
Use of (stable) isotopes serves as an internal standard (chemically identical)

Isotope labels

Labels can be incorporated via:

1 Culture media
2 Proteolytic cleavage
3 Derivatization of reactive groups on the proteins

Label introduction: techniques



Isotope ratio for quantification

Natural isotopic abundances of common elements

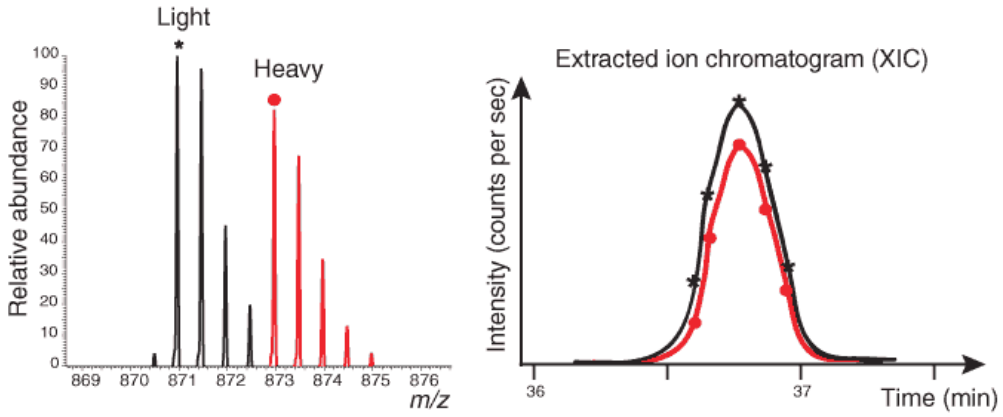
hydrogen	H	1	99.985
	D	2	0.015
carbon	^{12}C	12	98.9
	^{13}C	13	1.1
nitrogen	^{14}N	14	99.64
	^{15}N	15	0.36
oxygen	^{16}O	16	99.76
	^{17}O	17	0.04
	^{18}O	18	0.2
fluorine	^{19}F	19	100
phosphorus	^{31}P	31	100
sulpher	^{32}S	32	95.02
	^{33}S	33	0.76
	^{34}S	34	4.22
chlorine	^{35}Cl	35	75.77
	^{37}Cl	37	24.33
bromine	^{79}Br	79	50.5
	^{81}Br	81	49.5
iodine	^{127}I	127	100

Examples of isotope labels

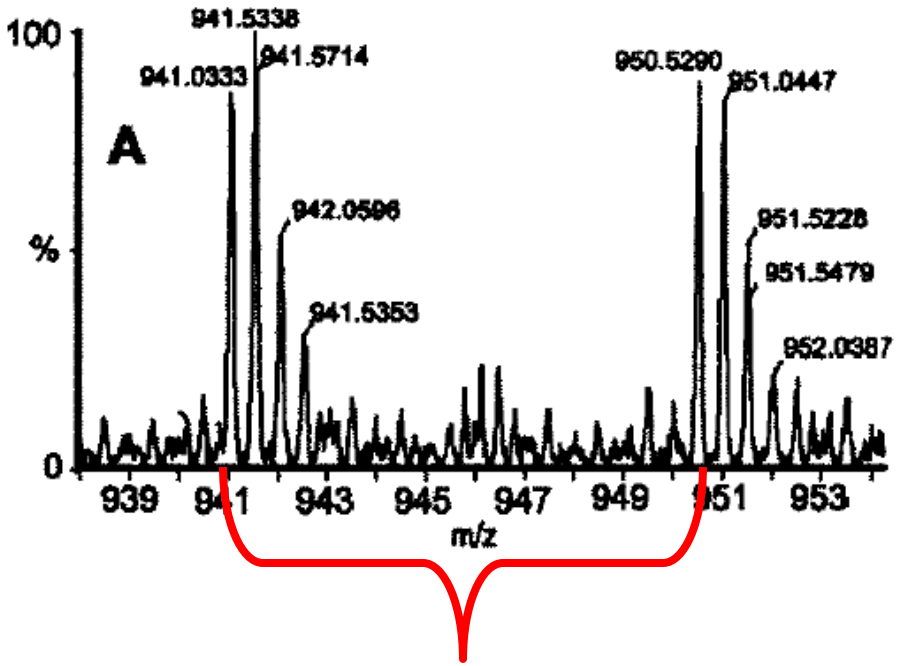
H/D	ICAT; D-labeled amino-acids (SILAC) Caution: D changes RP-HPLC retention
$^{12}C/^{13}C$	ICAT the sequel
$^{14}N/^{15}N$	Incorporated via growth media Can be used to calculate # of nitrogens
$^{16}O/^{18}O$	Incorporated by proteases Can be combined with other procedures

Isotope labels

- Extracted ion chromatograms (XIC)
  - Peak area of a selected *m/z* value

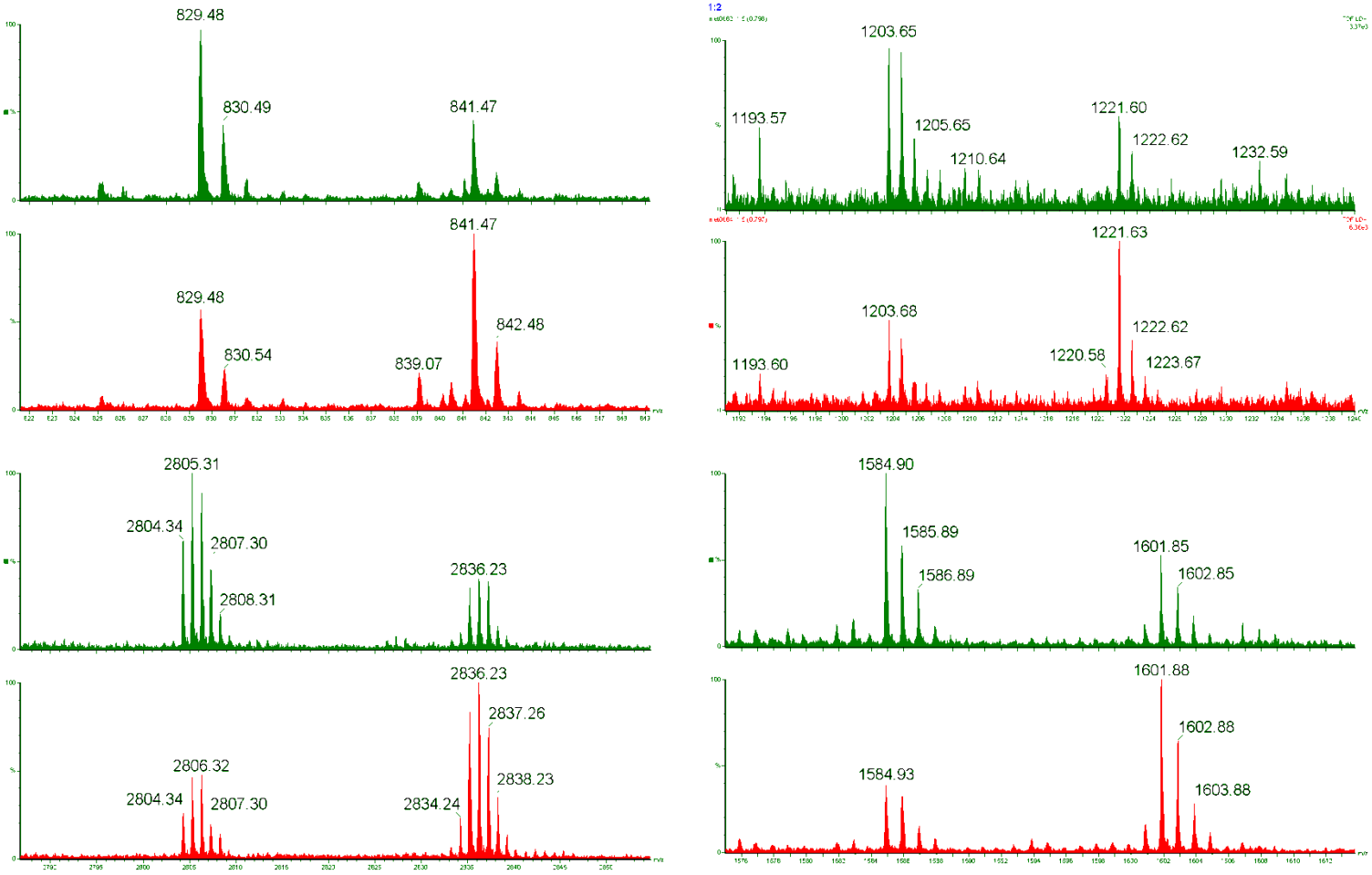


$^{14}N/^{15}N$: LCMS



Mass shift: number of nitrogen's in the peptide

Yeast cell wall proteins labeled with ^{15}N



Isotope label methods

Dynamic range	10^3 : > 10 both up and down
Reproducibility	Approximately 20% is attainable
Separating power	Thousands of PEPTIDES (not equal to proteins)
Bias	Less biased than gels

When to mix in the label?

Culture media:

-early in the procedure

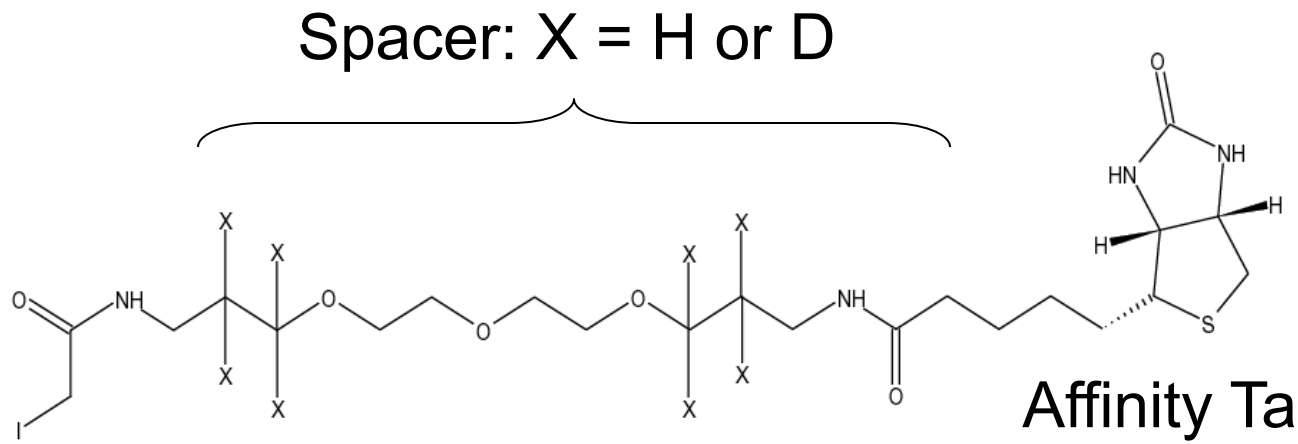
-identical treatment throughout the preparation process

-keeping standards standard may be difficult

Chemical probes/digestions

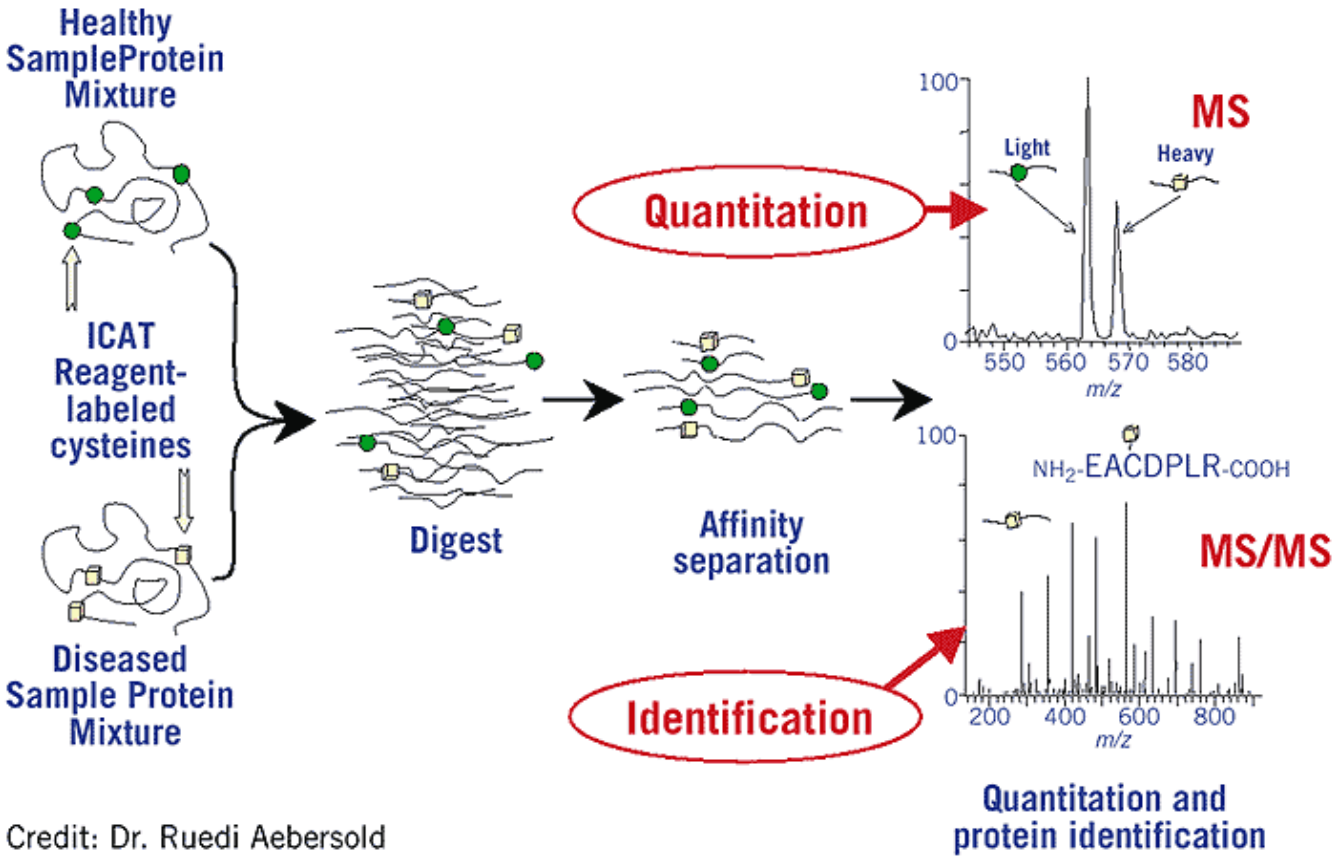
-irreproducibility's: check by inverse labeling

ICAT structure



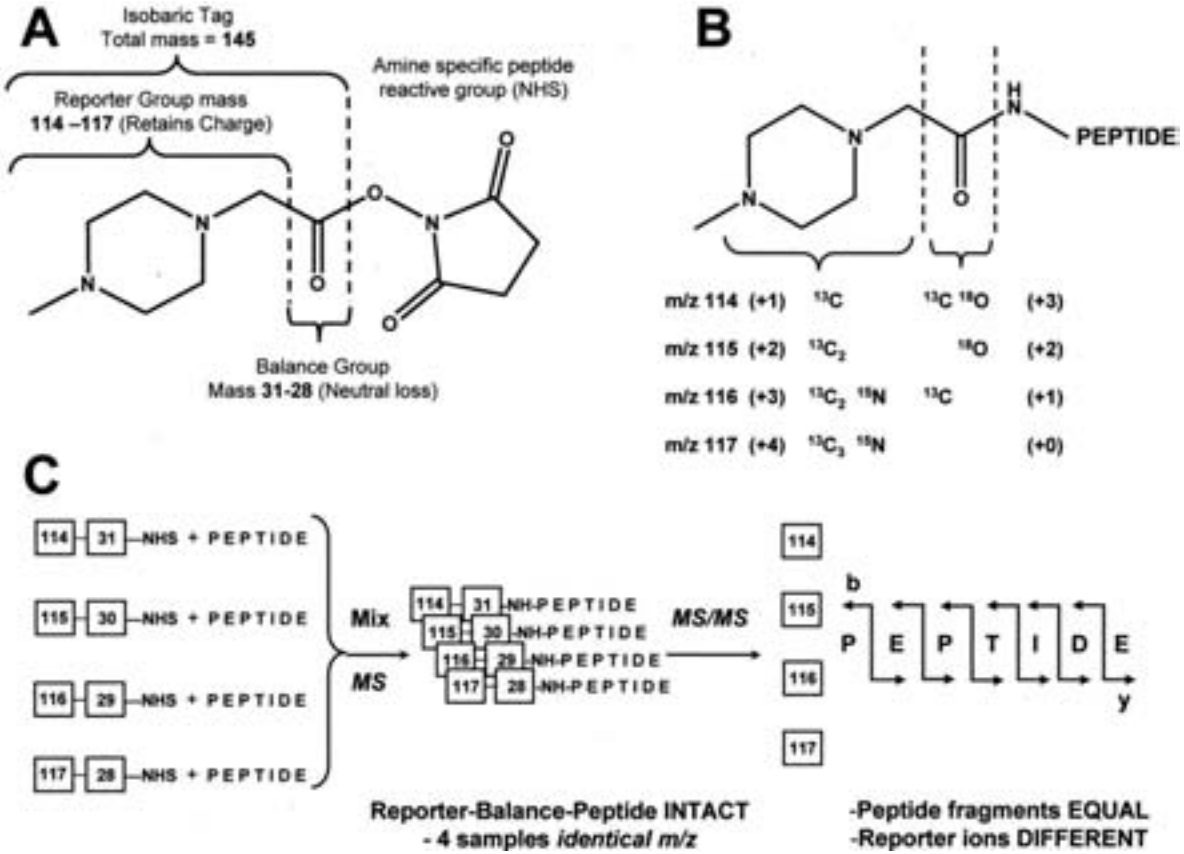
D0:+$C_{20}H_{35}N_4SO_5$ / -H
mass change = 442.225/cystein (mono) ; 442.5799 (av).
D8:+$C_{20}H_{27}D_8N_4SO_5$ / -H
mass change = 450.2752/cystein (mono) ; 450.63 (av).

