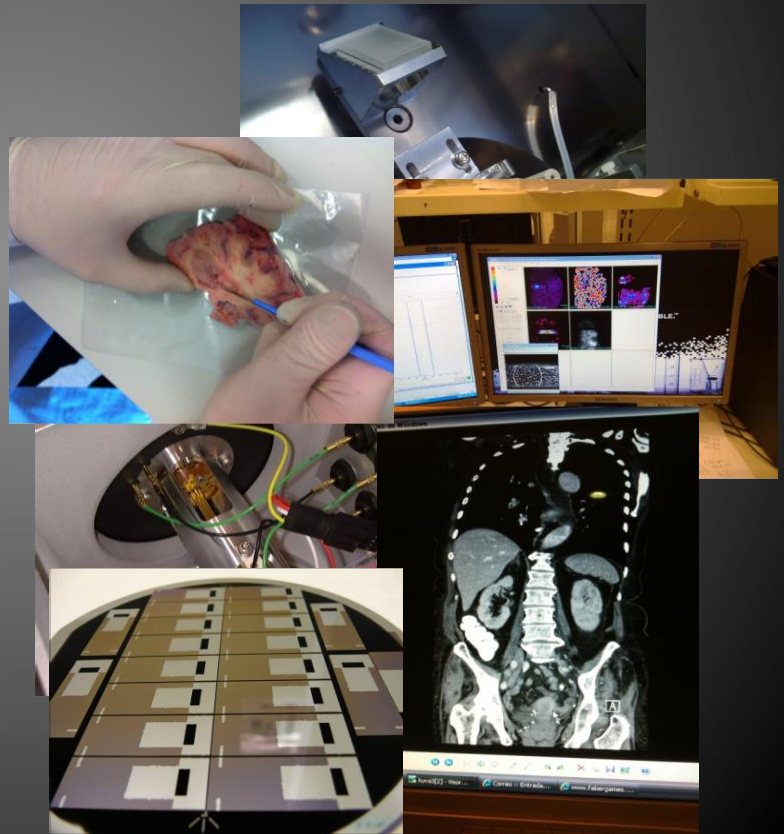


Analytical methods in Proteomics



Otaniemi, May 2012

Meilahti Clinical Proteomics Core Facility

Biomedicum Helsinki and Haartman Institute, 00014
University of Helsinki, Finland

E-Mail: marc.baumann@helsinki.fi

(<http://research.med.helsinki.fi/corefacilities/proteinchem>)

Services

- Proteomic services from Bedside-to-Bench
- High-throughput analysis of Clinical Samples (CSF, Saliva, Urine, Blood, Ascites, Tears etc.)
- Validation of Clinical Samples
- Validation of Clinical Biomarkers

- Basic Proteomics Analyses (1-2D-GE, 1-2D-LC)
- Ion Mobility Structural Analyses

- Array-based epitope mapping (Pep/Prot-Arrays)
- Pep-Array synthesis
- Imaging Mass Spectrometry (IMS) from tissue to cells
- Glycoproteomics
- Glycopeptidomics
- LC-MS/MS2 analysis of carbohydrates
- N-glycopeptide spectra analysis
- Core fucosylation analysis

Services



BIOCENTRUM HELSINKI

BF A member of the Finnish
Proteomics and Metabolomics
technology platform

ProtMet.net

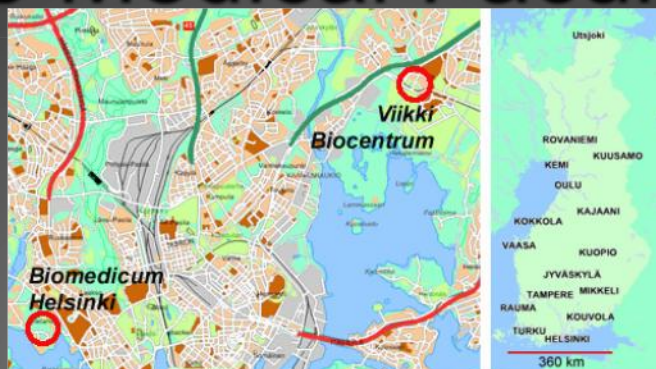


BIOMEDICUM



Over 1200 Researchers in only Medical Research
(Cancer, Genetics, Developmental Medicine, Neuroscience etc.)

The Medical Faculty



Proteomics

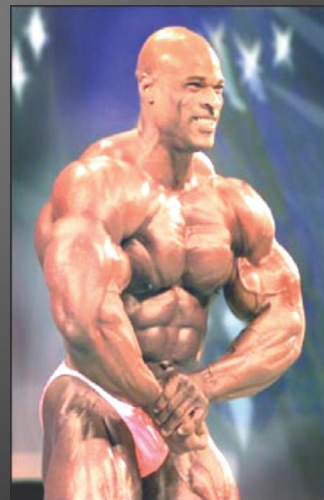
What is it all about??

2001: Human Genome Project Reveals

3,000,000,000 base pair nucleotides
= only about 25,000 genes



=



And that ...

Only 0.1% of each's persons DNA differs from any other person



=



So... what makes the Difference?

= Proteins

The study of the **Proteins**
expressed by a **Genome**

= Proteome



same Genome - different *Proteome*



Proteomic Research today

The main objectives today for clinical and general Proteomics:

- **Quantification** of all the proteins expressed in a cell or tissue proteome, body fluids e.g. blood, CSF etc. Searching for Biomarkers!
- **Functional study** of thousands of proteins in parallel, which protein is in contact to another protein and where? Searching for functionality!

For quantification purposes, standard **method** is 2DE electrophoresis or MudPIT separation followed by MS identification

For protein function studies, microarray based assays are used to study protein-protein and protein-ligand interactions

SEEING GRAVITY WAVES

21ST-CENTURY SLAVERY

SCIENTIFIC AMERICAN

APRIL 2002 \$4.95
WWW.SAONLINE.COM

Proteomics

Biotech's
Next Big
Challenge

PLUS:
Virtual Captions
for the Real World
Fighting Dad Death



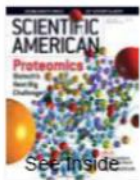
SEEING GRAVITY WAVES

21ST-CENTURY SLAVERY

APRIL 2002 \$4.95

SCIENTIFIC AMERICAN™

Permanent Address: <http://www.scientificamerican.com/article.cfm?id=proteins-rule>



Proteins Rule

Biotech's latest mantra is "proteomics," as it focuses on how dynamic networks of human proteins control cells and tissues

By Carol Ezzell | Wednesday, April 24, 2002 | 0

Move over, human genome, your day in the spotlight is coming to a

close. Researchers are now concentrating

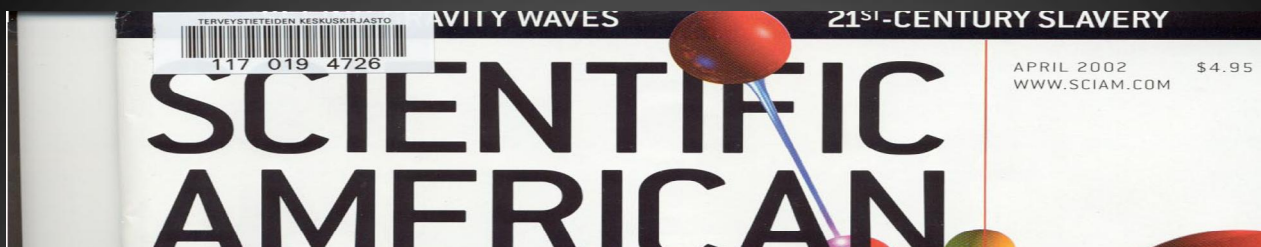
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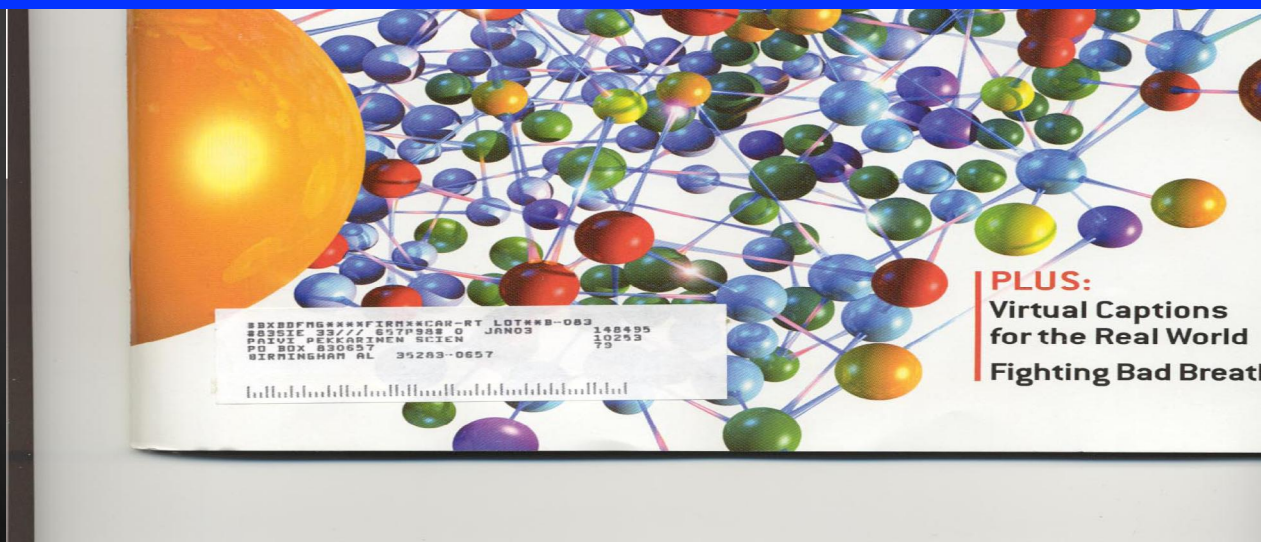
for the Real World
Fighting Bad Breath



The Post-Genome Project

Whether The Human Proteome Will Be Successfully Mapped In Three Years Depends On How You Define "Proteome"

By [Karen Hopkin](#) | August 17, 2001 | [0](#)



Vol. 14 No. 3

www.ddmag.com

Advantage
Business Media

DRUG

DISCOVERY & DEVELOPMENT

STRATEGIES & TECHNOLOGIES DRIVING DRUG DISCOVERY TO MARKET

A Long Way to the Bedside

Despite many
breakthroughs
Proteomics has not
translated yet to
patient care



■ March 2011

■ Policy and Projections
ESCAPE STRATEGY

■ Assay Development
METABOLOMICS

■ Drug Safety
FMT IMAGING

■ Informatics
DATA VISUALIZATION

■ How It Works
CLINICAL TRIAL DATA



MPI Research...
Your responsive
CRO partner.

See next page
for more information

How can we make Proteomics more suitable to the “real” life?

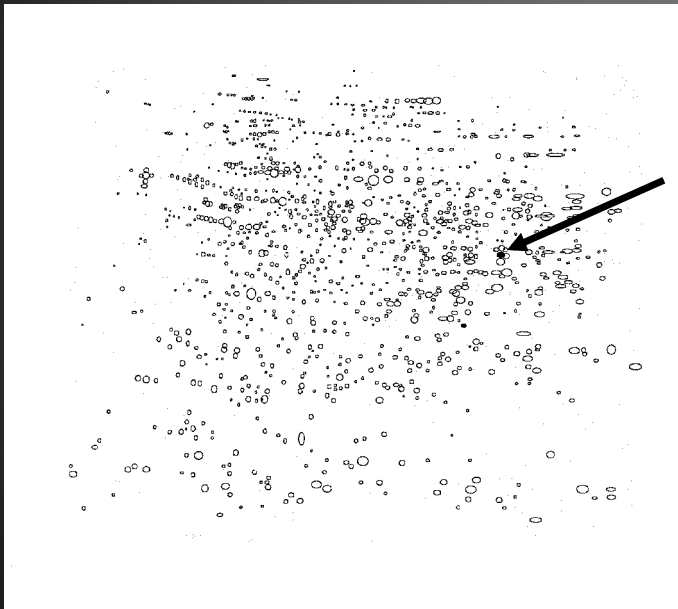
The technology...