ICAT procedure



Credit: Dr. Ruedi Aebersold
Institute for Systems Biology, Seattle, WA

iTRAQ



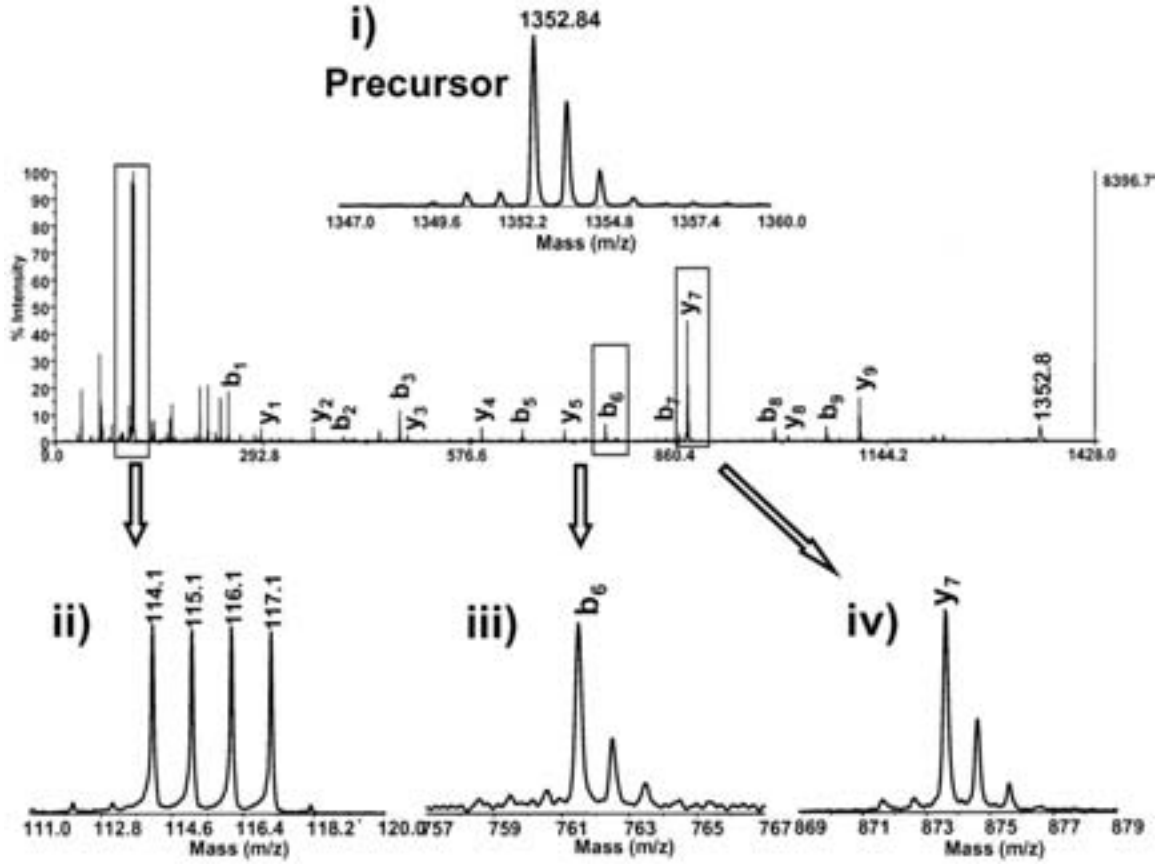
Signal is concentrated in MS overview:

High sensitivity

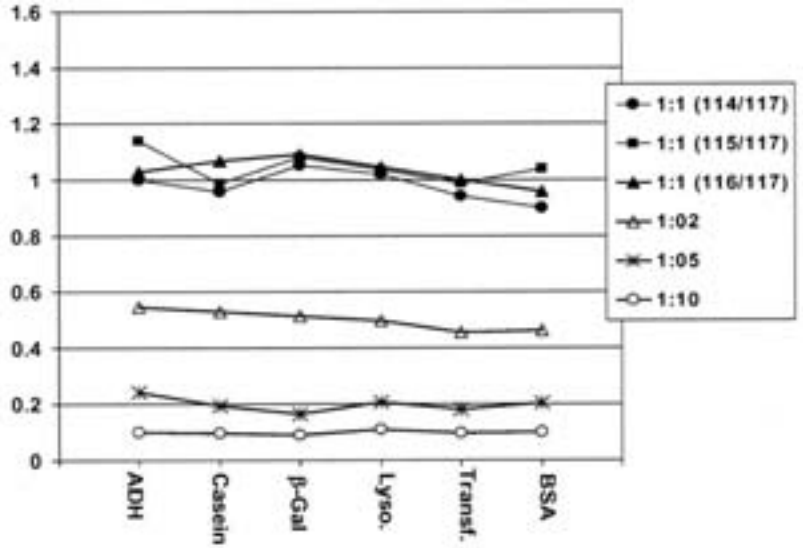
iTRAQ

Quantification on daughter ions in MSMS:

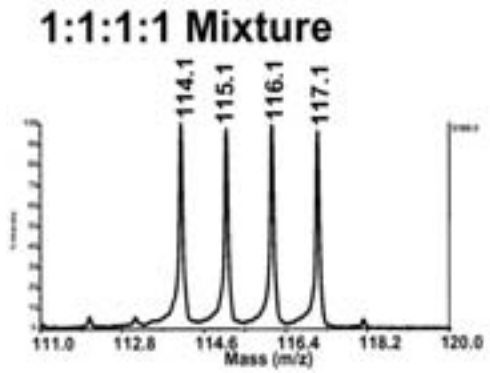
Improved S/N ratio

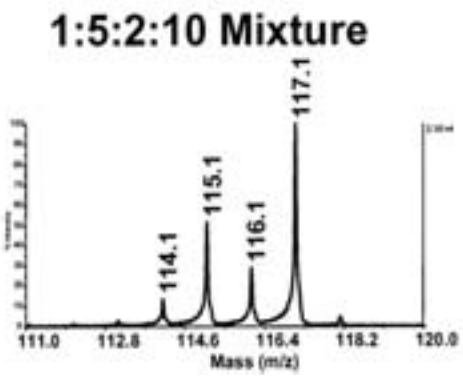


iTRAQ



Ratio	Mean	SD
1:1	1.03	0.16
1:2	0.514	0.12
1:5	0.204	.045
1:10	0.097	0.023





Mass spectrometry-based proteomics

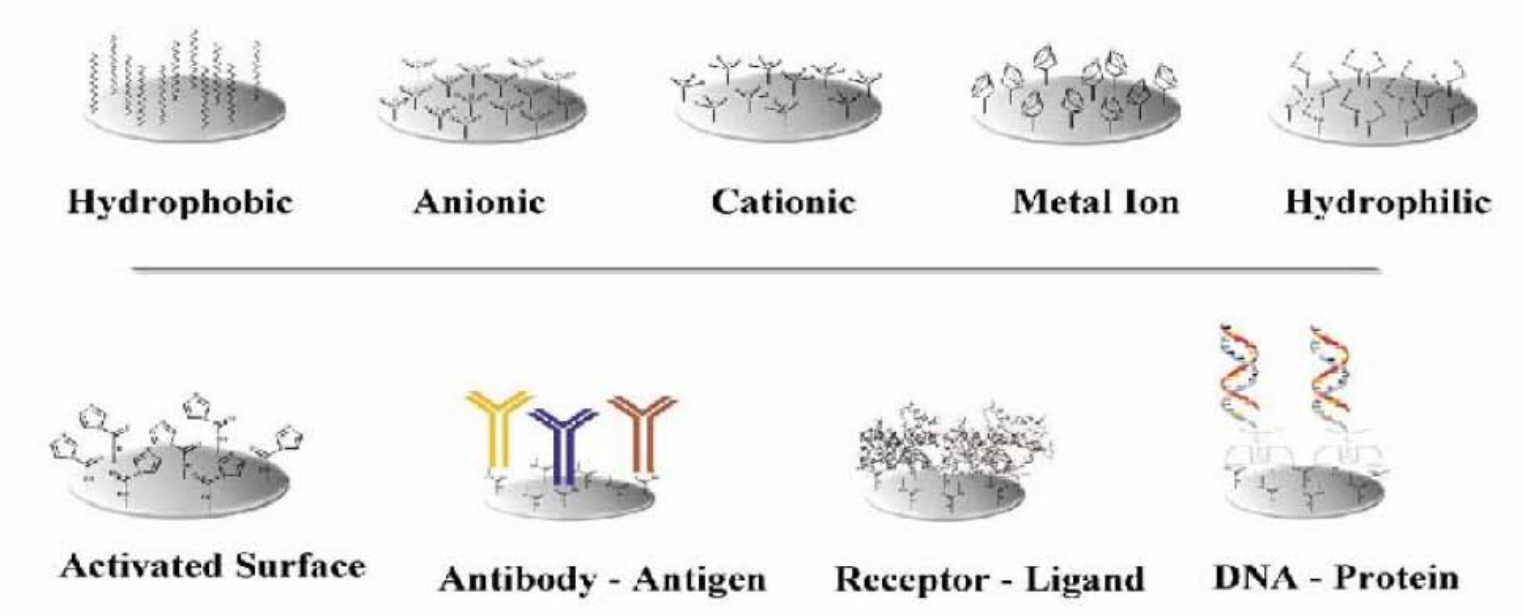
Ruedi Aebersold* & Matthias Mann†

- 1. Basics of proteomic mass spectrometry
- 2. Quantitative proteomics
- 3. New developments, Case studies

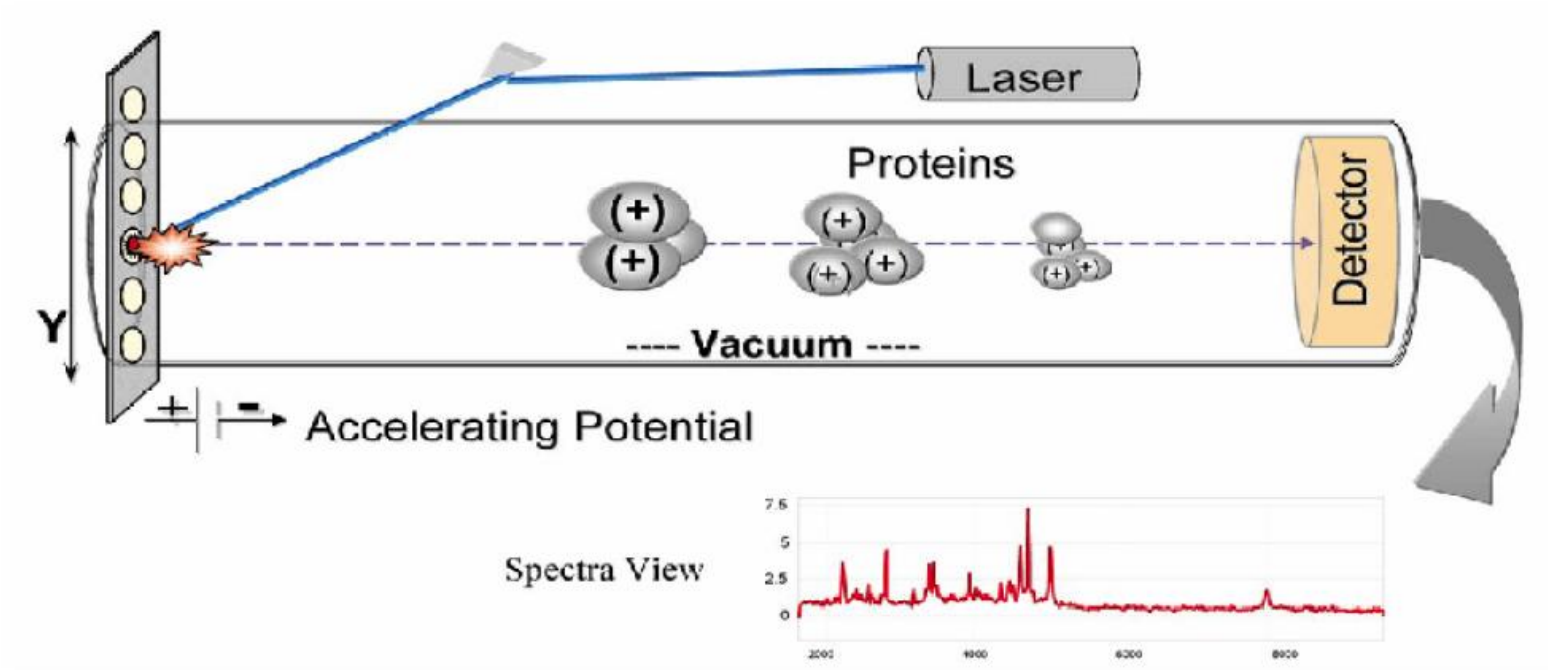
Some recent developments

- Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS): protein profiling and biomarker identification

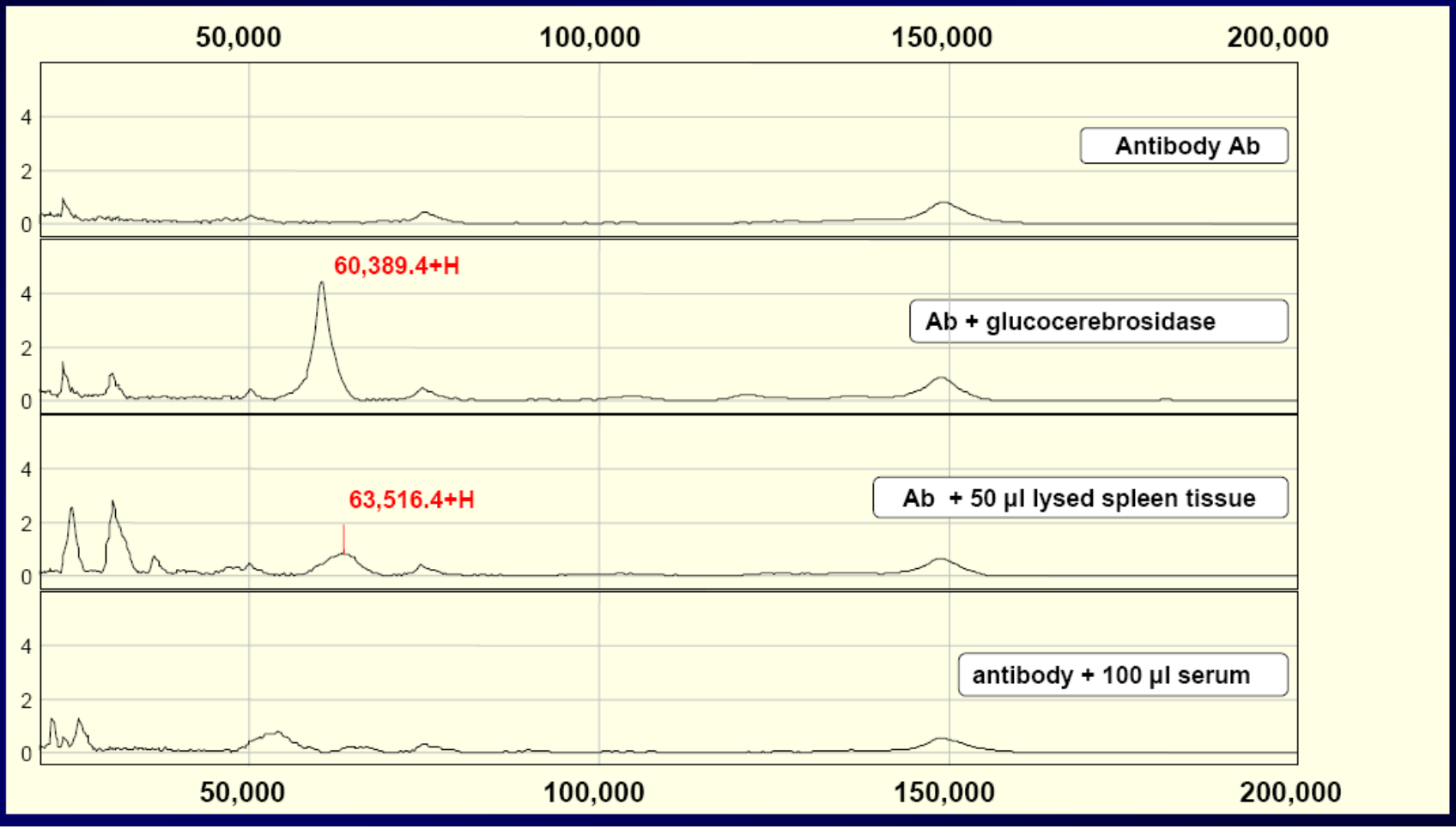
SELDI-TOF-MS : Chemically modified MALDI targets are used to retain a group of proteins



SELDI-TOF-MS : The SELDI mass spectrometer



Capture of glucocerebrosidase by antibody immobilized on PS20 chips

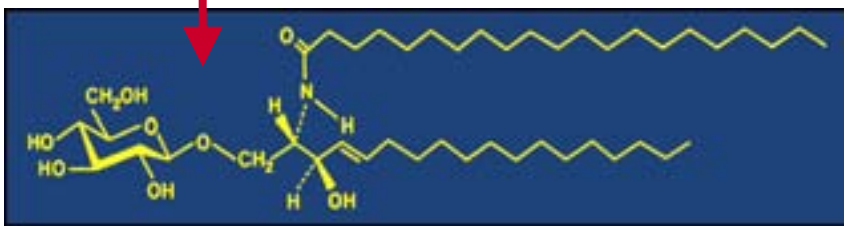


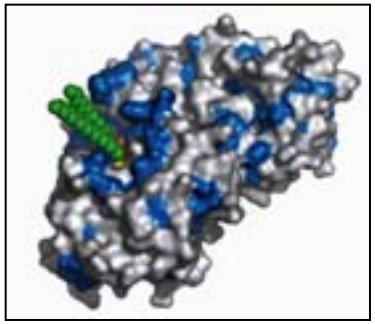
Gaucher Disease

Lysosomal storage disorder

Deficiency in lysosomal glucocerebrosidase

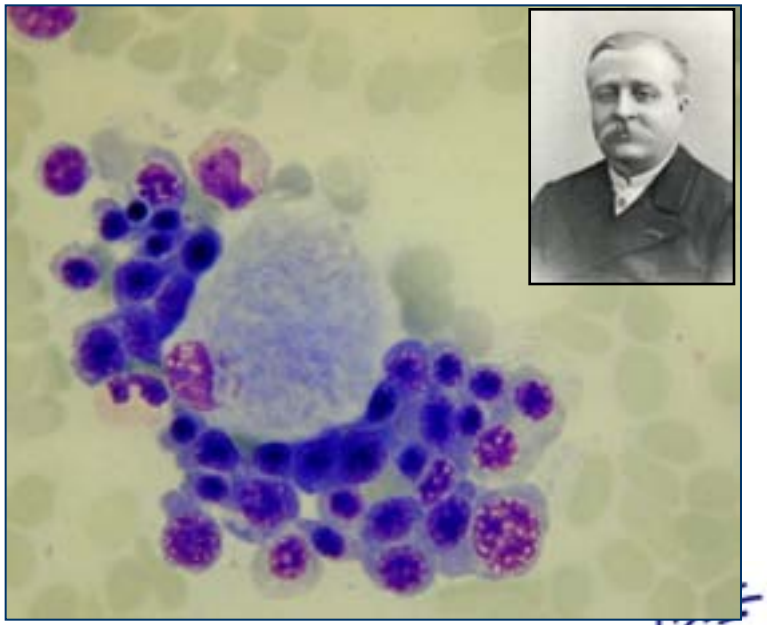
Substrate : glucosylceramide



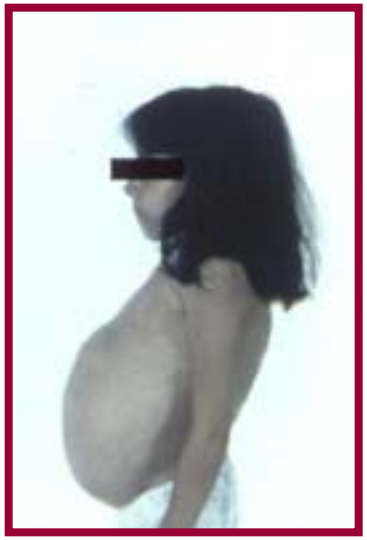


Flux of glycosphingolipids is largest in monocyte-macrophage system

These cells become major storage sites (Gaucher cells >> mainly found in liver, spleen and bone marrow)



Treatment of lysosomal storage diseases

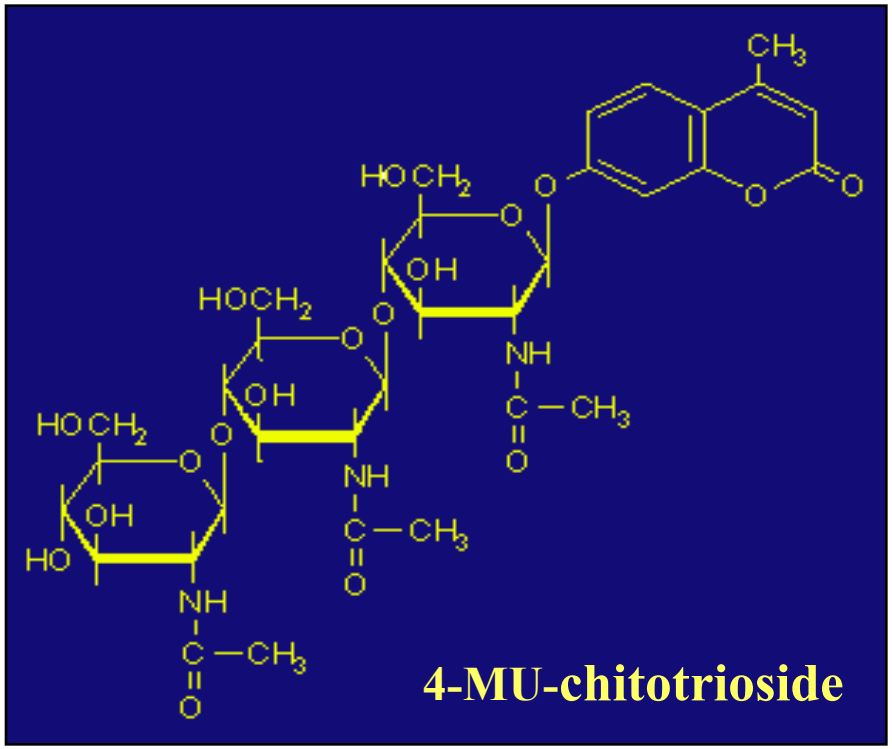


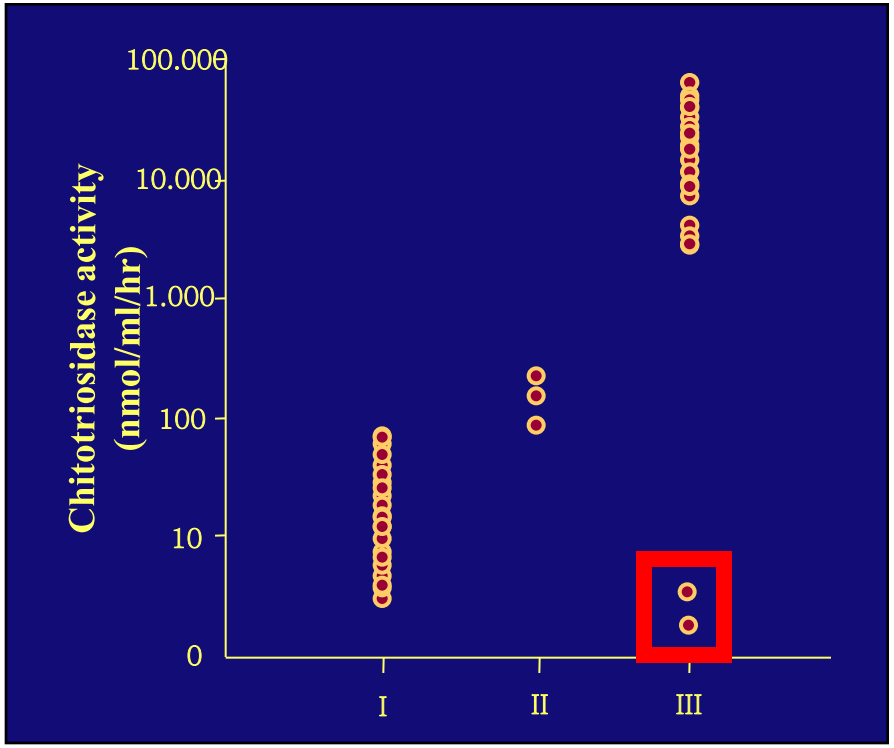
Discovery of chitotriosidase

Gaucher disease : search for markers

Patient plasma : 1000-fold increased 4-MU-chitotrioside hydrolysis

Unfortunately 10-20 % of people deficient for this enzyme





Search for novel surrogate markers

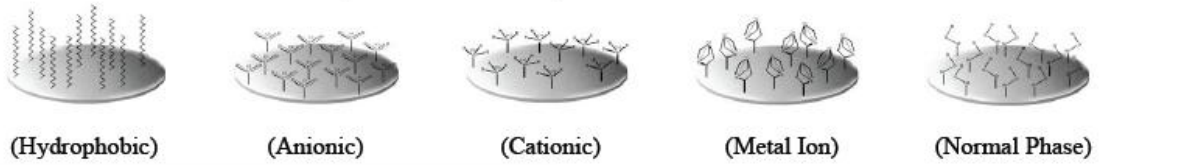
SELDI-TOF-MS

Surface enhanced laser disorption ionisation

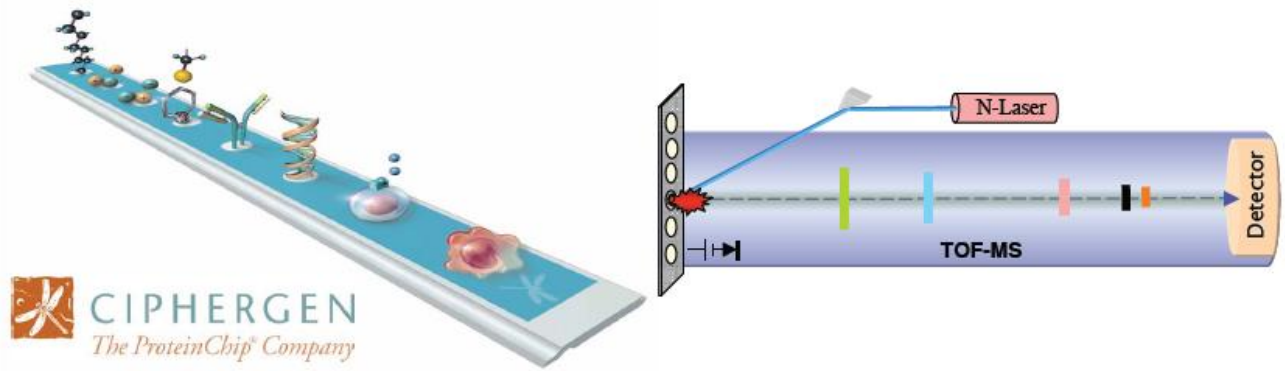
Time of flight

Mass spectrometry

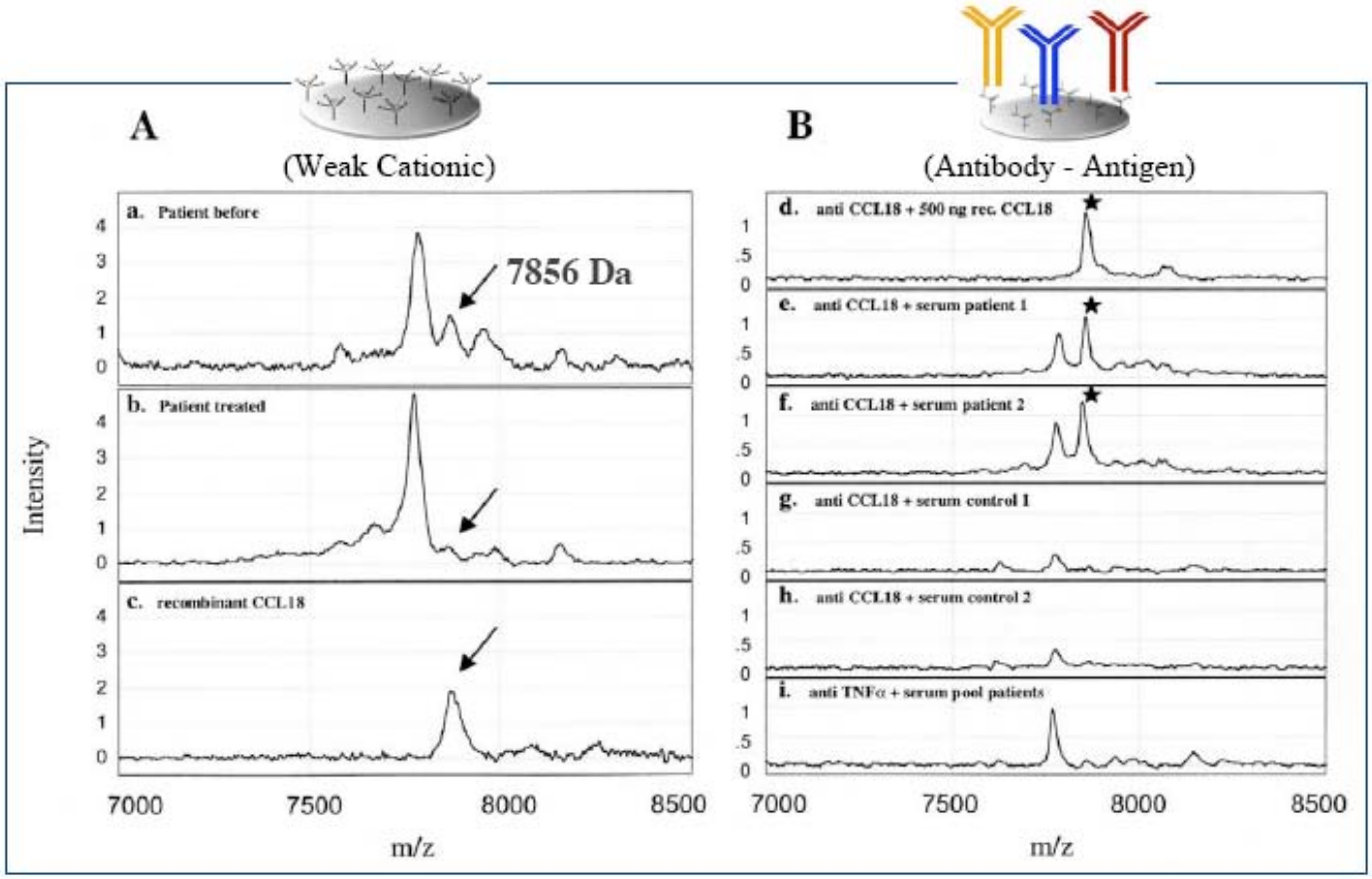
Chemical Surfaces – Protein Expression Profiling:



(Hydrophobic) (Anionic) (Cationic) (Metal Ion) (Normal Phase)



SELDI-TOF mass spectrometry profiling of Gaucher plasma

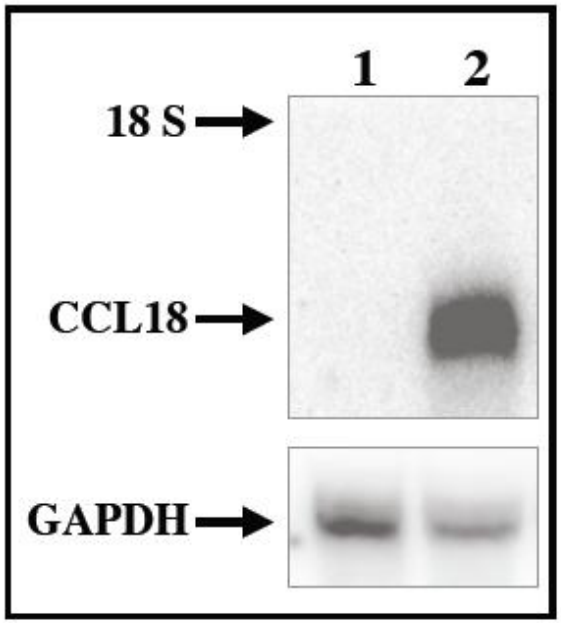


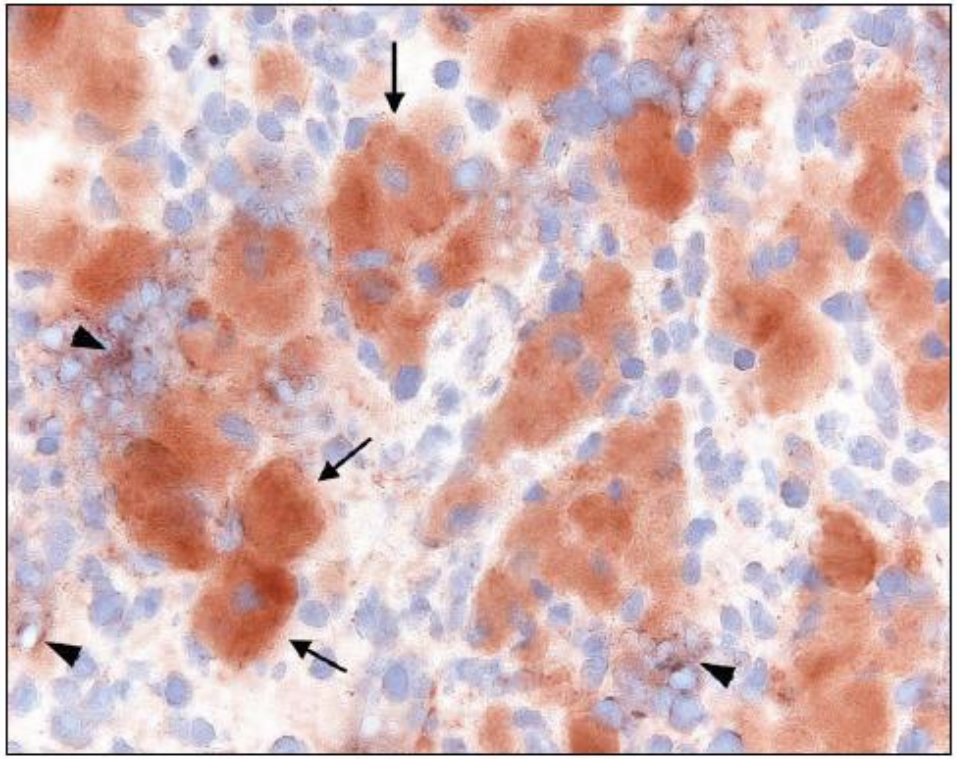
Molecular mass and basic pI similar to CCL18 (PARC)

Identified to be upregulated in Gaucher spleen

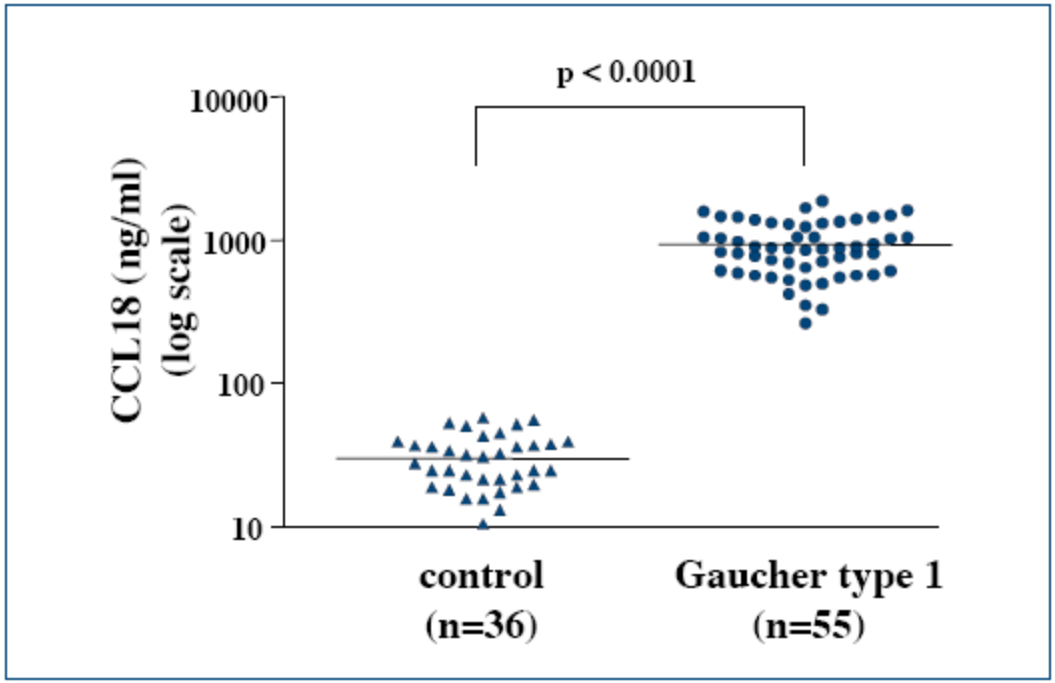
(Moran et al. (2000) Blood 96:1969-1978)

Expression of CCL18 in Gaucher spleen





Plasma CCL18 levels in controls and Gaucher patients



Sandwich ELISA: Median control plasma level 33 ng/ml (10-72 ng/ml)

Median Gaucher plasma level 948 ng/ml (237-2285 ng/ml)

CCL18 levels on average 30 fold elevated compared to control

No Overlap with control values

Post-translational modifications

Journal of Molecular and Cellular Cardiology 53 (2012) 354–368

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

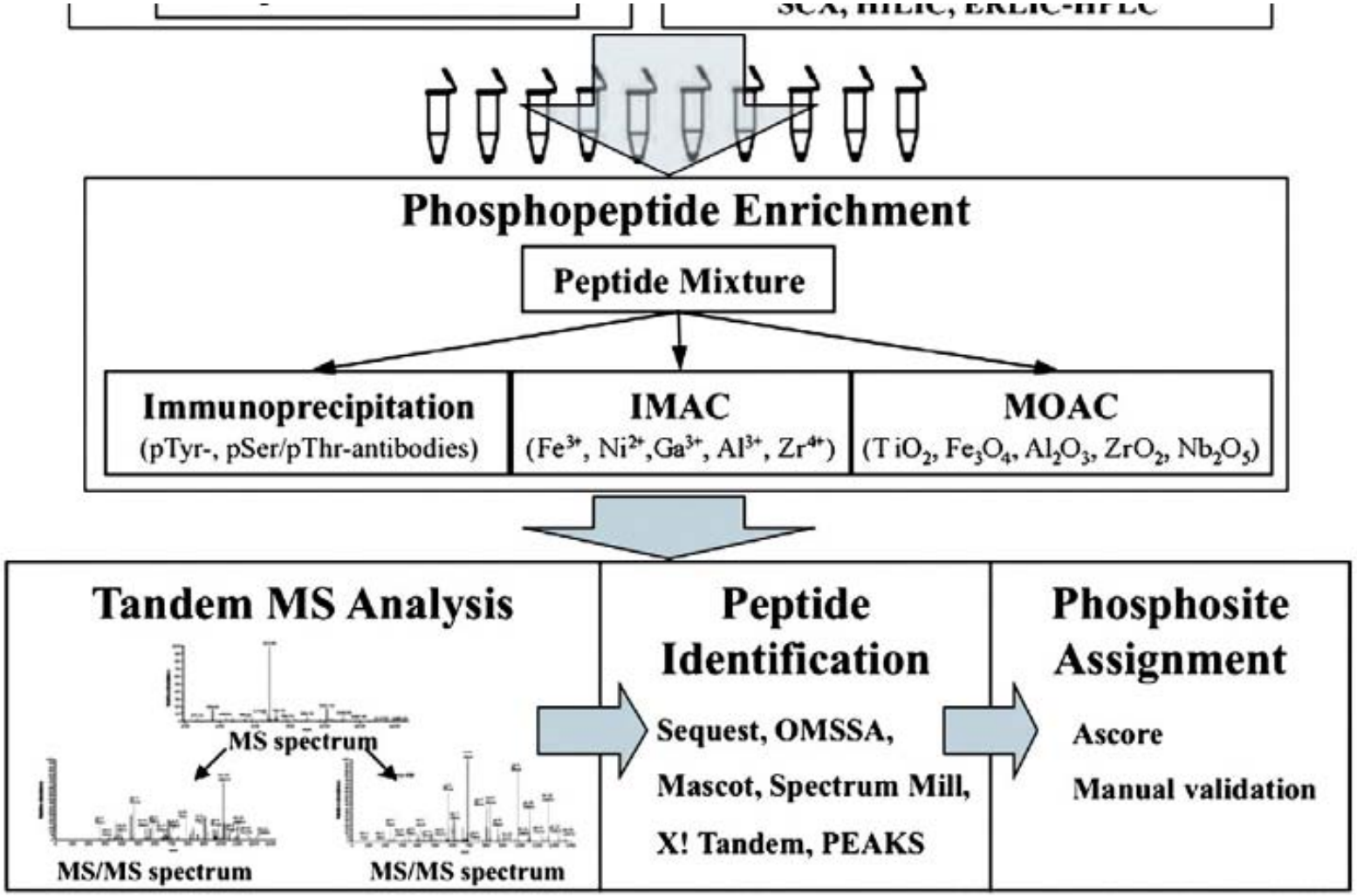
journal homepage: www.elsevier.com/locate/yjmcc