2D gel electrophoresis

2D liquid chromatography

Micro arrays

We need a technology to find changes in Proteome



A protein Array

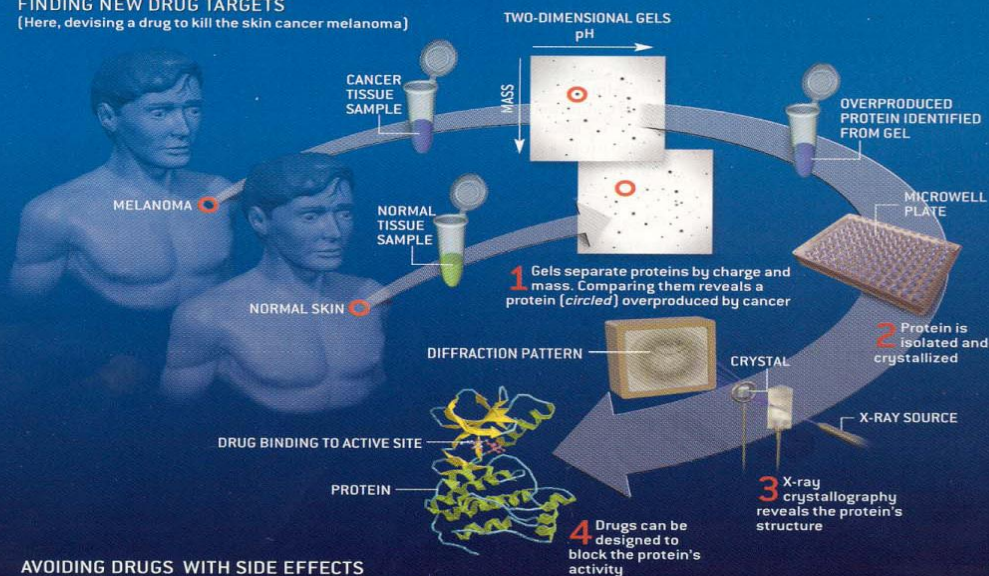
Administration of a drug known to bind to an orphan receptor

- Changes in expression level of 23 proteins

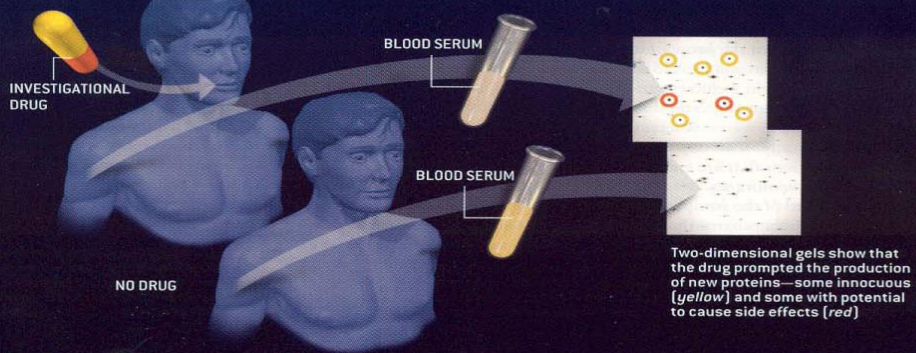
An example

HOW PROTEOMICS CAN HELP DRUG DEVELOPMENT

FINDING NEW DRUG TARGETS (Here, devising a drug to kill the skin cancer melanoma)



AVOIDING DRUGS WITH SIDE EFFECTS (Here, determining whether an investigational drug prompts production of possibly harmful proteins)

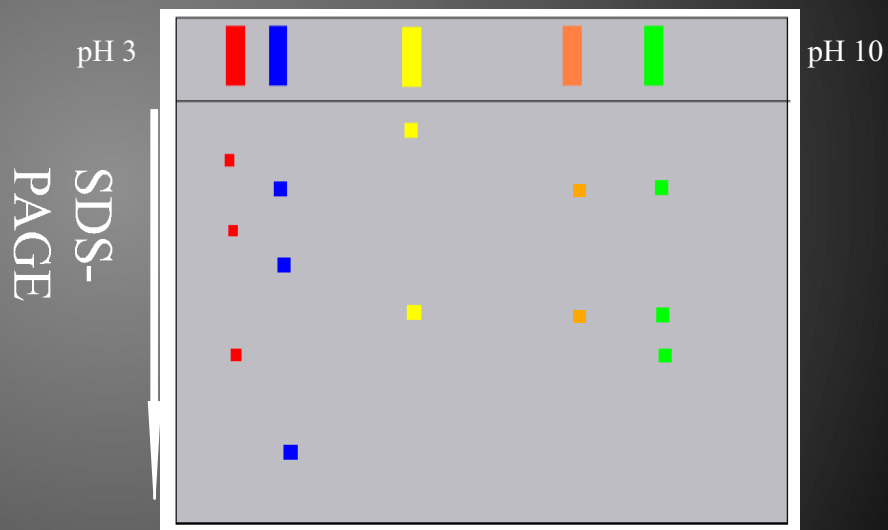


IN FILMS (preceding pages)

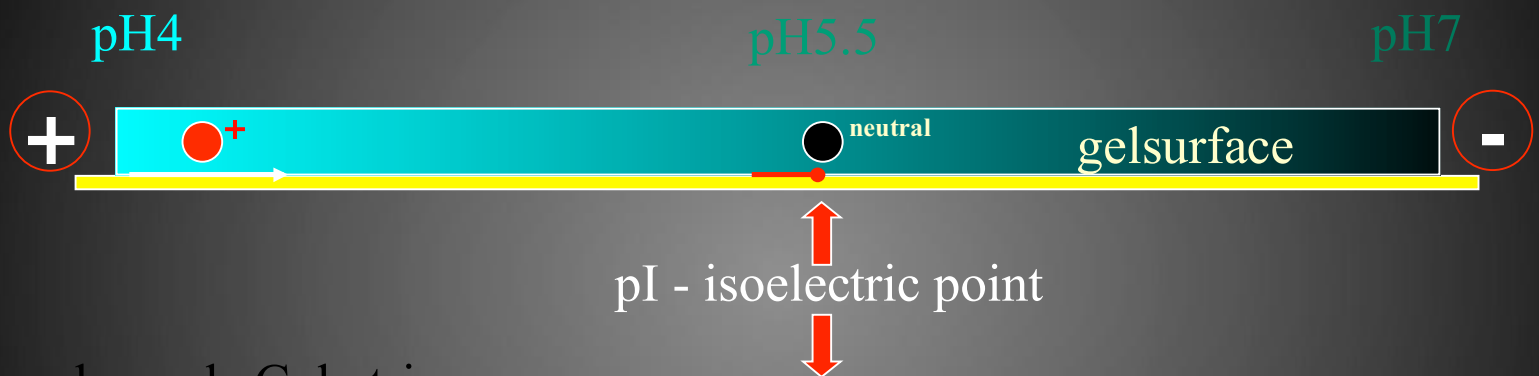
IN FILMS: PROTEIN/DRUG STRUCTURE COUR

Two-dimensional gel electrophoresis (2D) could do it?

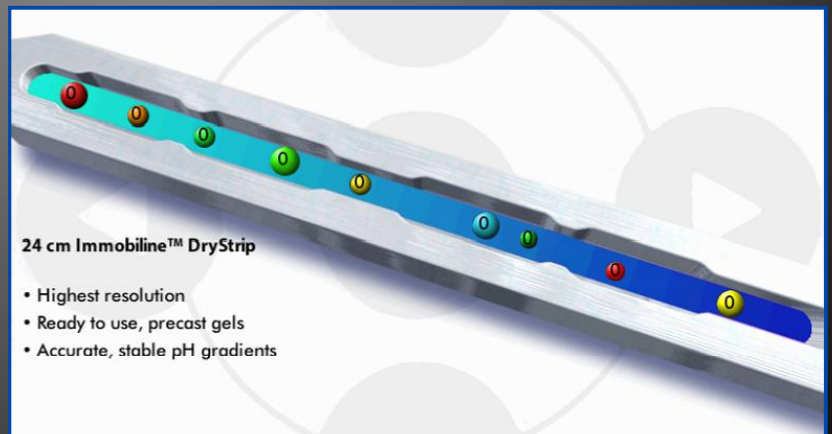
- 1st dimension, IEF, Proteins are separated according to their isoelectric point (IP)
- 2nd dimension, SDS-PAGE, Proteins are separated according to their molecular mass
- Efficient: More than a thousand proteins resolved in E-Coli cell lysates and ~8000 in brain lysates



1st Dimension - Isoelectric Focusing



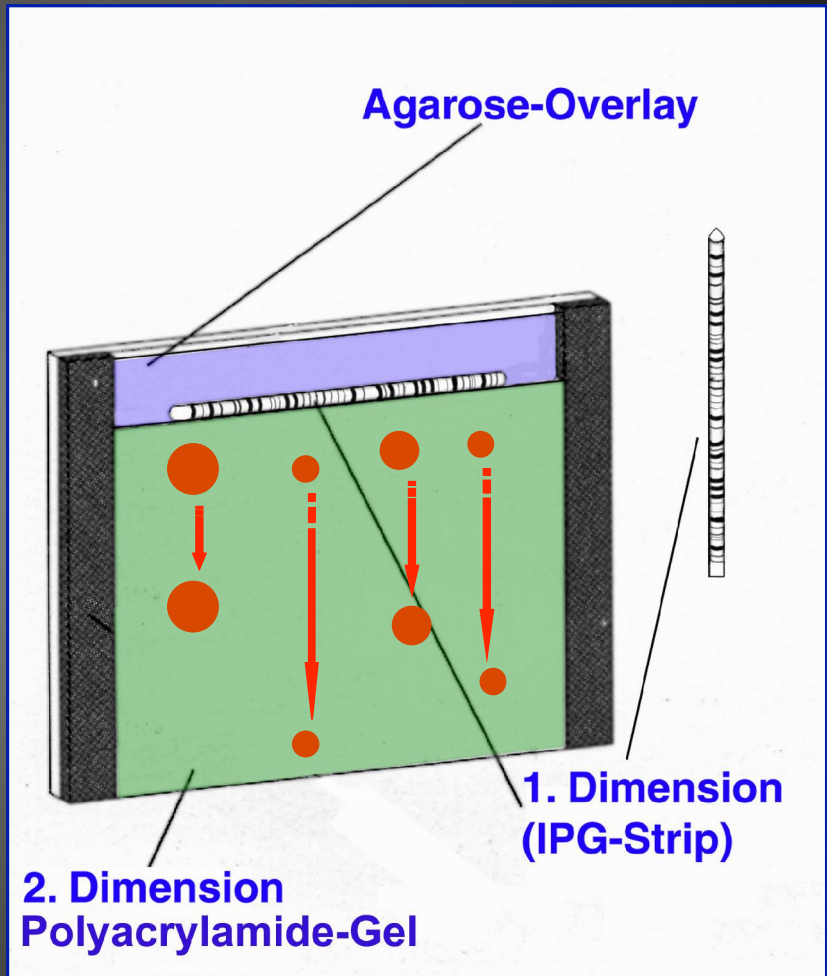
ready made Gel-strips



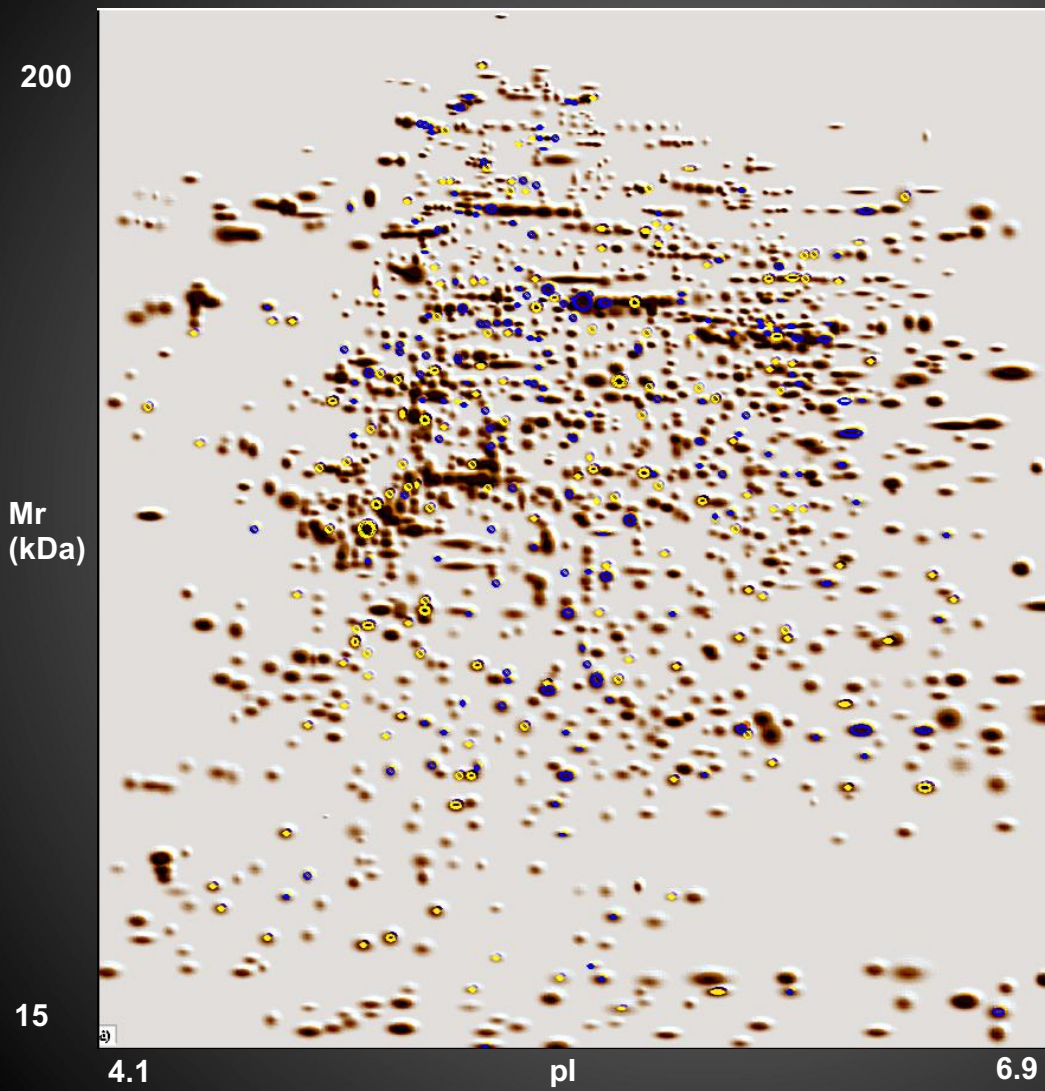
2nd Dimension - Isoelectric Focusing

2DE

M_w



Protein Fingerprint: 2-DE



About 2000
proteins

CyDye DIGE Fluor dyes

Minimal labelling dyes

- Label 50 μg of protein
- 3 colors: CyTM2, Cy3, Cy5
- MW matched ($\sim 450\text{Da}$)
- Charge matched (positive)
- Label ϵ -amino group of lysine
- Sensitivity - 0.025 ng
- Linear dynamic range over 4 orders of magnitude