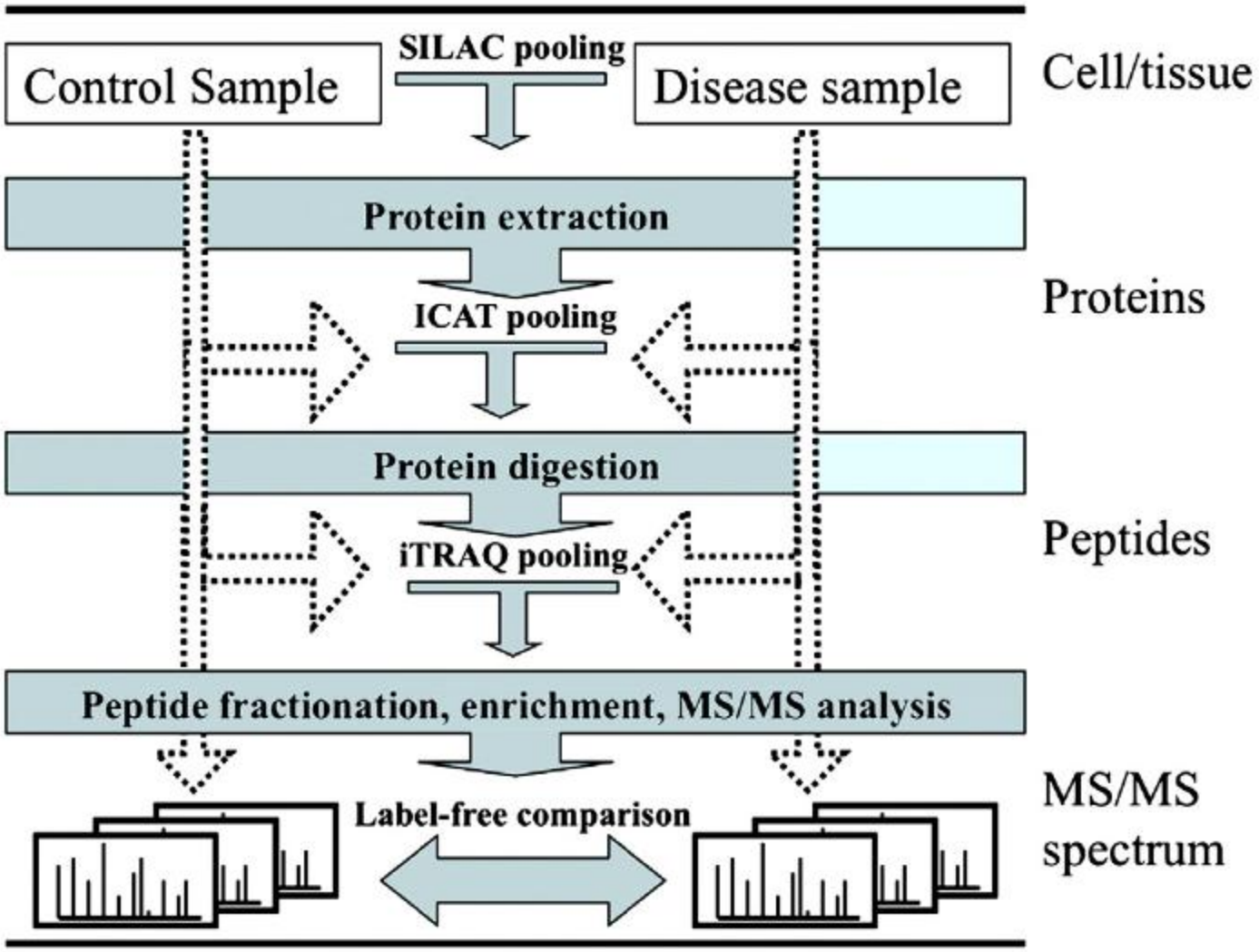
Review article

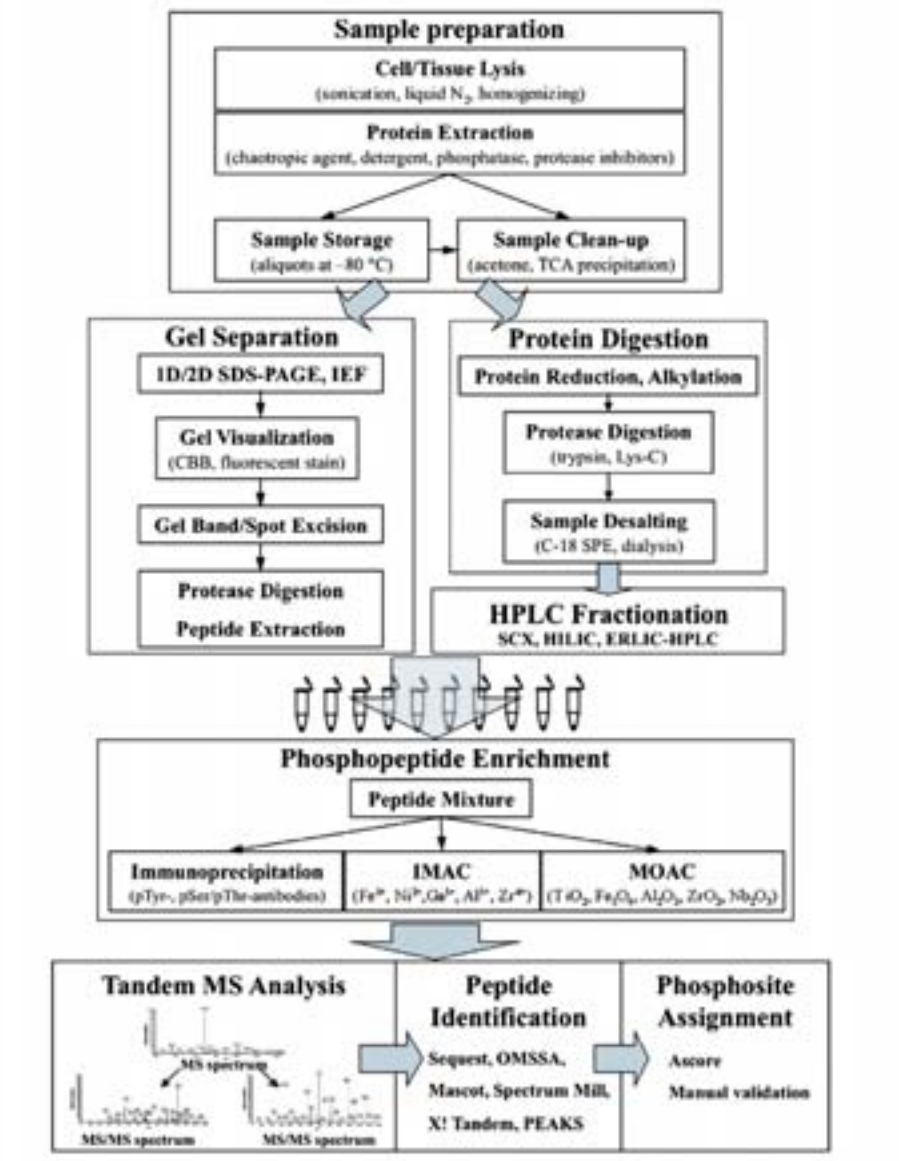
Phosphoproteomics and molecular cardiology: Techniques, applications and challenges

Zeyu Sun [a], Karyn L. Hamilton [b,d], Kenneth F. Reardon [a,b,c,*]

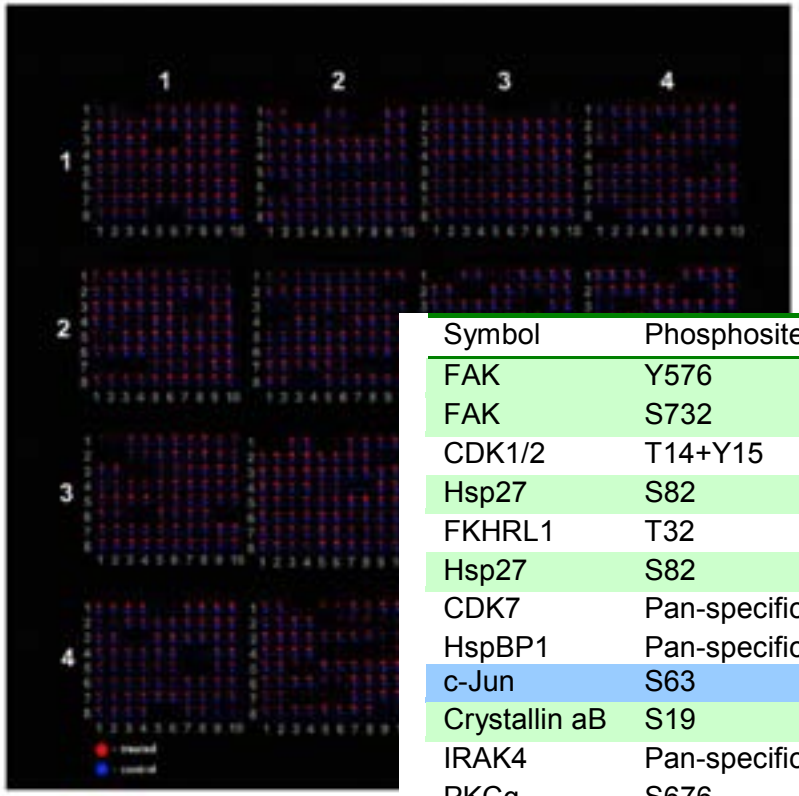
[a] Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO, 80523, USA
[b] Graduate Program in Cell and Molecular Biology, Colorado State University, Fort Collins, CO, 80523, USA
[c] School of Biomedical Engineering, Colorado State University, Fort Collins, CO, 80523, USA
[d] Department of Health and Exercise Science, Colorado State University, Fort Collins, CO, 80523, USA


Phosphopeptide Enrichment
Peptide Mixture
Immunoprecipitation
(pTyr-, pSer/pThr-antibodies)
IMAC
(Fe^{3+}, Ni^{2+},Ga^{3+}, Al^{3+}, Zr^{4+})
MOAC
(TiO_2, Fe_3O_4, Al_2O_3, ZrO_2, Nb_2O_5)
Tandem MS Analysis
MS spectrum
MS/MS spectrum
MS/MS spectrum
Peptide Identification
Sequest, OMSSA,
Mascot, Spectrum Mill,
X! Tandem, PEAKS
Phosphosite Assignment
Ascore
Manual validation


Control Sample
SILAC pooling
Disease sample
Cell/tissue
Protein extraction
Proteins
ICAT pooling
Protein digestion
Peptides
iTRAQ pooling
Peptide fractionation, enrichment, MS/MS analysis
Label-free comparison
MS/MS spectrum


Sample preparation
Cell/Tissue Lysis
(sonication, liquid N_2, homogenizing)
Protein Extraction
(chaotropic agent, detergent, phosphatase, protease inhibitors)
Sample Storage
(aliquots at –80 °C)
Sample Clean-up
(acetone, TCA precipitation)
Gel Separation
1D/2D SDS-PAGE, IEF
Gel Visualization
(CBB, fluorescent stain)
Gel Band/Spot Excision
Protease Digestion
Peptide Extraction
Protein Digestion
Protein Reduction, Alkylation
Protease Digestion
(trypsin, Lys-C)
Sample Desalting
(C-18 SPE, dialysis)
HPLC Fractionation
SCX, HILIC, ERLIC-HPLC
Phosphopeptide Enrichment
Peptide Mixture
Immunoprecipitation
(pTyr-, pSer/pThr-antibodies)
IMAC
(Fe^{3+}, Ni^{2+},Ga^{3+}, Al^{3+}, Zr^{4+})
MOAC
(TiO_2, Fe_2O_3, Al_2O_3, ZrO_2, Nb_2O_5)
Tandem MS Analysis
MS spectrum
MS/MS spectrum
MS/MS spectrum
Peptide Identification
Sequest, OMSSA,
Mascot, Spectrum Mill,
X! Tandem, PEAKS
Phosphosite Assignment
Ascore
Manual validation

Kinome analysis:



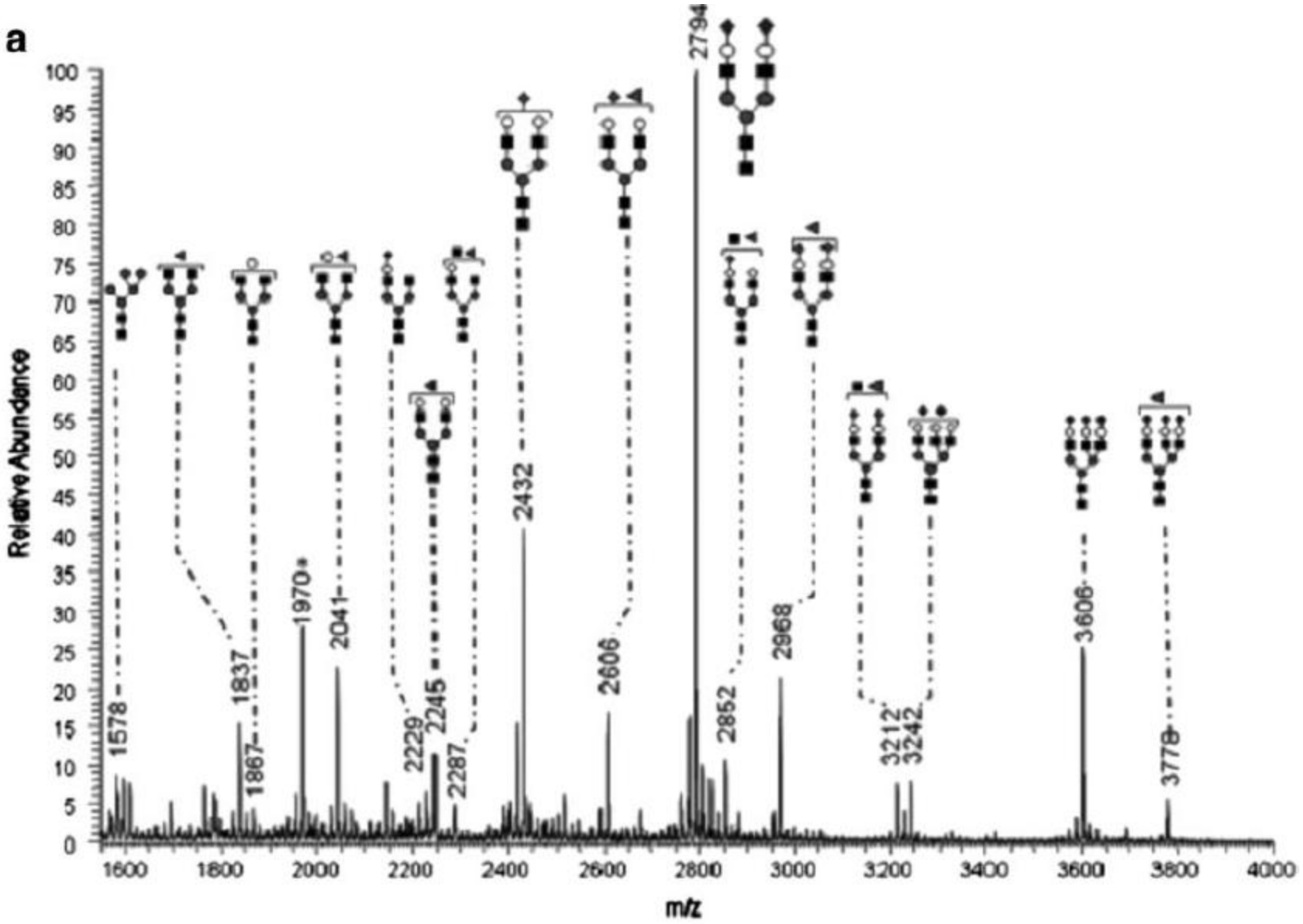
Symbol	Phosphosite	Bayes.p	Fold
FAK	Y576	0.0005	-3.02
FAK	S732	0.0008	-2.81
CDK1/2	T14+Y15	0.0091	-1.67
Hsp27	S82	0.0150	-1.93
FKHRL1	T32	0.0155	-3.65
Hsp27	S82	0.0169	-2.41
CDK7	Pan-specific	0.0203	-1.45
HspBP1	Pan-specific	0.0217	-1.49
c-Jun	S63	0.0324	-3.54
Crystallin aB	S19	0.0351	-1.57
IRAK4	Pan-specific	0.0426	-4.10
PKCq	S676	0.0472	-1.39
Ksr1	Pan-specific	0.0489	-1.68
Rb	S780	0.0498	-1.62
PP6C	Pan-specific	0.0586	-1.34
Tau	S518	0.0595	-1.28
EGFR	Y1148	0.0602	-1.94
ERK5	T218+Y220	0.0618	-1.44

Symbol	Phosphosite	Bayes.p	Fold
PKCg	T514	0.0036	2.24
Abl	Y412	0.0048	1.62
RSK1/2	S363/S369	0.0117	3.16
Src	Pan-specific	0.0179	1.61
HO2	Pan-specific	0.0267	2.04
ATF2	T51+T53	0.0273	1.44
p38a	Pan-specific	0.0336	1.48
PKCb2	T641	0.0380	2.42
STAT5A	Y694	0.0418	3.41
S6Ka	T389	0.0462	2.53
Bad	S75	0.0506	1.42
EGFR	Pan-specific	0.0570	1.54
PKCg	Pan-specific	0.0575	2.04
FAK	Y397	0.0667	1.81
Erk4	Pan-specific	0.0668	1.33
Kit	Y730	0.0690	1.20