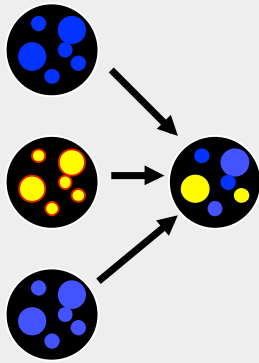
Achieving accurate quantitative data

Ettan™ DIGE system

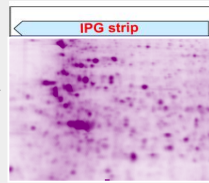
Pooled internal
standard
label with Cy™2

Protein extract 1
label with Cy3

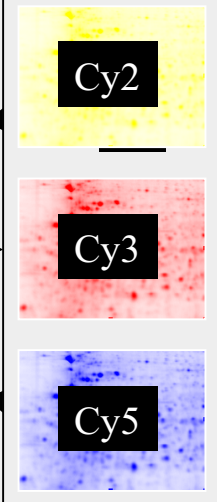
Protein extract 2
label with Cy5



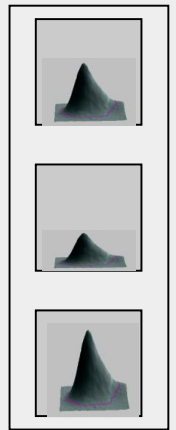
Mix labelled
extracts



2-DE
separation

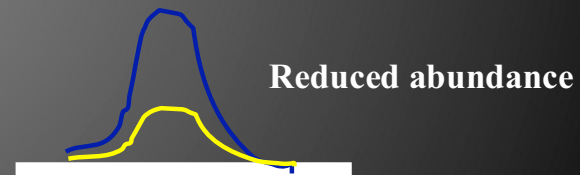
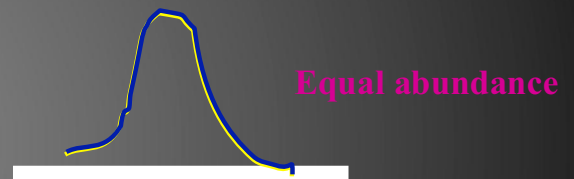
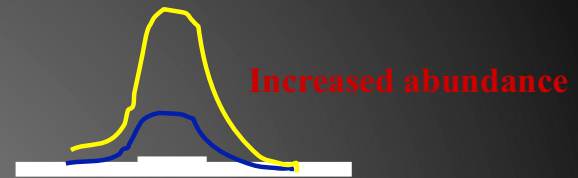
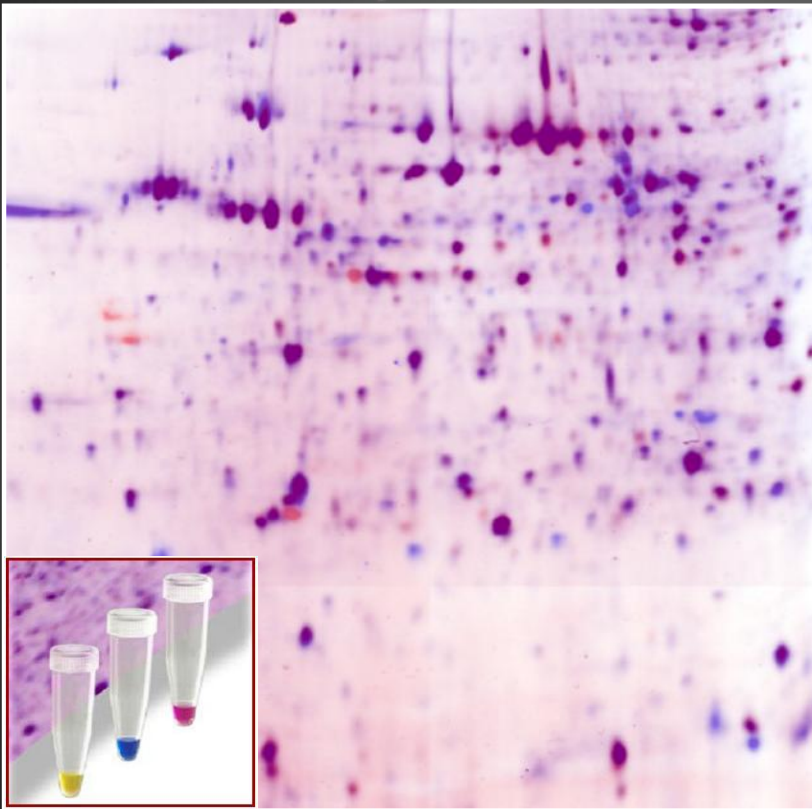


Typhoon™
Variable Mode
Imager



DeCyder™
Differential
Analysis
Software

Overlay of normal and patient protein samples



Normal control = CyTM3 labelled - Blue

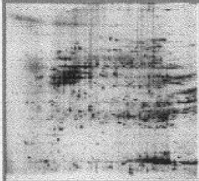
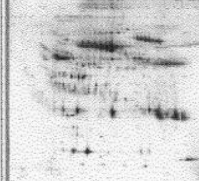
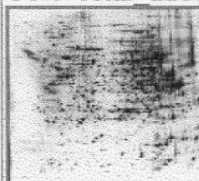

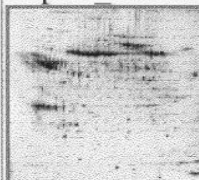
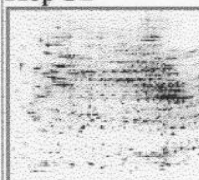
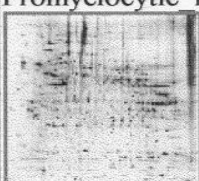
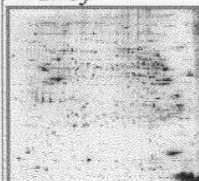
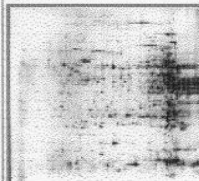
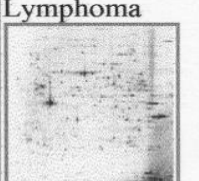
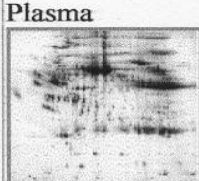
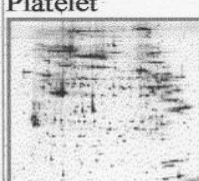
Patient A sample = Cy5 Labelled - Red

EttanTM DiGE
the quantitative approach to do Proteomics



2D Databases exist!

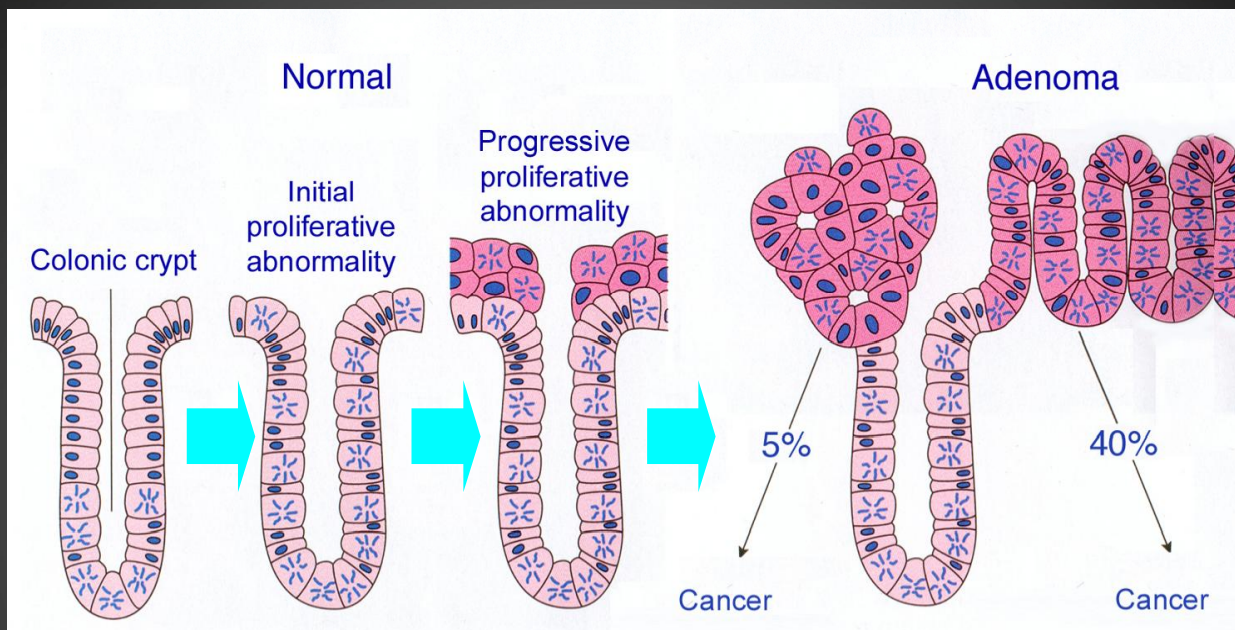
HUMAN
Homo sapiens

Colorectal epithelia_cells 	Cerebrospinal Fluid 	Colorectal adenocarcinoma_c 
Erythroleukemia_Cell 	HepG2 Secreted Proteins 	HepG2 
Promyelocytic leukemia_cells 	Kidney 	Liver 
Lymphoma 	Plasma 	Platelet 

The simple way to test “real”
Proteomics?

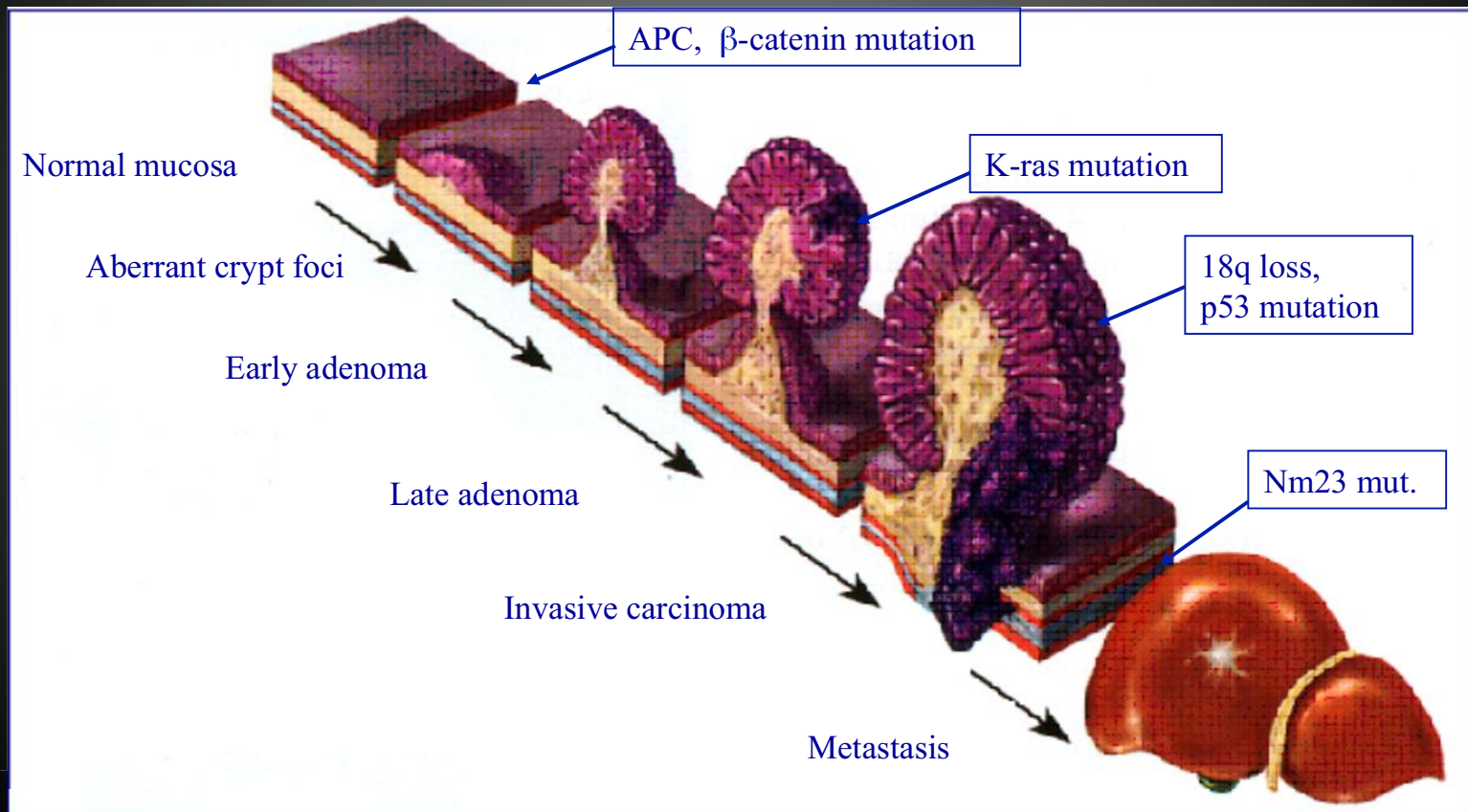
An example from the clinics...

Colorectal Crypt - Physiology and Transformation

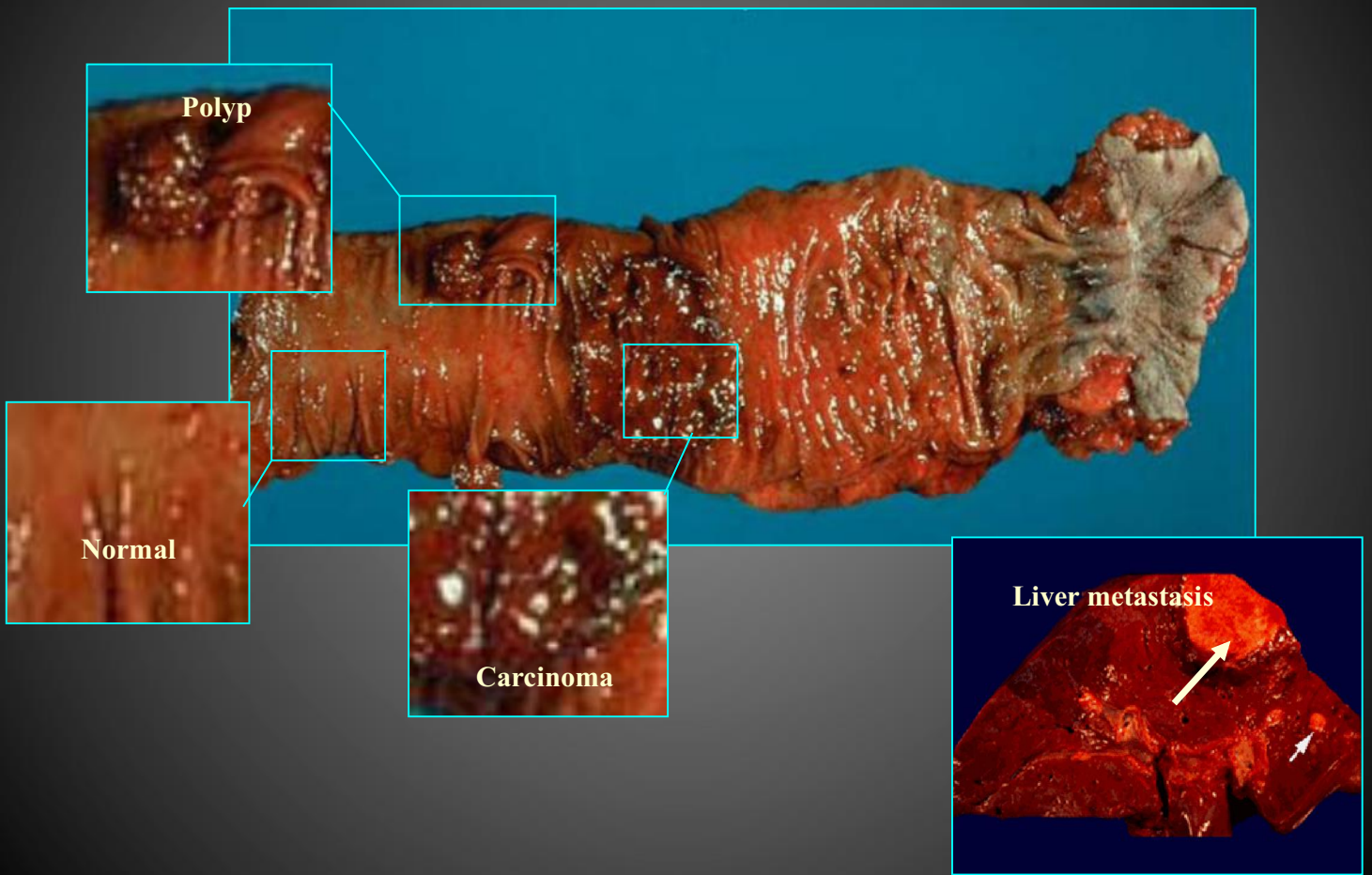


- Stem cells are located in the lower crypt poles, colonocytes are migrating to the surface of the crypt
- Continually replacement in the toxic and mechanically stressing environment of the gut
- Hyperproliferation is believed to precede adenoma formation
- Loss of APC function leads to deregulated crypt homeostasis and is thought to be the initiating event in adenoma formation

Correlation of Histological Progression and Molecular Changes in Colorectal Cancer

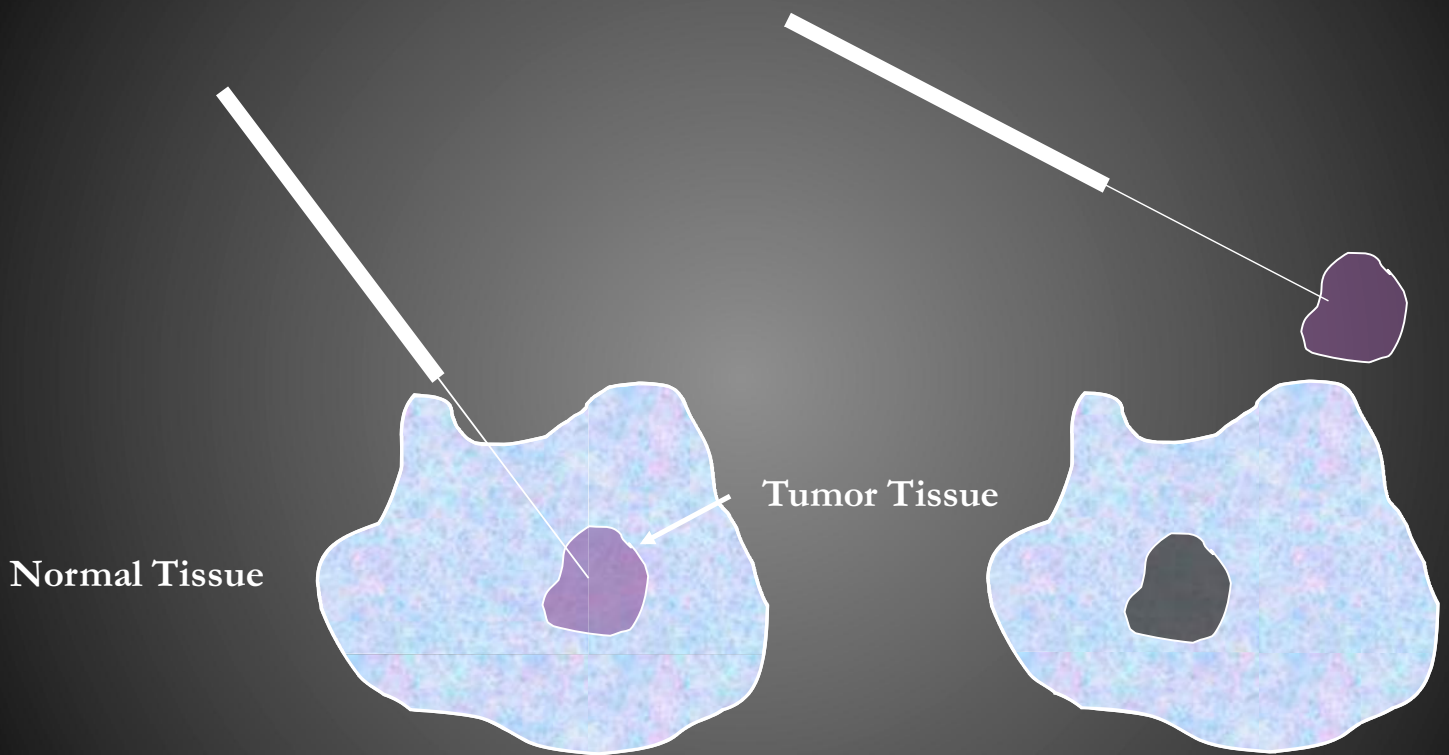


Samples



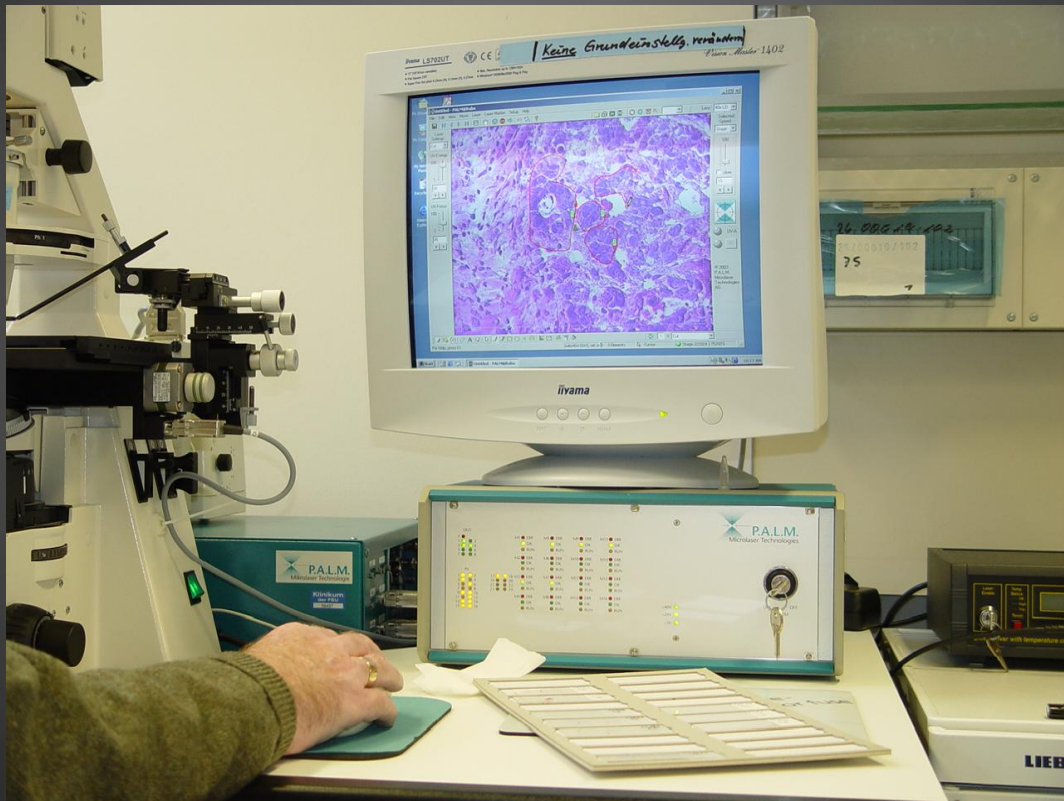
Tissue microdissection

Why Tissue Microdissection?



You would like to isolate only the targeted diseased tissue

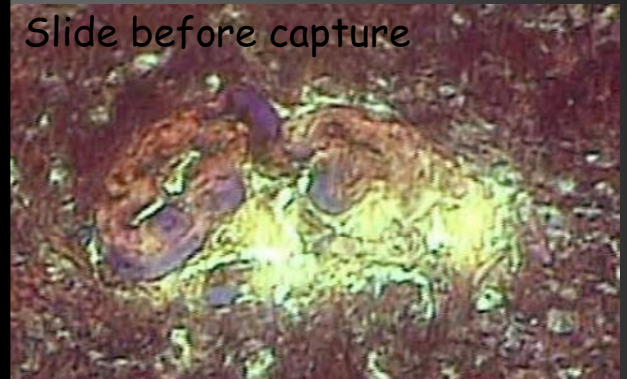
Laser-based microdissection:



Laser Capture Microdissection (Arcturus Autopix)

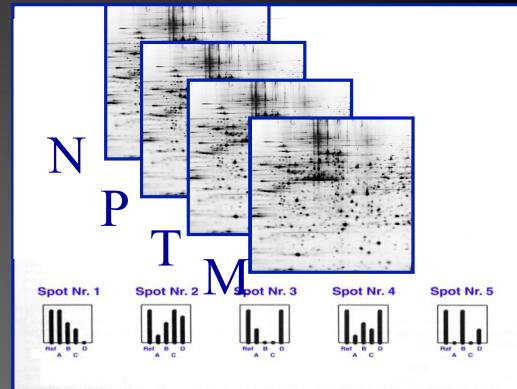
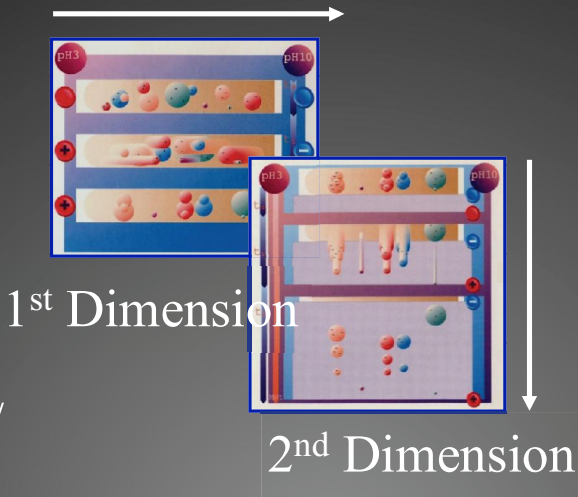
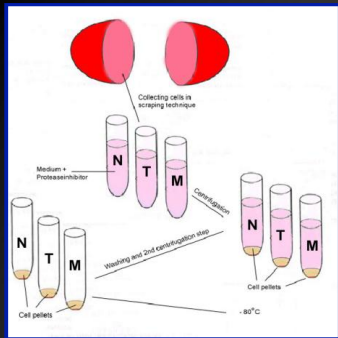