Boon, Blood 2010

Glycomics using mass spectrometry

Manfred Wuhrer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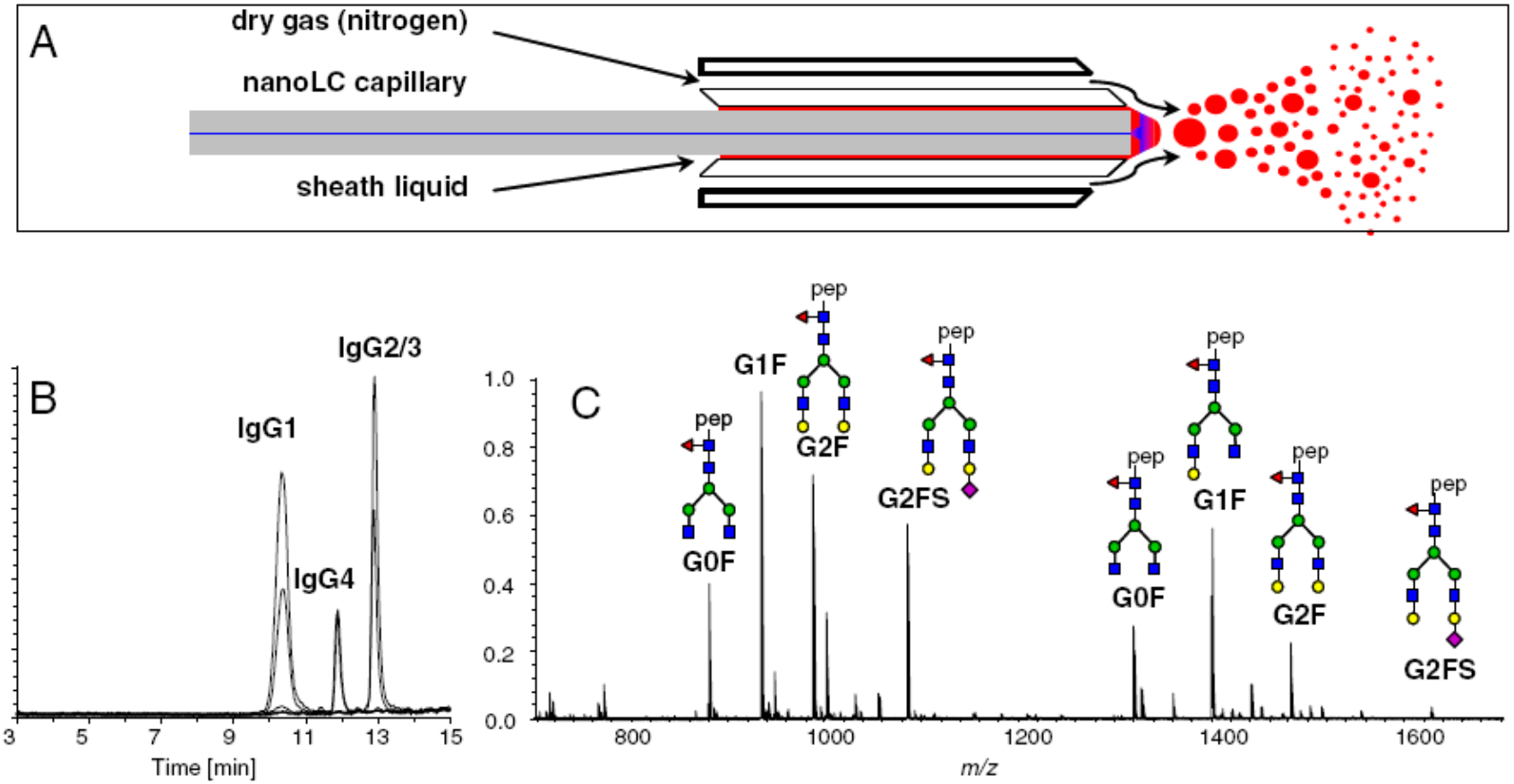
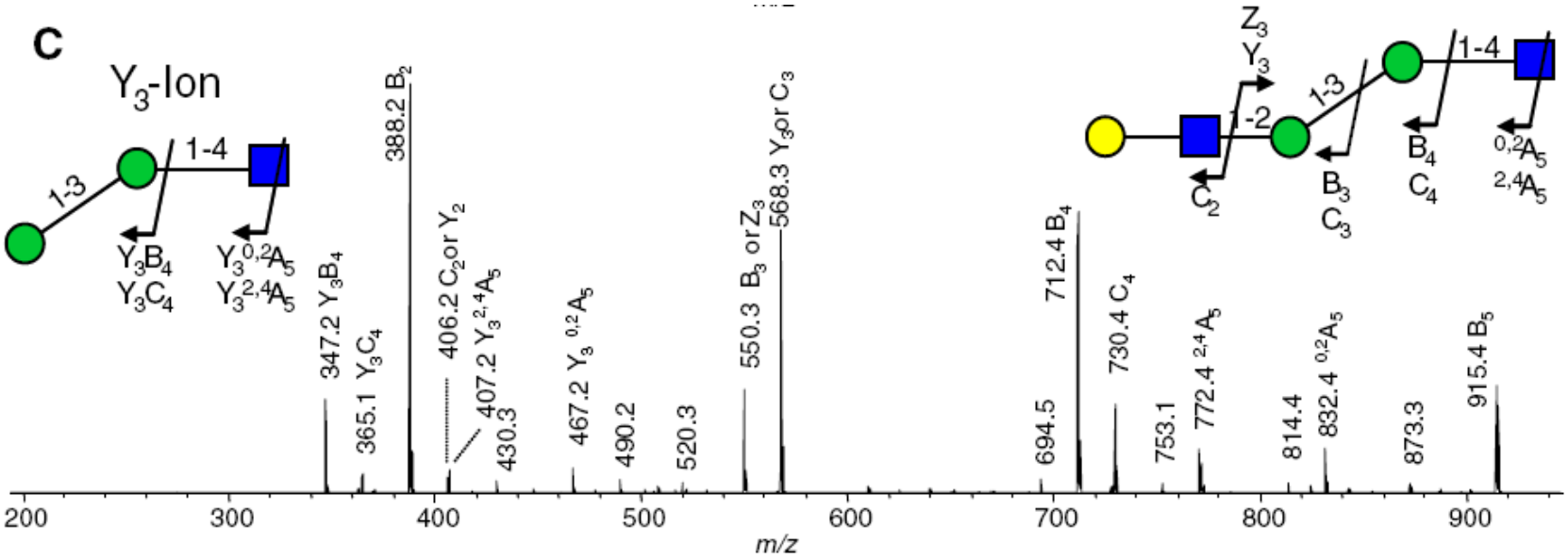
3778

Glycosylation of IgG changes during disease: biomarkers



Glycosylation structure determination using MS-MS



blood

2009 114: 723-732
Prepublished online Apr 15, 2009;
doi:10.1182/blood-2009-02-205930

Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures

Marianna Prokopi, Giordano Pula, Ursula Mayr, Cécile Devue, Joy Gallagher, Qingzhong Xiao, Chantal M. Boulanger, Nigel Westwood, Carmen Urbich, Johann Willeit, Marianne Steiner, Johannes Breuss, Qingbo Xu, Stefan Kiechl and Manuel Mayr

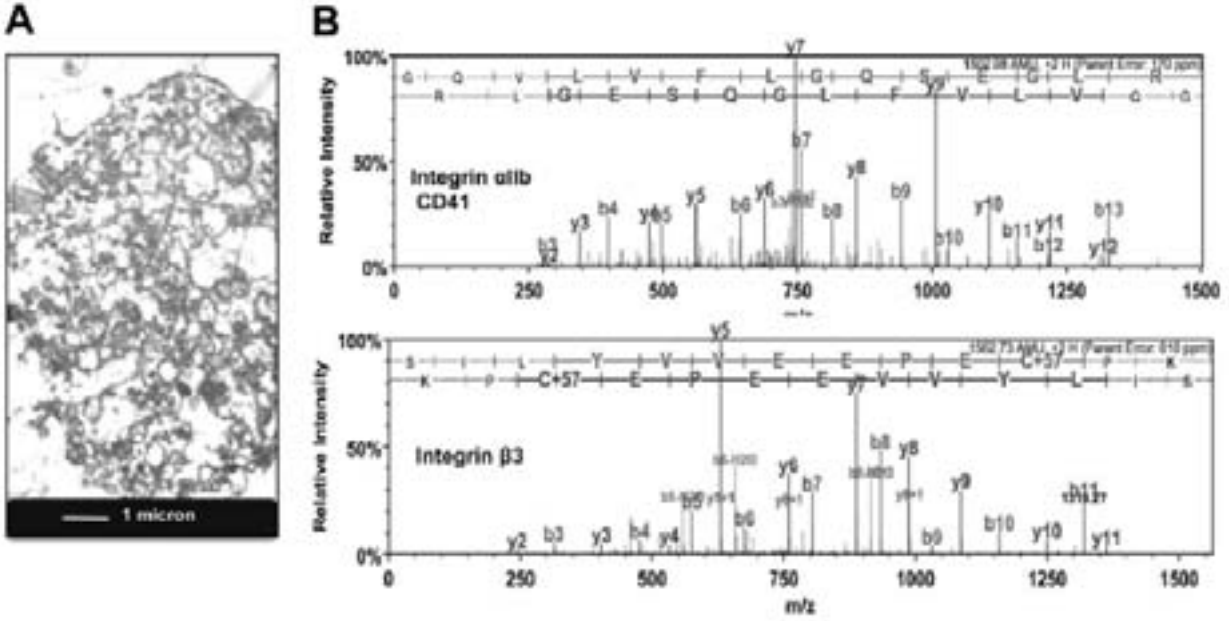


Figure 1. Presence of platelet proteins in MPs of EPC cultures. (A) Transmission electron microscopy. Image of MPs harvested from the conditioned medium of EPC cultures. (B) Proteomic analysis. Product ion spectra of doubly charged tryptic peptides identified as the platelet integrin αIIb (GQVLVFLGQSEGLR) and integrin β3 (SILYVVEEPECPK).

EPC = monocyte with ingested platelets!

Table 1. Membrane proteins identified in microparticles derived from EPC cultures

Protein name	SWISS PROT accession name	MW, kDa	Spectra, n
Integrins, alpha chain			
Integrin alpha-IIb precursor, CD41 antigen	ITA2B_HUMAN	113	156
Integrin alpha-6 precursor, CD49f antigen	ITA6_HUMAN	127	21
Integrin alpha-M precursor, CD11b antigen	ITAM_HUMAN	127	13
Integrin alpha-2 precursor, CD49b antigen	ITA2_HUMAN	129	6
Integrin alpha-X precursor, CD11c antigen	ITAX_HUMAN	128	5
Integrins, beta chain			
Integrin beta-3 precursor, CD61 antigen	ITB3_HUMAN	87	78
Integrin beta-2 precursor, CD18 antigen	ITB2_HUMAN	85	49
Integrin beta-1 precursor, CD29 antigen	ITB1_HUMAN	88	18
Other surface receptors			
Platelet glycoprotein Ib alpha chain CD42b-alpha/CD42b antigen	GP1BA_HUMAN	69	21
Leukocyte antigen MIC3, CD9 antigen	CD9_HUMAN	25	20
Platelet glycoprotein IX, CD42a antigen	GPIX_HUMAN	19	19
4F2 cell-surface antigen heavy chain, CD98 antigen	4F2_HUMAN	58	18
Platelet glycoprotein Ib beta chain CD42b-beta/CD42c antigen	GP1BB_HUMAN	22	15
Hyaluronate receptor, CD44 antigen	CD44_HUMAN	82	15
Intercellular adhesion molecule 3	ICAM3_HUMAN	59	13
Transferrin receptor protein 1, CD71 antigen	TFR1_HUMAN	85	13
Platelet endothelial cell adhesion molecule, CD31 antigen	PECA1_HUMAN	83	9
Leukocyte common antigen, CD45 antigen	CD45_HUMAN	147	7
Leukocyte surface antigen, CD47 antigen	CD47_HUMAN	35	6

EPC = monocyte with ingested platelets!

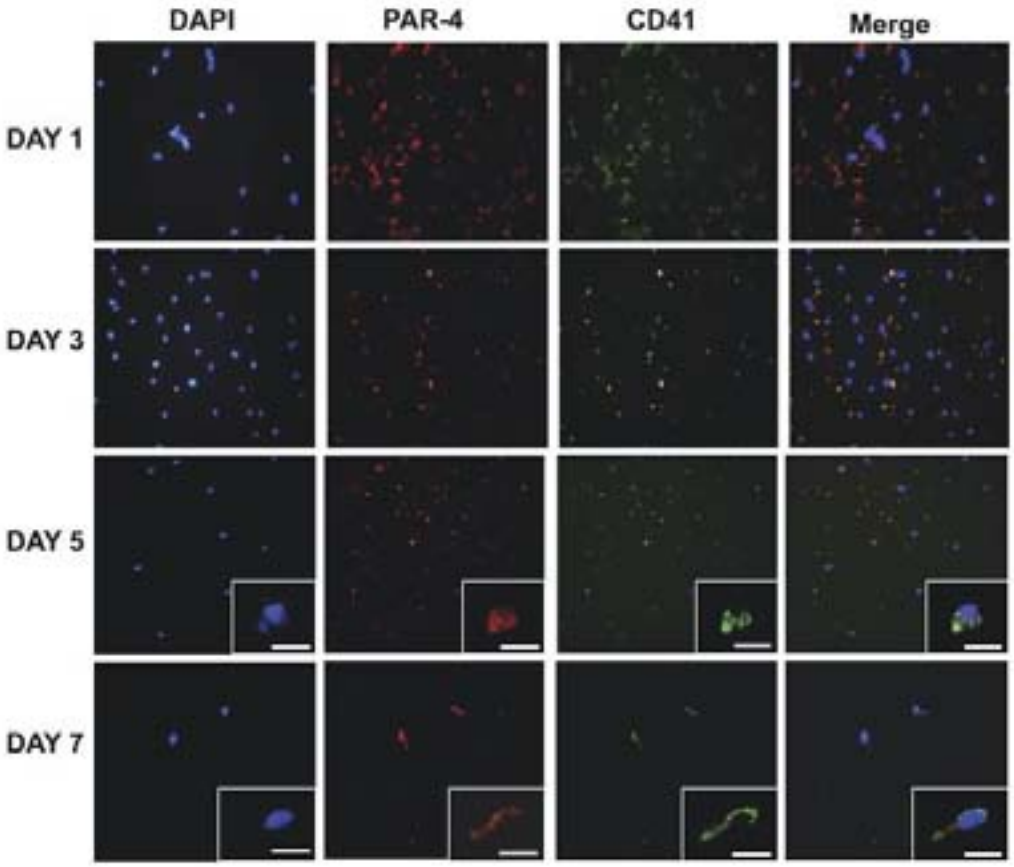


Figure 4. Cellular uptake of platelet MPs. Intact platelets stained positive for PAR-4 and integrin αIIb (CD41) among the PBMNCs counterstained with DAPI (day 1). Over time, the platelets disintegrated, but platelet proteins remained detectable in EPC cultures (day 3) and were taken up by the adherent cell population (day 5, inset). By day 7, most cells stained positive for platelet markers (insets: scale bar represents 25 μm).