Capturing of wanted tissue section to the slide



MALDI MS analysis directly from the tissue captured on the cap membrane

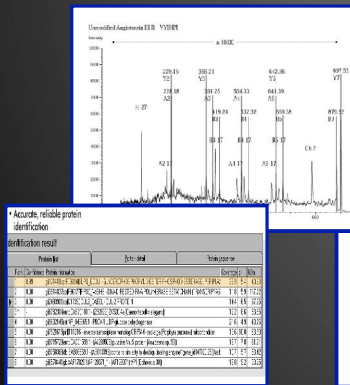




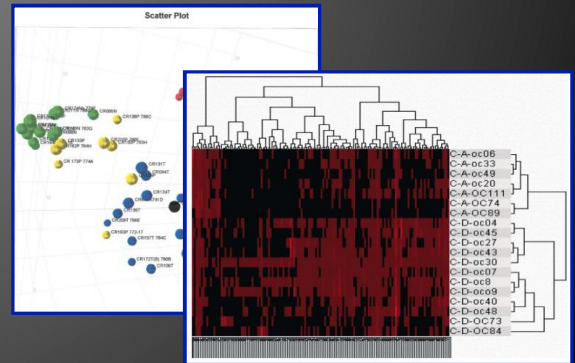
Sample enrichment/
preparation

PC based matching

2-DE Algorithm



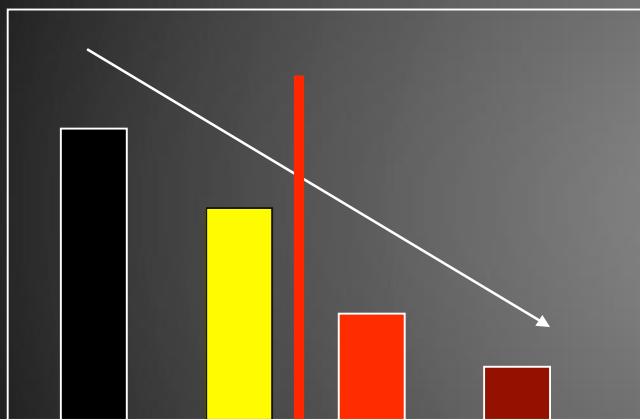
Mass spectrometry identification



Statistics

Deviating Proteins (n=112) Identification of 72

N=26

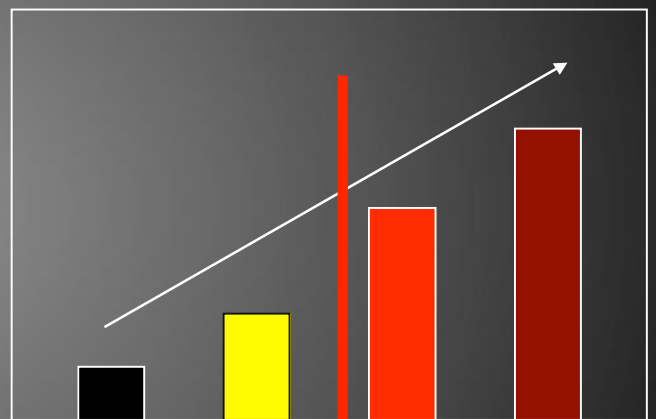


N P T M
benign maligne

Controls:

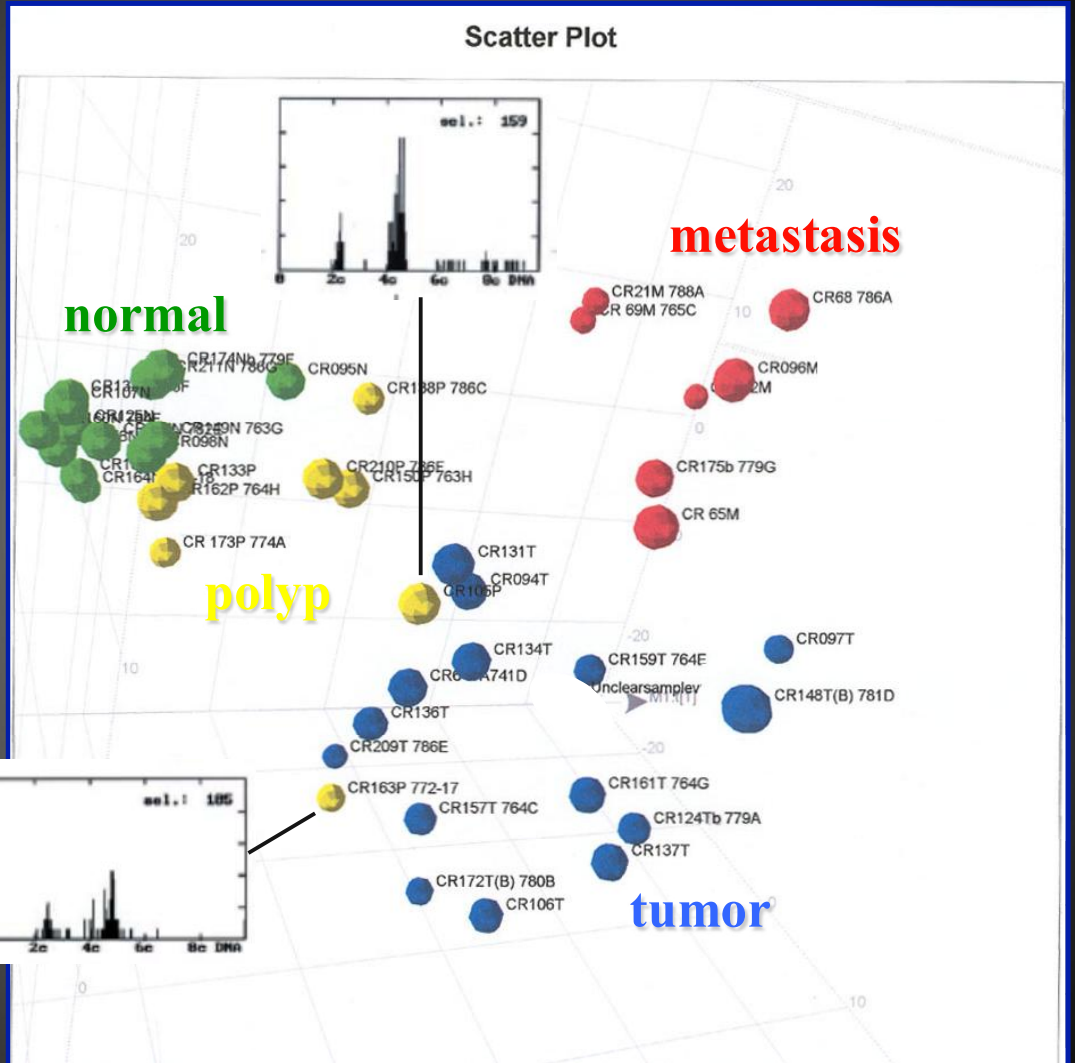
normal liver tissue
HCT116 and Lovo cell lines

N=46

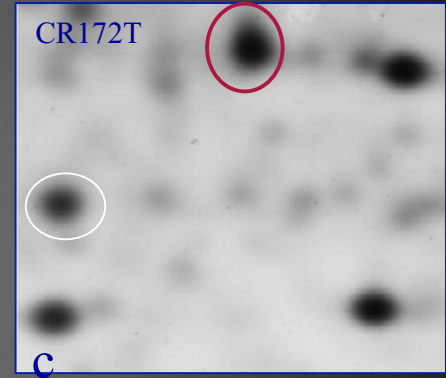
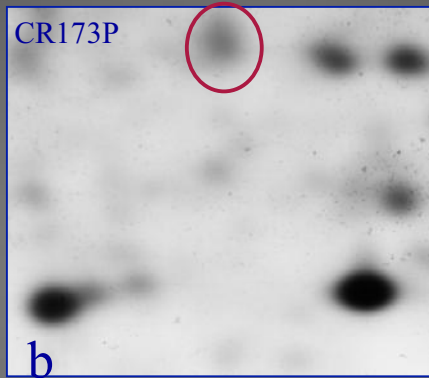
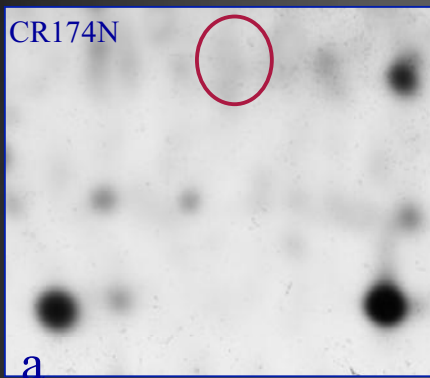


N P T M
benign maligne

Principal Component Analysis (t1,t2,t3)

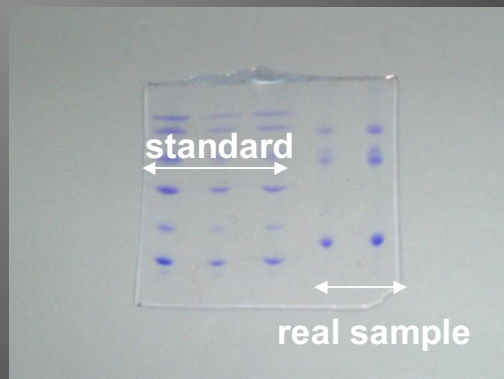
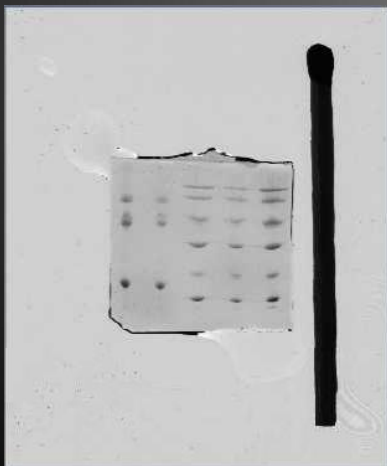
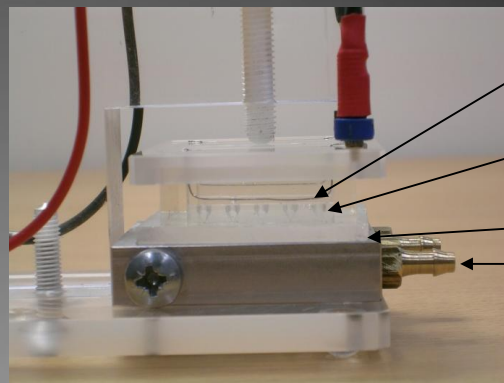


*Intra-individual expression differences
of Cytokeratin 20 in patient 14.*

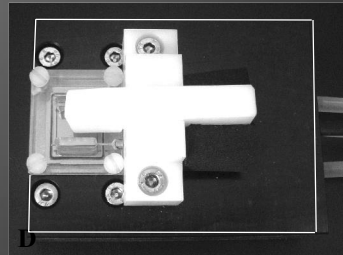
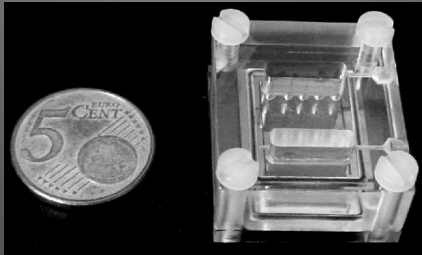
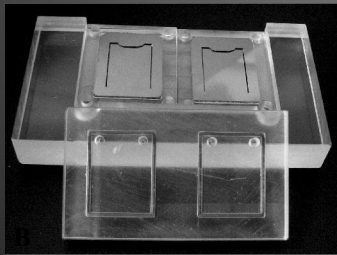
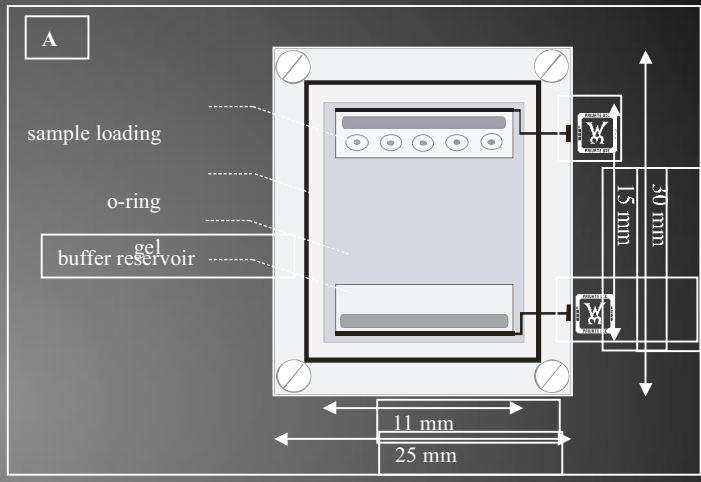
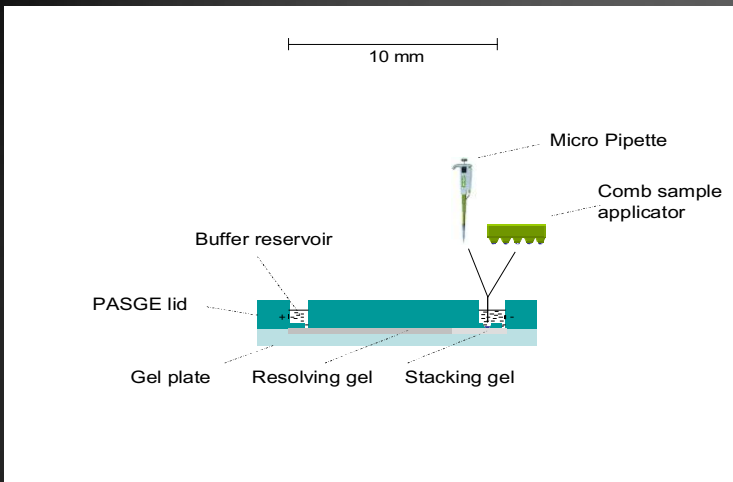


The left gel segment (a) is zoomed from the normal mucosa,
gel b represents the patients polyp and
gel c is the corresponding segment of the same patients
adenocarcinoma

Micro gel devices

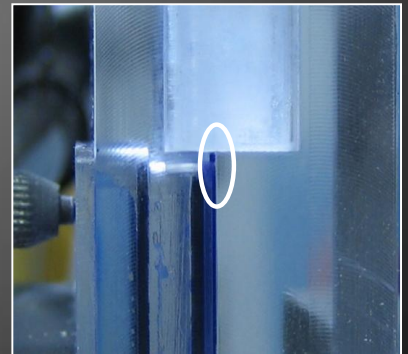
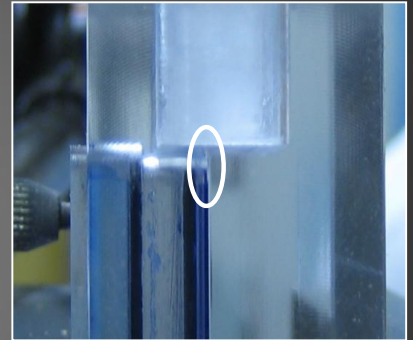
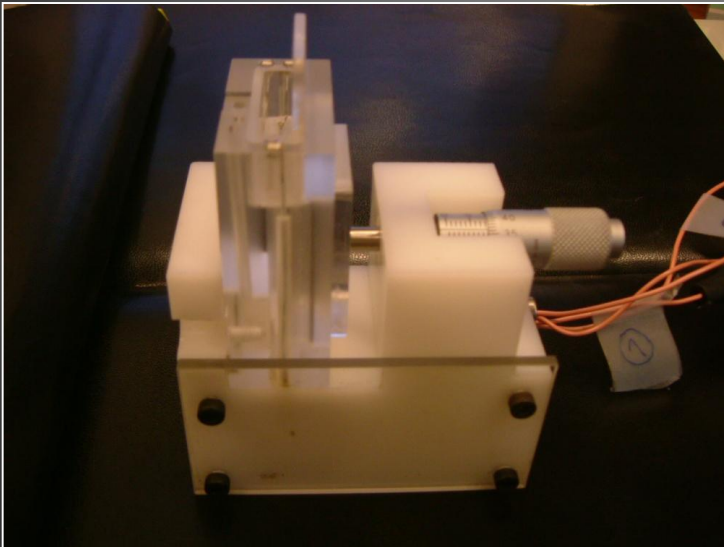


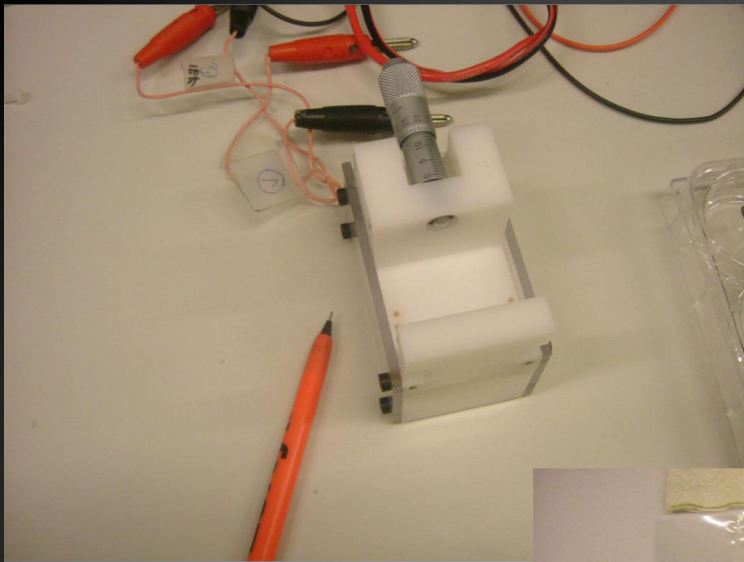
Running time 10 minutes



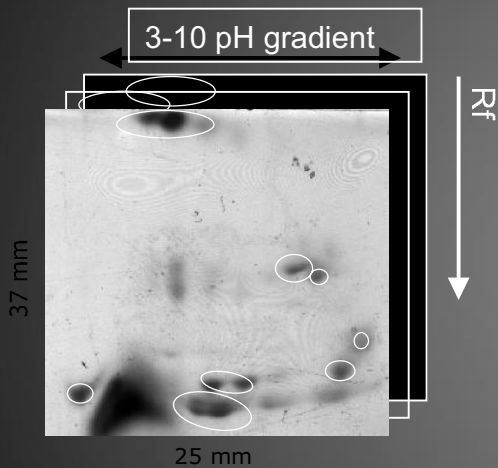
Automated 2D devices

The compress system





2-D map of IEF standards



Repeatability of 2-DE runs

Rf values (%) [*]		pI position errors(%) [*]	
STDV	6,1	STDV	2,5
max	15	max	6
min	0,8	min	0,6

^{*} comparison of 3 gels

- 2-DE separation completed in **approx. 80 min**
- Limit of detection is **approx. 65 ng**

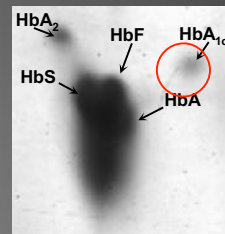
Running time 20-30 minutes

Examples for the Clinics

- Performance

- Native IEF and native PAGE

- 5 variants of hemoglobin
- pH 6.7 -7.7



- Native IEF and SDS-PAGE

- standard IEF proteins
- pH 3-10



- Denatured IEF and SDS-PAGE

- GFAP protein variants expression differences
- in control and Alzheimer diseased patients
- pH 4-6

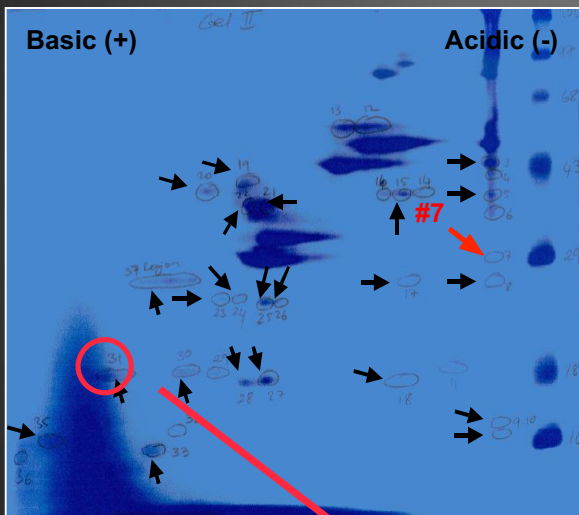


control



AD

2D-PAGE Direct In-situ Digest



100 fmol

running the gel

- staining each spot of interest (→)

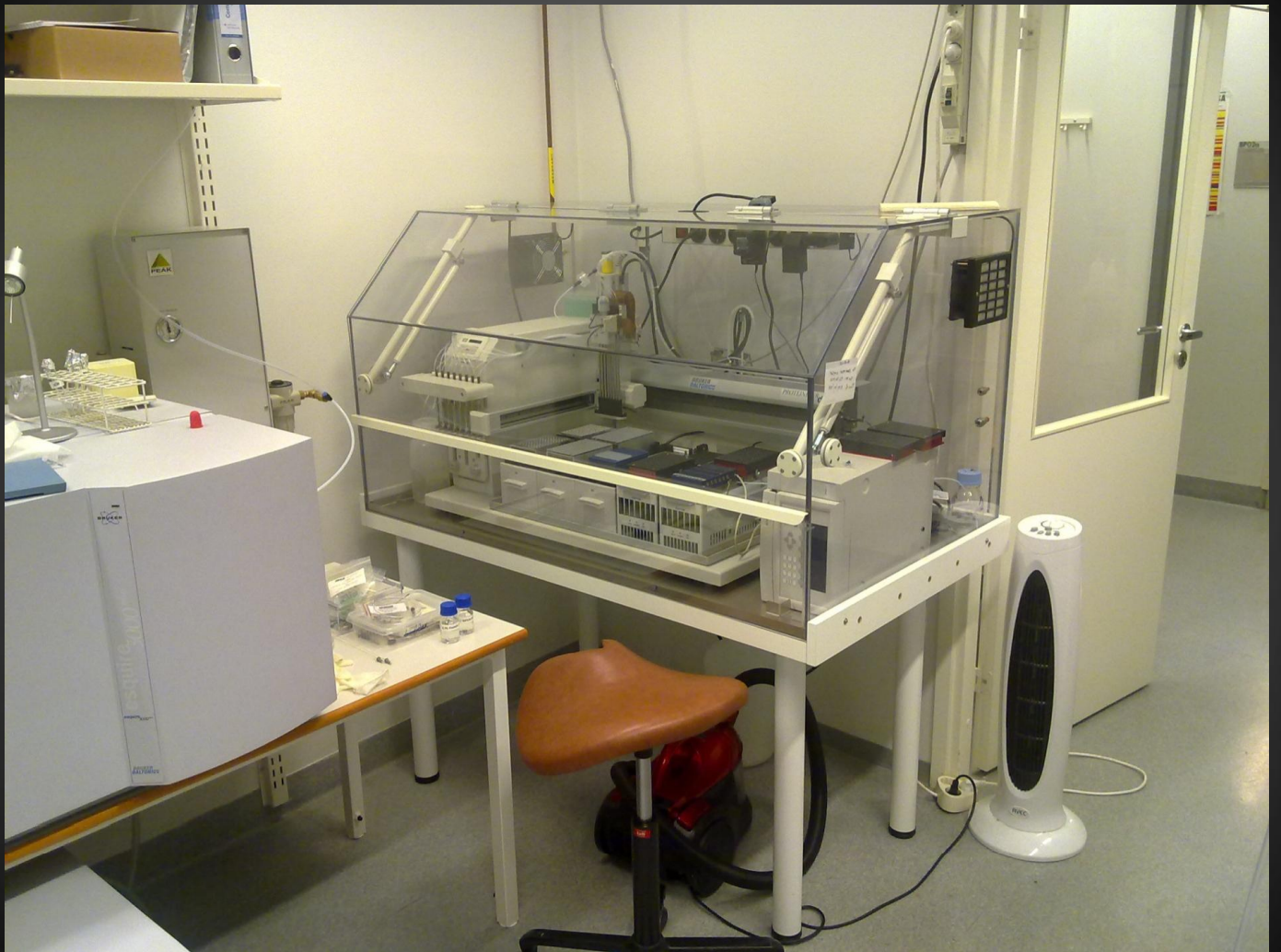
- excise

- *in-gel* digestion



PEPTIDE ANALYSIS

Trypsin digest




Brucker Daltonik GmbH - Microsoft Internet Explorer

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map II

The MALDI AutoPrep Robot System


Automated MALDI sample preparation of robot systems - including the washing or purification with Millipore 8-channel liquid handler.

MAP II and MAP II/8 use disposable plates. Samples can be placed in capped plate formats are supported and MALDI target.

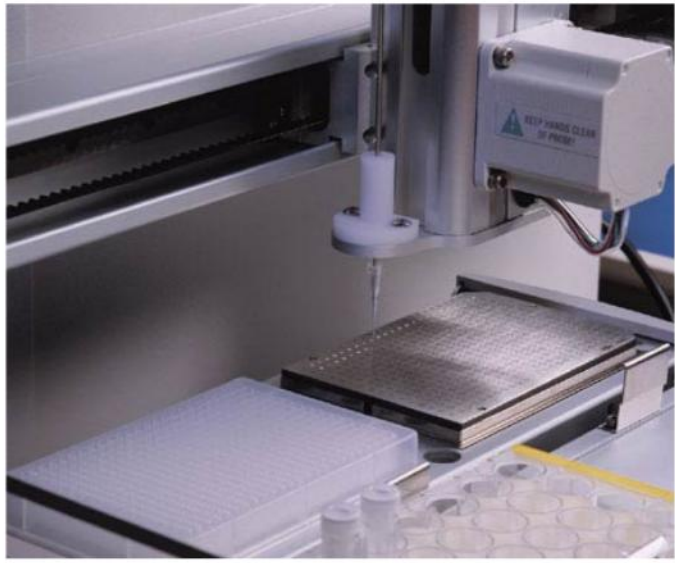
One single sample spreadsheet controls sample preparation, measurement control and the FLEX III MALDI-Ti guiding the path from sample preparation to measurement.

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<http://www.bruker-daltonik.de/images/mapii5.jpg> - Microsoft Internet Explorer



BRUKER DALTONICS
Enabling Life Science Tools Based On Mass Spectrometry™

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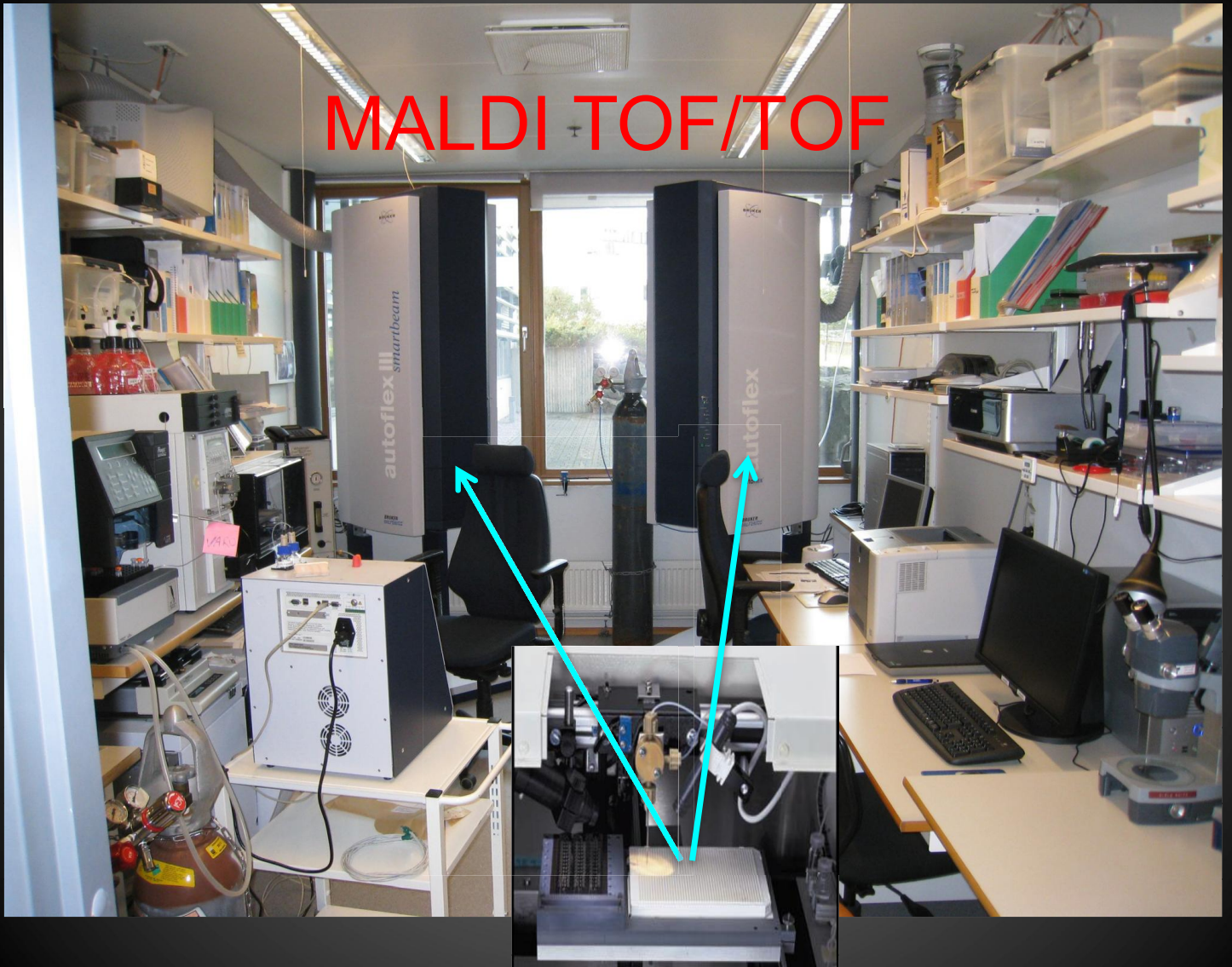
Brucker Daltonik GmbH - Mi... USB Bridge Cable Applicat... Microsoft PowerPoint - [P... <http://www.bruker-da...> 5:17 PM