Journal of Molecular and Cellular Cardiology 50 (2011) 982–990

Contents lists available at ScienceDirect

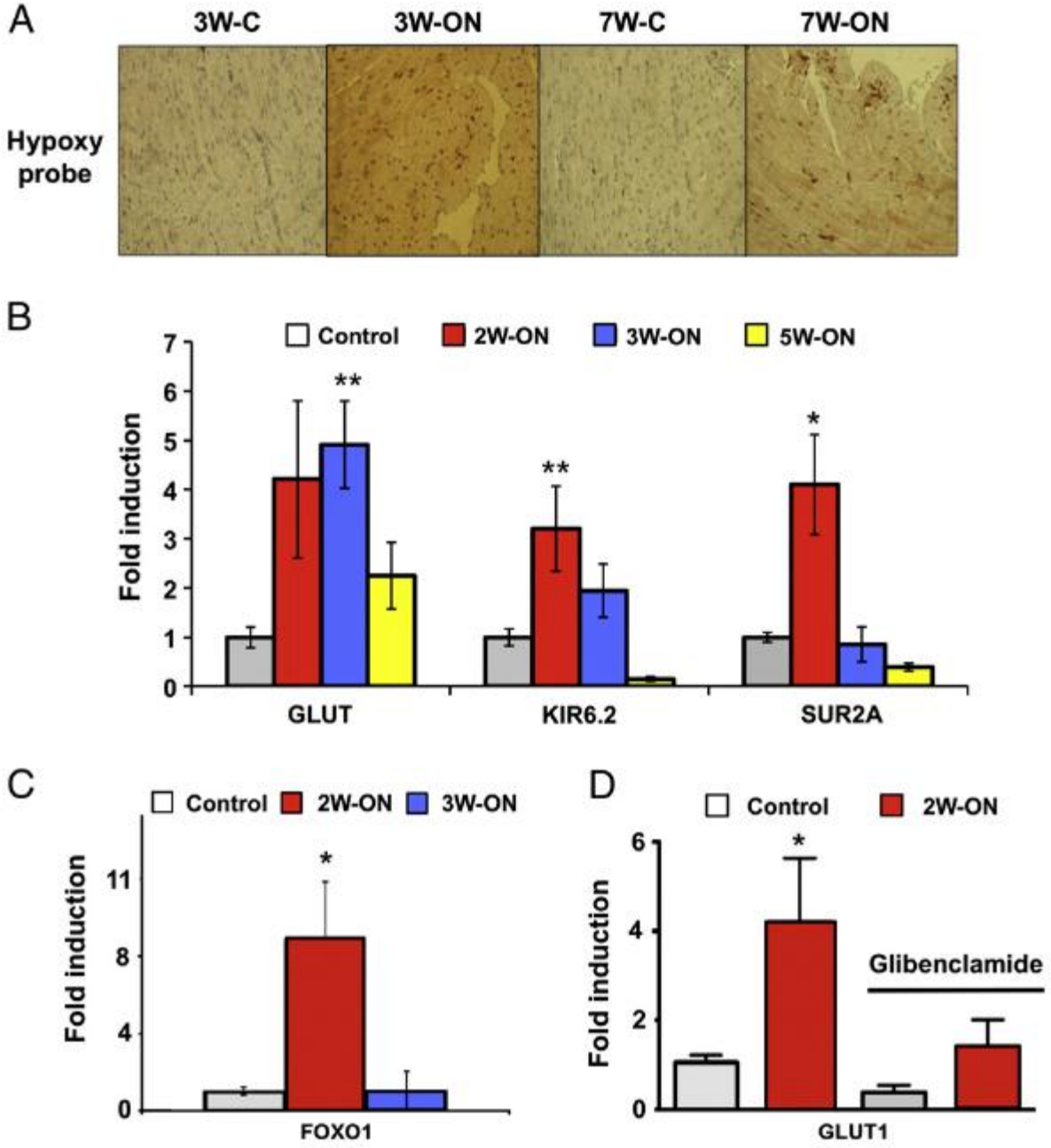
Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Metabolic homeostasis is maintained in myocardial hibernation by adaptive changes in the transcriptome and proteome

Manuel Mayr [a,*], Dalit May [b], Oren Gordon [b], Basetti Madhu [c], Dan Gilon [d], Xiaoke Yin [a], Qiuru Xing [a], Ignat Drozdov [e], Chrysanthi Ainali [e], Sophia Tsoka [e], Qingbo Xu [a], John Griffiths [c], Anton Horrevoets [f], Eli Keshet [b]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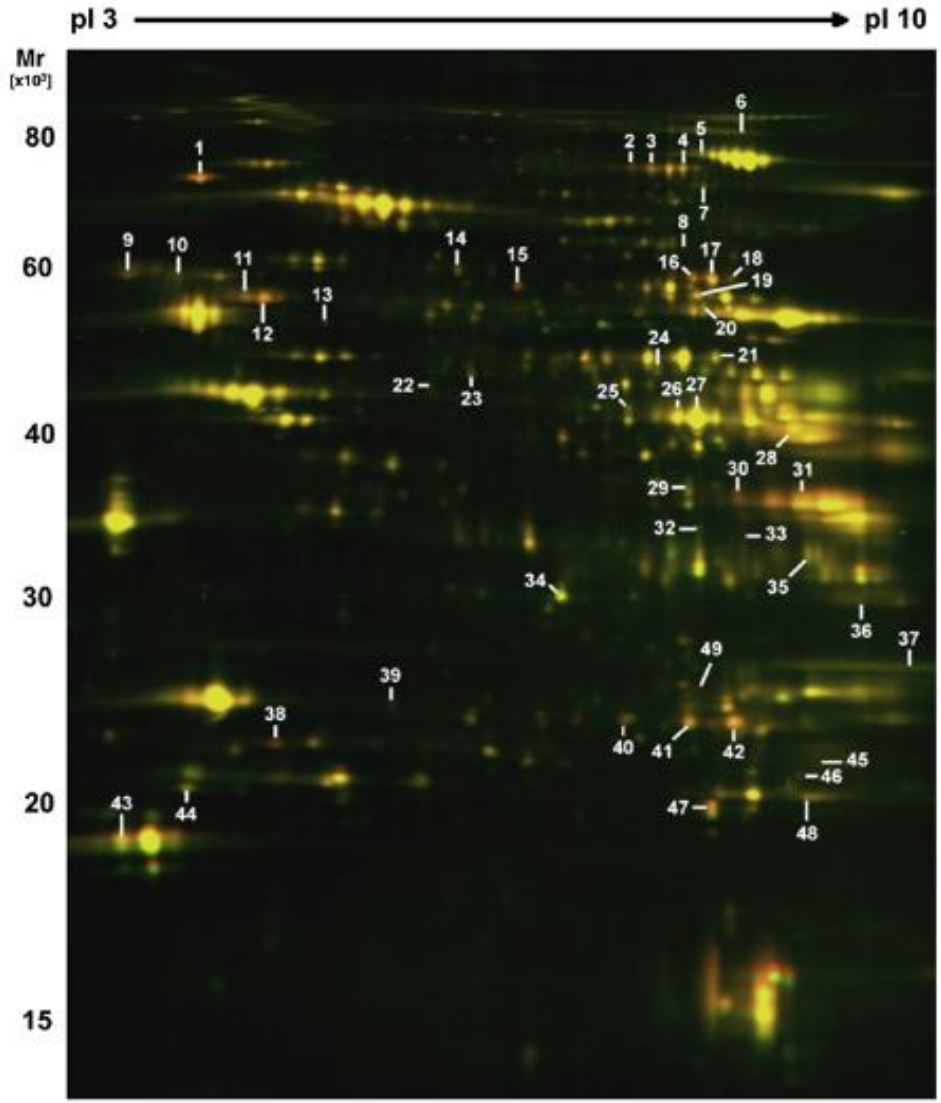
Proteomics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metabolomics

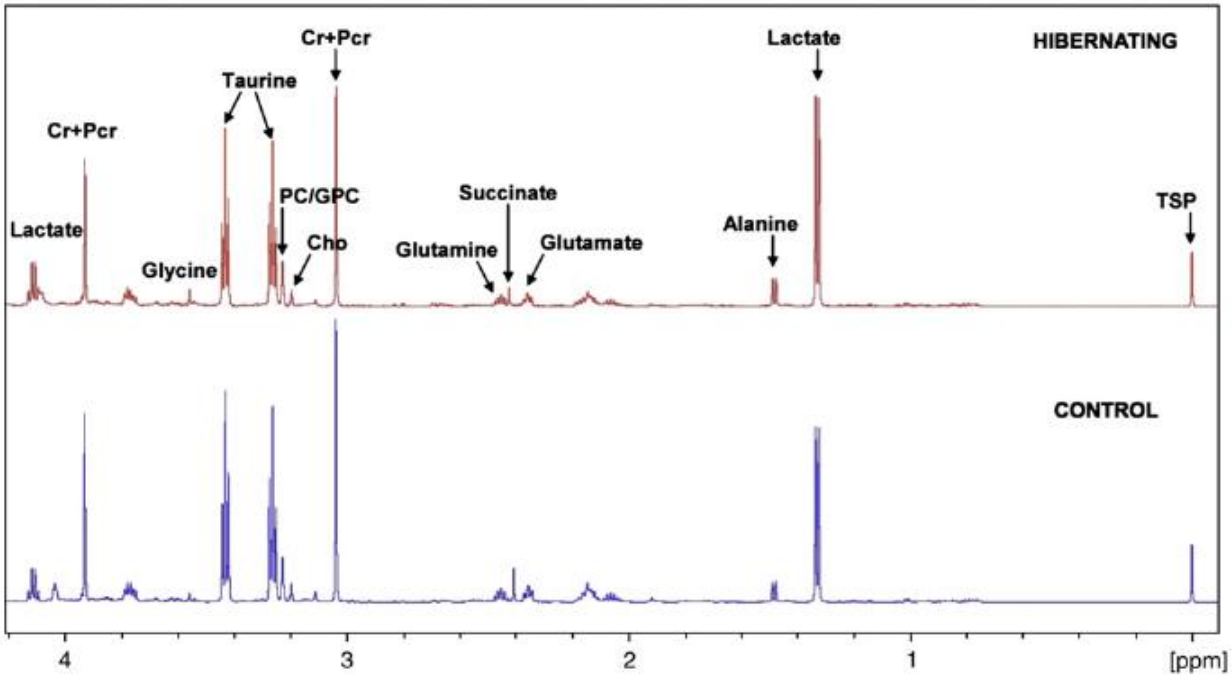


Fig. 5. High-resolution ^{1}H-NMR spectroscopy of cardiac tissue extracts. Representative spectra of the aliphatic region (−0.05 to 4.2 ppm) from control (bottom) and hibernating hearts (top). Quantitative metabolite data are presented in Table 2.

Nothing changed at metbolite level!!: Homeostasis

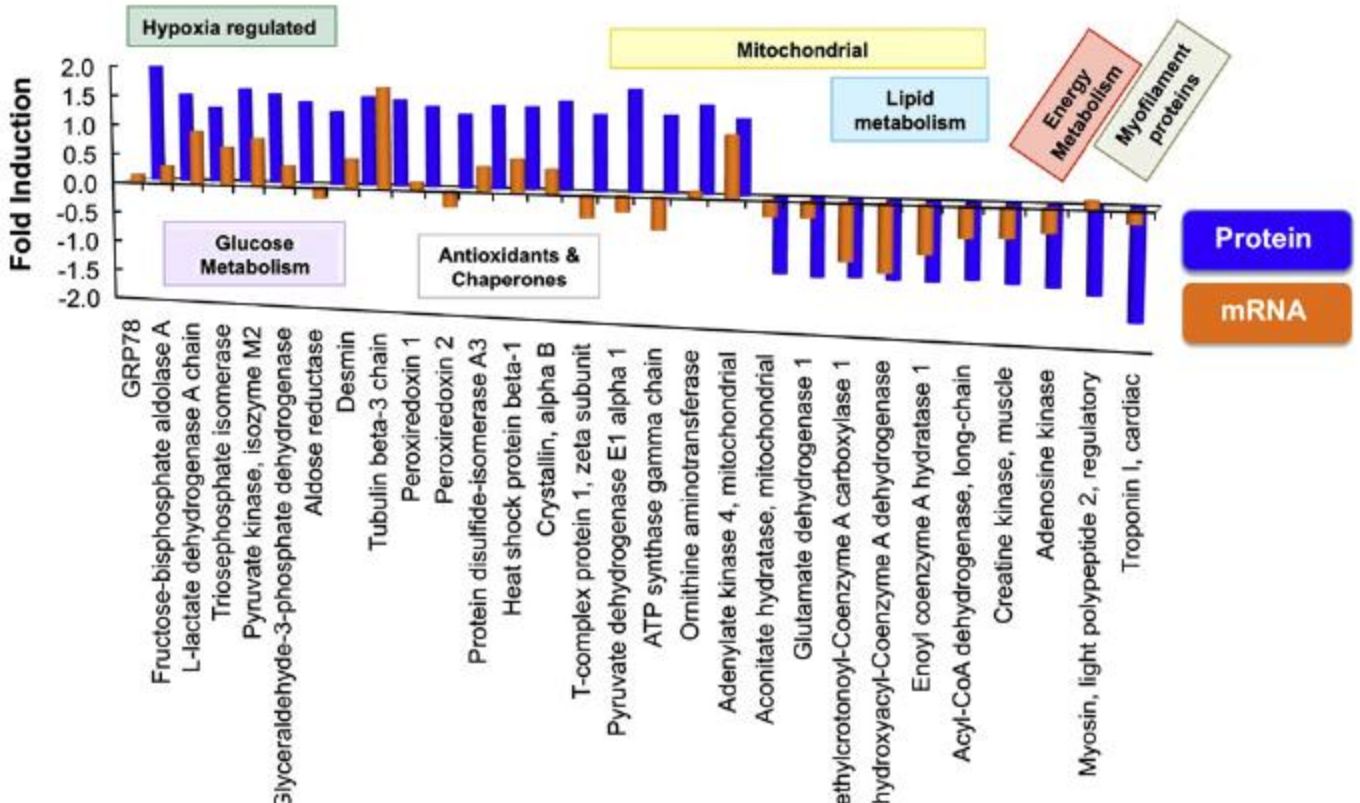
Combined tri-omics: increased sensitivity

Table 2
Metabolite changes by ^{1}H-NMR in cardiac tissue extracts.

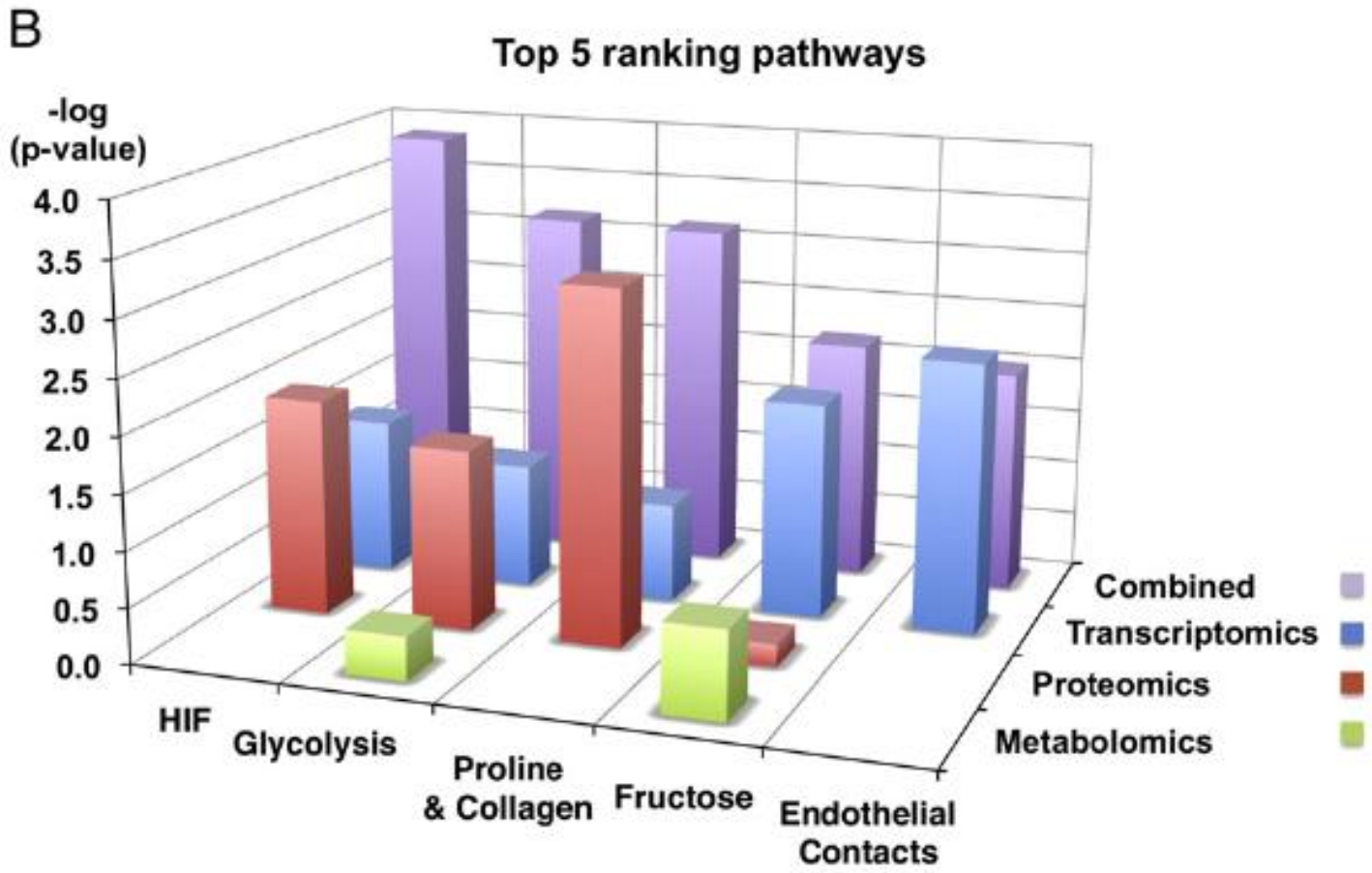
	Control ($n=3$)	Hibernating ($n=5$)	Fold change	P (t-test)
Leucine	0.101 (±0.005)	0.075 (±0.005)	**0.74**	**0.016**
Isoleucine	0.414 (±0.138)	0.374 (±0.107)	0.90	0.828
Valine	0.105 (±0.011)	0.086 (±0.008)	0.82	0.214
Isovalerate	0.123 (±0.034)	0.143 (±0.048)	1.16	0.774
Beta-OH butyrate	0.145 (±0.030)	0.126 (±0.026)	0.87	0.654
Lactate	10.383 (±0.784)	11.689 (±0.648)	1.12	0.255
Alanine	1.680 (±0.273)	1.719 (±0.106)	1.02	0.878
Acetate	0.337 (±0.053)	0.310 (±0.090)	0.92	0.835
Glutamate	3.752 (±0.258)	2.563 (±0.126)	**0.68**	**0.003**
Succinate	1.234 (±0.343)	1.087 (±0.119)	0.88	0.638
Glutamine	2.873 (±0.315)	2.000 (±0.186)	**0.69**	**0.042**
Aspartate	0.266 (±0.097)	0.346 (±0.073)	1.30	0.534
Choline	0.077 (±0.005)	0.051 (±0.004)	**0.66**	**0.006**
Phosphocholine	0.173 (±0.027)	0.129 (±0.013)	0.75	0.145
Carnitine	0.546 (±0.091)	0.562 (±0.033)	1.03	0.845
Taurine	22.11 (±1.937)	16.01 (±0.936)	**0.72**	**0.018**
Glycine	0.572 (±0.033)	0.704 (±0.082)	1.23	0.282
Creatine	8.349 (±0.937)	6.051 (±0.461)	**0.72**	**0.047**
Glycolic acid	0.583 (±0.026)	0.572 (±0.055)	0.98	0.882
Glucose	0.218 (±0.100)	0.309 (±0.061)	1.42	0.438
Fumerate	0.085 (±0.023)	0.073 (±0.012)	0.86	0.622
Tyrosine	0.134 (±0.068)	0.036 (±0.004)	0.27	0.098
Phenylalanine	0.051 (±0.005)	0.043 (±0.003)	0.84	0.217
Adenosine pool	3.419 (±0.357)	2.808 (±0.244)	0.82	0.193
NAD + NADH	0.344 (±0.093)	0.360 (±0.047)	1.05	0.875
Formate	0.306 (±0.015)	0.300 (±0.039)	0.98	0.912