Prespotted AnchorChip targets dedicated for Clinical Proteomics

- > no cross contamination
- > no memory effects
- > Easy storing and archiving of targets
- > Re-visiting of samples
- > One calibrant, four matrix spots for samples
- > 10-100 fold increased sensitivity, in situ sample purification



MALDI TOF/TOF



High Sample Throughput for the Post-Genomic Era



MALDI-TOF-MS



The TWISTER

Typical mass spectrum from a protein band trypsin digest

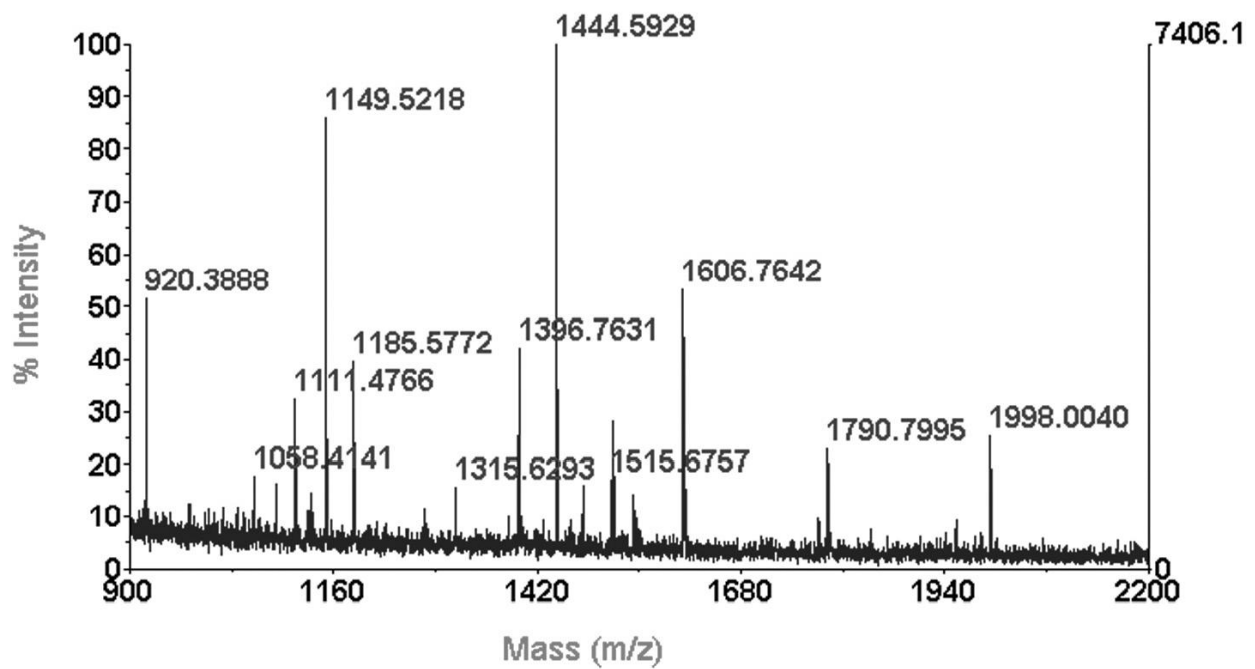
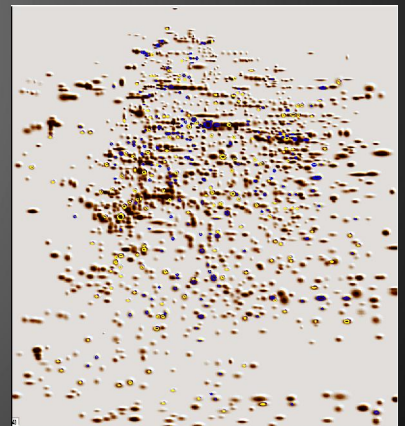


Fig. 5a

Automated Database Search (MASCOT)

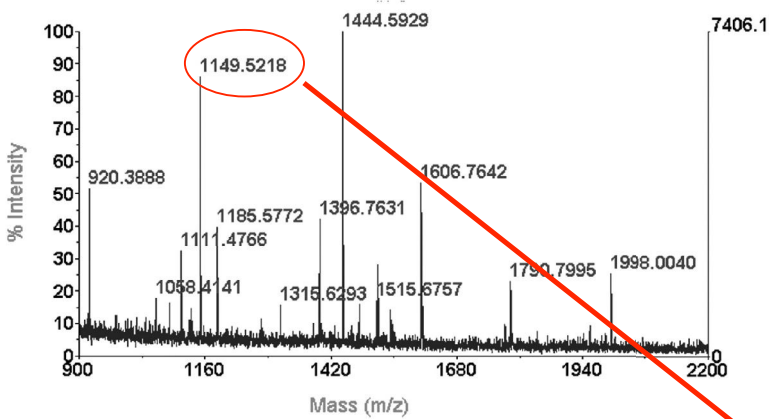
Number 1 match: tumor necrosis factor type 1 receptor associated protein TRAP-1 (Mr): 76030.27

1	RALRRAPALA	AVPGGKPILC	PRR FTAQLGP	RNPAPWSLQA	GRLFSTQTAE
51	D KEEPLHSII	SSTESVOGST	SK HEFQAETK	KLLD IVARSL	YSEK EVFIRE
101	LISNASDALE	KLRHKLVS	DG QALPEMEIHL	QTNAEKGTIT	IQDTGIGMTQ
151	EELVSNLGTI	ARSGSK AFID	ALQNQAEASS	KIIGQ FGVGF	YSAFMVADRV
201	EVYSRSAAPG	SLGYQWLS	DG SGVFEIAEAS	GVRTGTKIII	HLKSDCKEFS
251	SEARVRDVVT	KYSNFVSFPL	YLNRRMNTL	QAIWMDPKD	VGEWQHEEFY
301	RYVAQAHDKP	RYTLHYK TDA	PLNIR SIFYV	PDMKPSMFDV	S RELGSSVAL
351	YSR KVLIQTK	ATDILPKWLR	FIRGVVDS	ED IPLNLSR ELL	QESALIR KLR
401	DVLQQR LIKF	FIDQSK KDAE	KYAK FFEDYG	LFMREGIVTA	TEQEVKEDIA
451	KLLRYESSAL	PSGQLTSLSE	YASR MRAGTR	NIYYLCAPNR	HLAEHSPYYE
501	AMK KKDTEVL	FCFEQFDELT	LLHLREFDKK	KLISVETDIV	VDHYKEEKFE
551	DRSPA A ECLS	EK E TEELMAW	MRNVLGSRVT	NVKVTLR LDT	HPAMVTVLEM
601	GAARHFLRMQ	QLAK TQEERA	QLLQPTLEIN	PRHALIKKLN	HCAQASLAWL
651	SCWWIRYTRT	P			



Total coverage: **33.4%**

Even a single peptide is enough
for Protein identification!

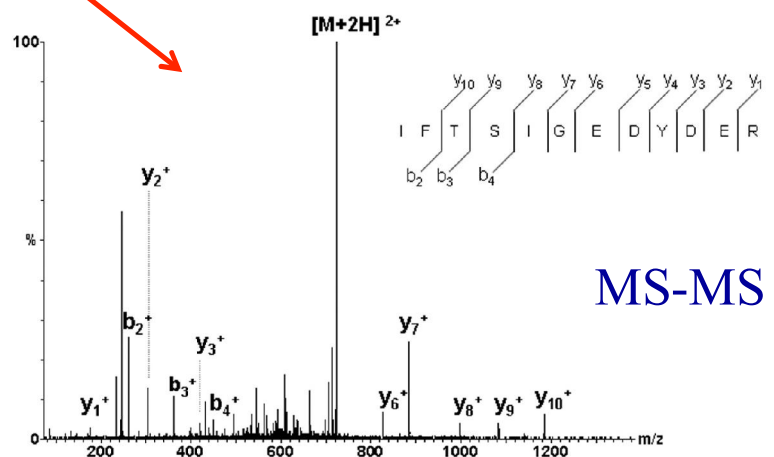


MALDI MS TOF/TOF or
Quadrupole MS TOF/TOF

Fragmentation of a single peptide in
the Mass Spectrometer

>>>> Database search

>>>> Identification of the whole protein



MS-MS

Automated Database Search (MASCOT)

Number 1 match: tumor necrosis factor type 1 receptor associated protein TRAP-1 (Mr): 76030.27

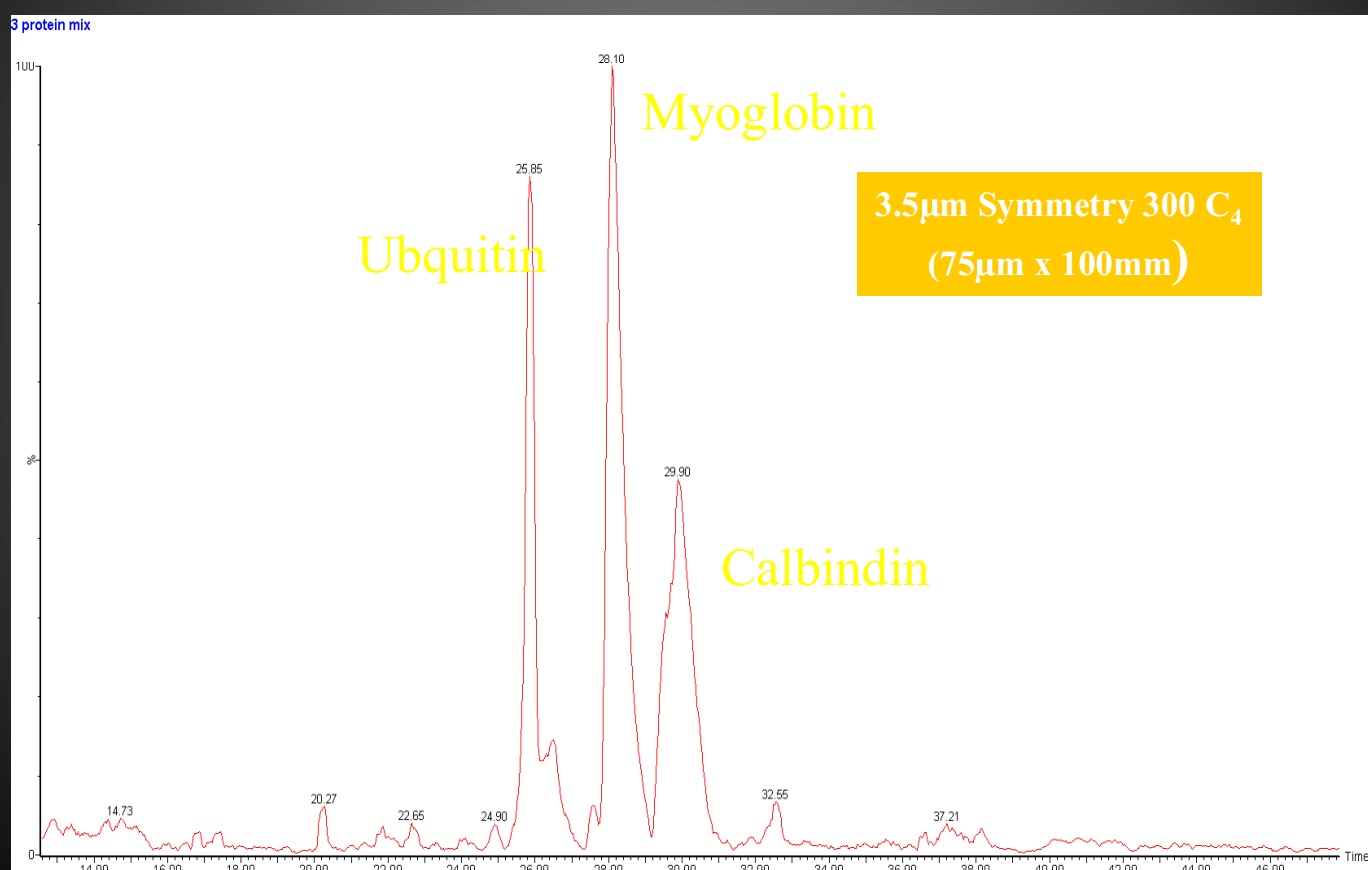
1	RALRRAPALA	AVPGGKPLLC	PRRTTAQLGP	RRNPAWSLQA	GRLFSTQTAE
51	DKEEPLHSII	SSTESVQGST	SKHEFQAETK	KLLDIVARSL	YSEKEVFIRE
101	LIISNASDALE	KLRHKLVS DG	QALPEMEIHL	QTNAEKGTIT	IQDTGIGMTQ
151	EELVSNLGTI	ARSGSKAFLD	ALQNQAEASS	HIIGQFGVGF	YSAFMVADRV
201	EVYSRSAAPG	SLGYQWLS DG	SGVFEIAEAS	GVRTGTKIII	HLKSDCKEFS
251	SEARVRDVVT	KYSNFVSFPL	YLNRRMNTL	QAIWMDPKD	VGEWQH EEFY
301	RYVAQAHDKP	RYTLHYKTDA	PLNIRSIFYV	PDMKPSMFDV	SRELGS SVAL
351	YSRKVLIQTK	ATDILPKWLR	FIRGVV DSED	IPLNLSRELL	QESALIRKLR
401	DVLQQRLIKF	FIDQSKKDAE	KYAKFFEDYG	LFMREGIVTA	TEQEVKEDIA
451	KLLRYESSAL	PSGQLTSLSE	YASRM RAGTR	NIYYLCAPNR	HLAEHSPYYE
501	AMKKKDTEVL	FCFEQFDEL T	LLHLREFDKK	KLISVETDIV	VDHYKEEKFE
551	DRSPAAECLS	EKETEELMAW	MRNVLGS RVT	NVKVTLRLDT	HPAMVTVLEM
601	GAARHFLRMQ	QLAKTQEERA	QLLOPTLEIN	PRHALIKKLN	HCAQASLAWL
651	SCWWIRYTRT	P			