Data presented are given in μmol/g wet weight (mean ± SE), $n=3$ for control and $n=5$ for hibernating hearts. P-values for differences between the two groups were derived from unpaired t-tests (bold numbers highlight significant differences $P<0.05$).

transcriptomics



Combined tri-omics: increased sensitivity



Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®

Local Atherosclerotic Plaques Are a Source of Prognostic Biomarkers for Adverse Cardiovascular Events

Dominique P.V. de Kleijn, Frans L. Moll, Willem E. Hellings, Gonen Ozsarlak-Sozer, Peter de Bruin, Pieter A. Doevendans, Aryan Vink, Louise M. Catanzariti, Arjan H. Schoneveld, Ale Algra, Mat J. Daemen, E.A. Biessen, W. de Jager, Huoming Zhang, Jean-Paul de Vries, Erling Falk, Sai K. Lim, Peter J. van der Spek, Siu Kwan Sze and Gerard Pasterkamp

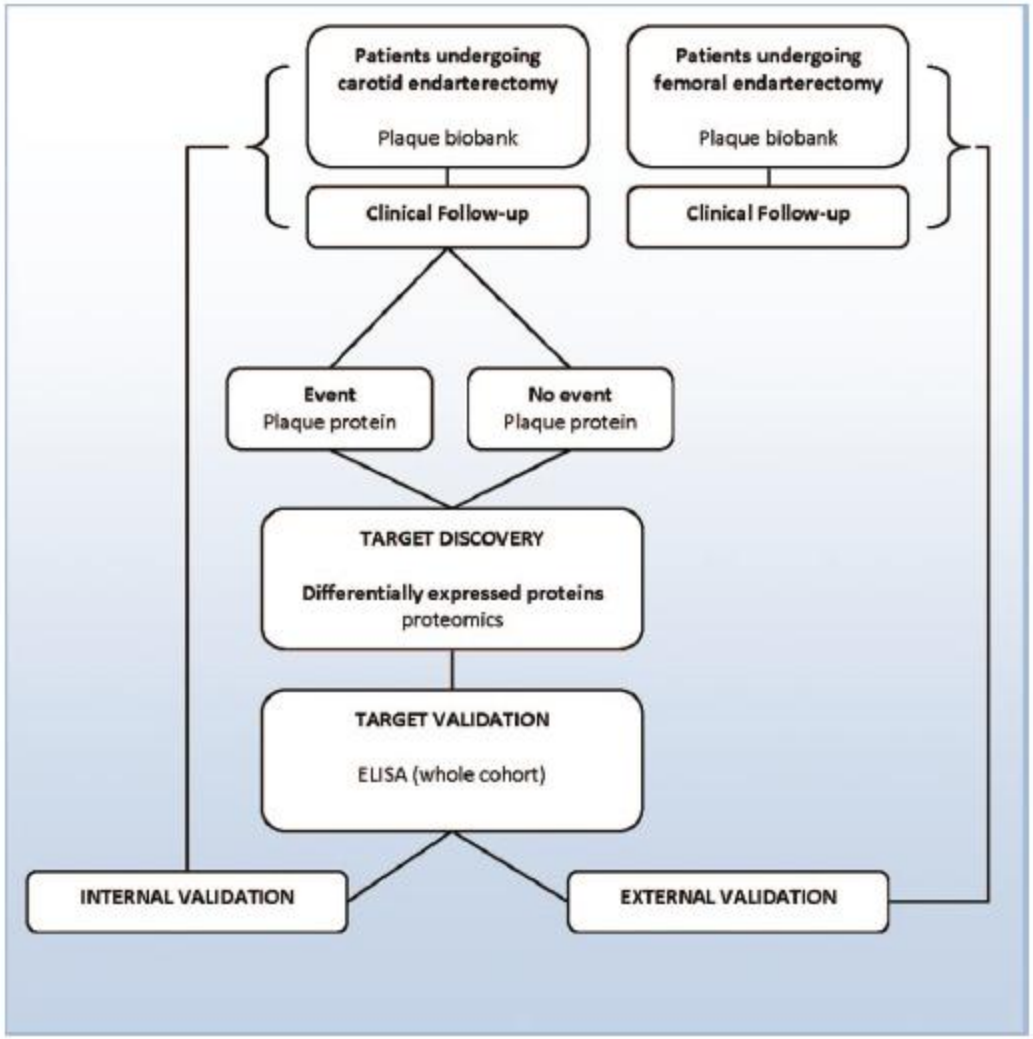
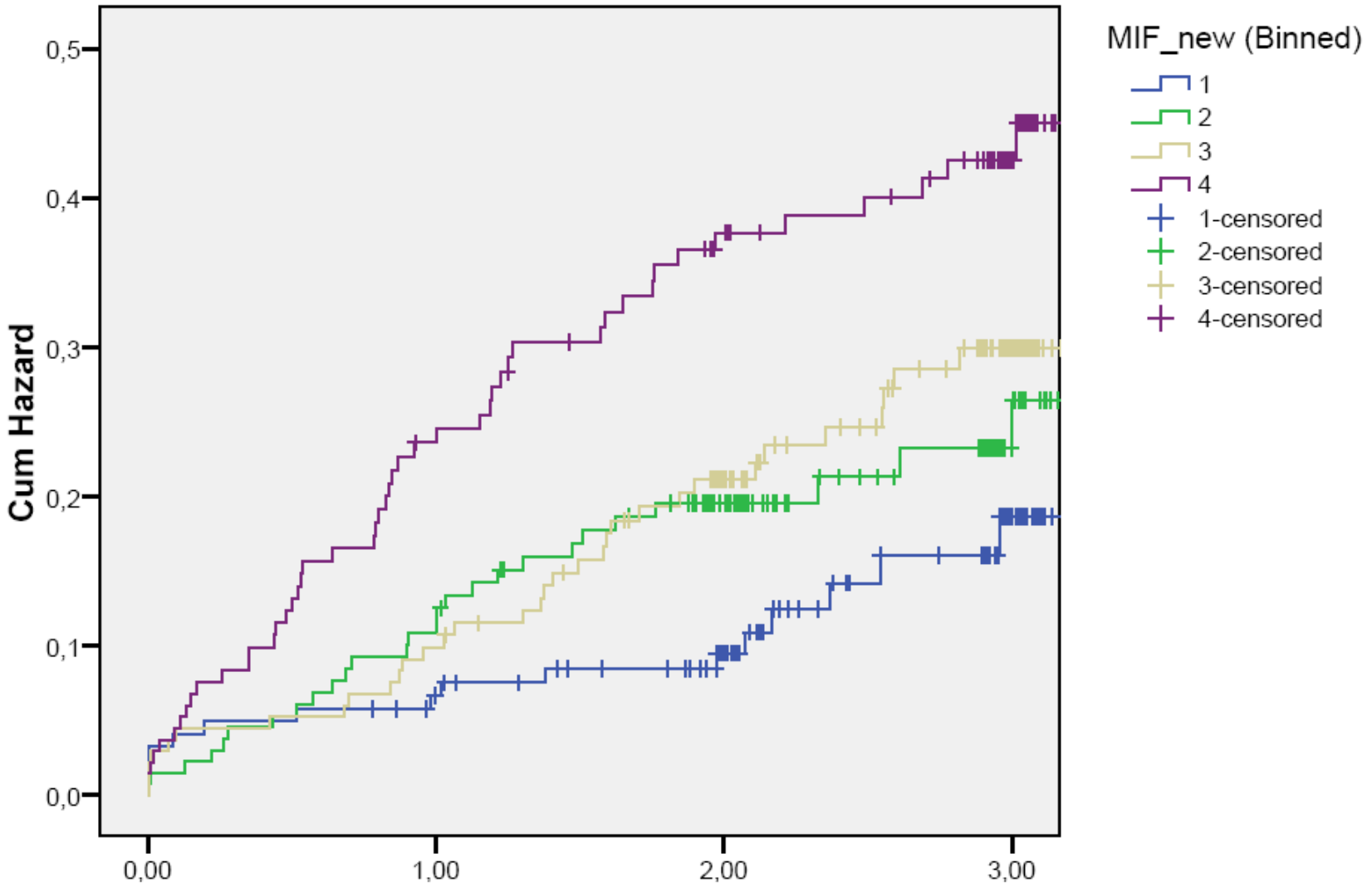


Figure 1. Outline of the study design.



Web-figure 3 Hazard curves of carotid plaque Macrophage Migration Inhibitory Factor (MIF) levels in the same cohort as used for OPN carotid plaque measurements. MIF levels

Carotid OPN predicts all vascular events!

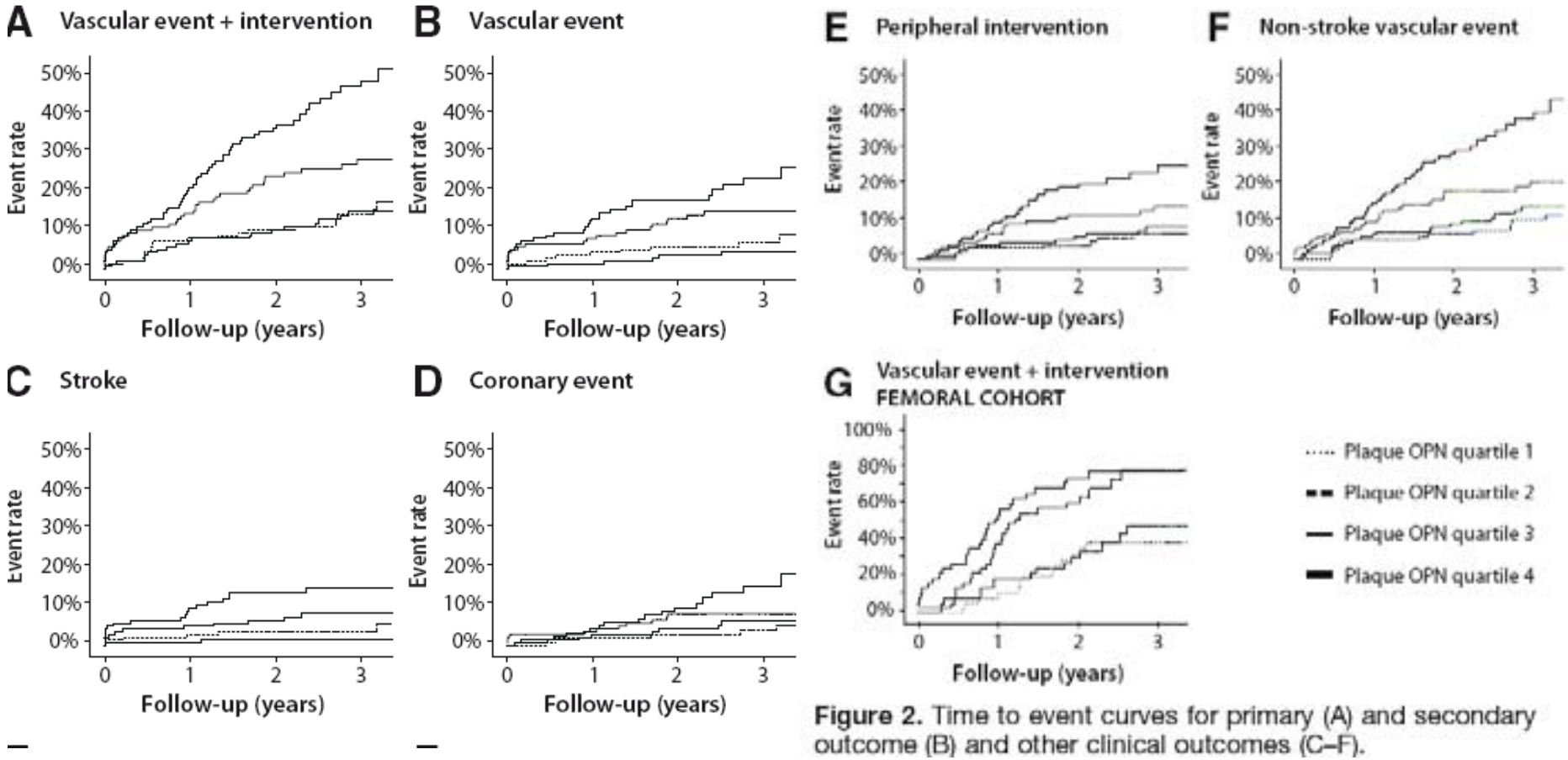


Figure 2. Time to event curves for primary (A) and secondary outcome (B) and other clinical outcomes (C–F).

Table 4. Comparison Plaque and Blood OPN Levels

	OPN in Plaque				OPN in Blood			
Vascular Event and Intervention	N Patients at Risk at Baseline	N Patients With Event	HR (95% CI)	*P*	N Patients at Risk at Baseline	N Patients With Event	HR (95% CI)	*P*
Q1 OPN	143	20	Ref		64	11	Ref	
Q2 OPN	144	20	Ref		77	11	Ref	
Q3 OPN	144	38	2.3 (1.4–3.8)	0.001	76	17	1.4 (0.8–2.7)	0.255
Q4 OPN	143	59	4.0 (2.5–6.5)	<0.001	88	29	2.2 (1.2–3.8)	0.006
Total	574	137			305	68		