Sequence successfully identified

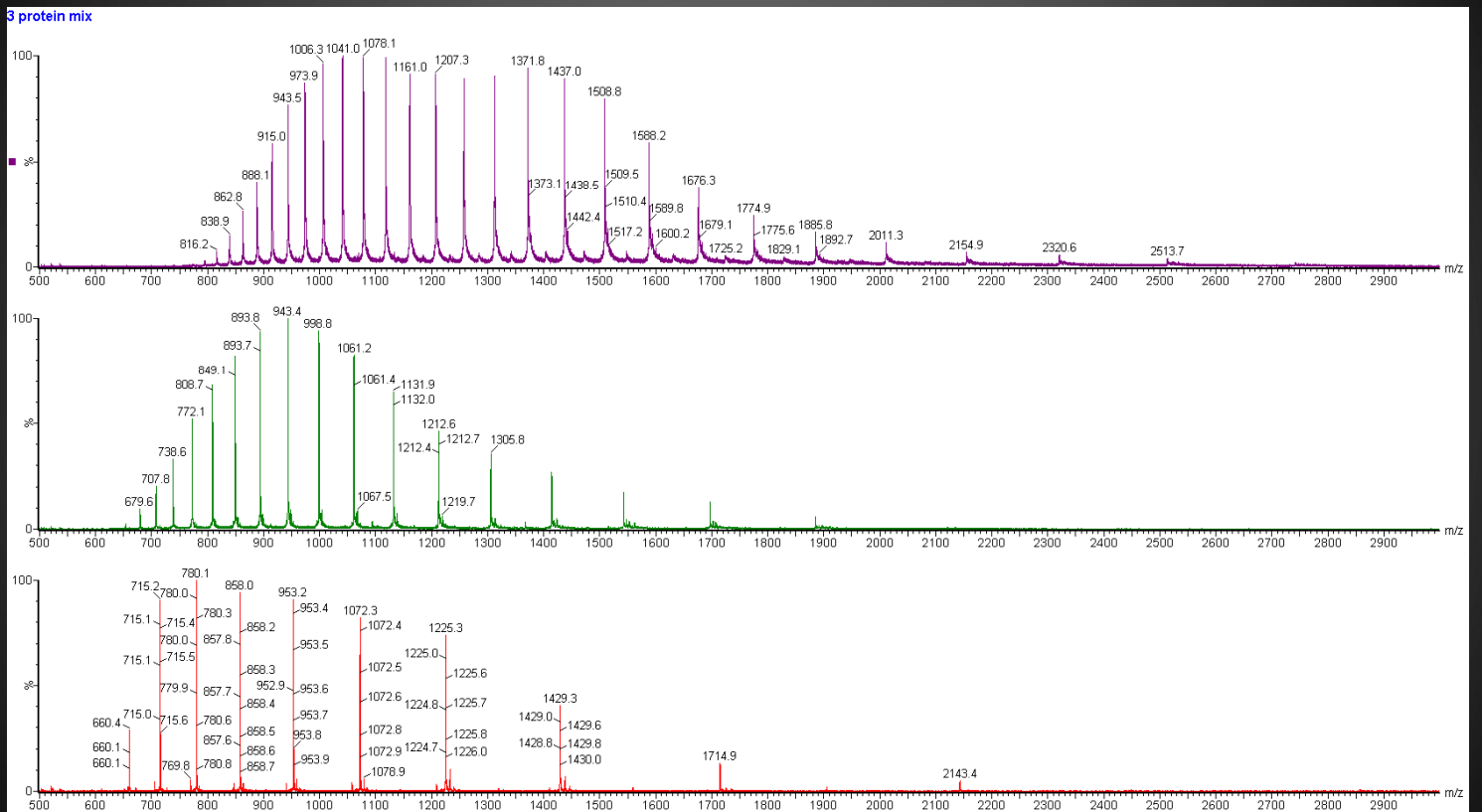
Applications

Top-Down UPLC-ETD sequencing of Proteins

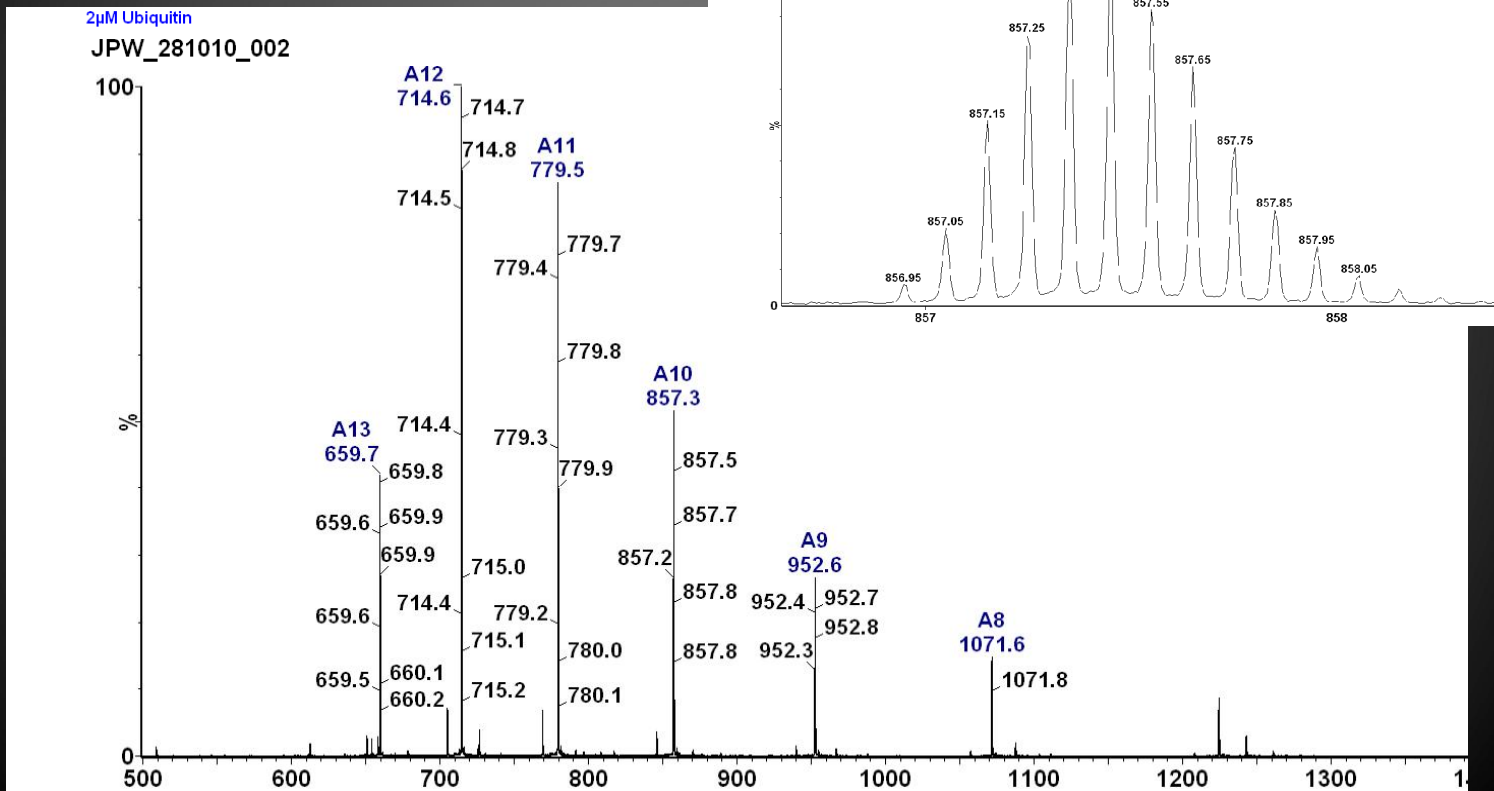
3 Protein Mixture: Ubiquitin ($5\mu\text{M}$), Myoglobin ($10\mu\text{M}$) and Calbindin ($20\mu\text{M}$)



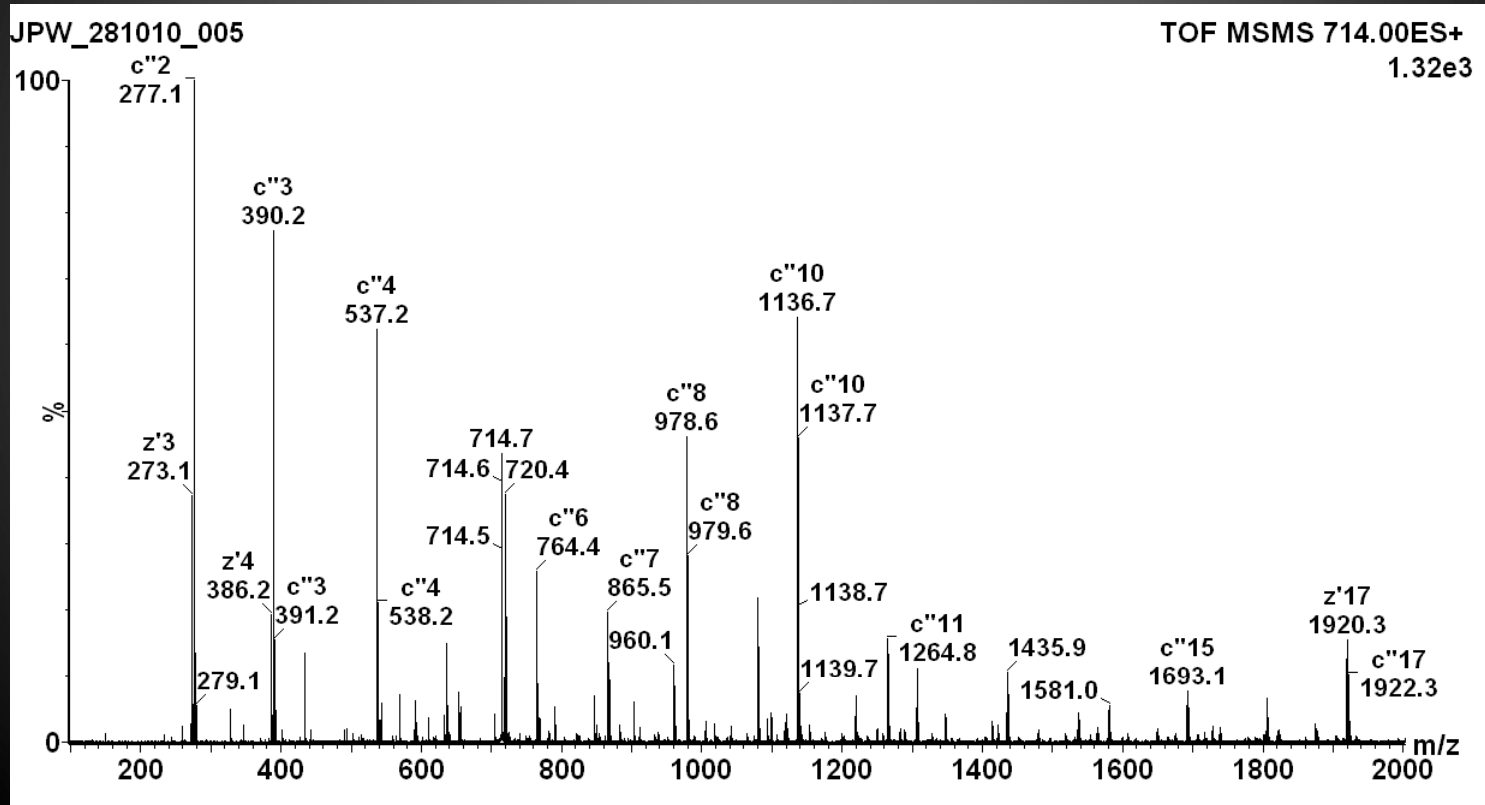
Normal Protein MS



Ubiquitin (5 μ M) MS



Intact Tandem MS of Bovine Ubiquitin ETD of m/z 714, $[M+12H]^{12+}$



UBIQ_BOVIN

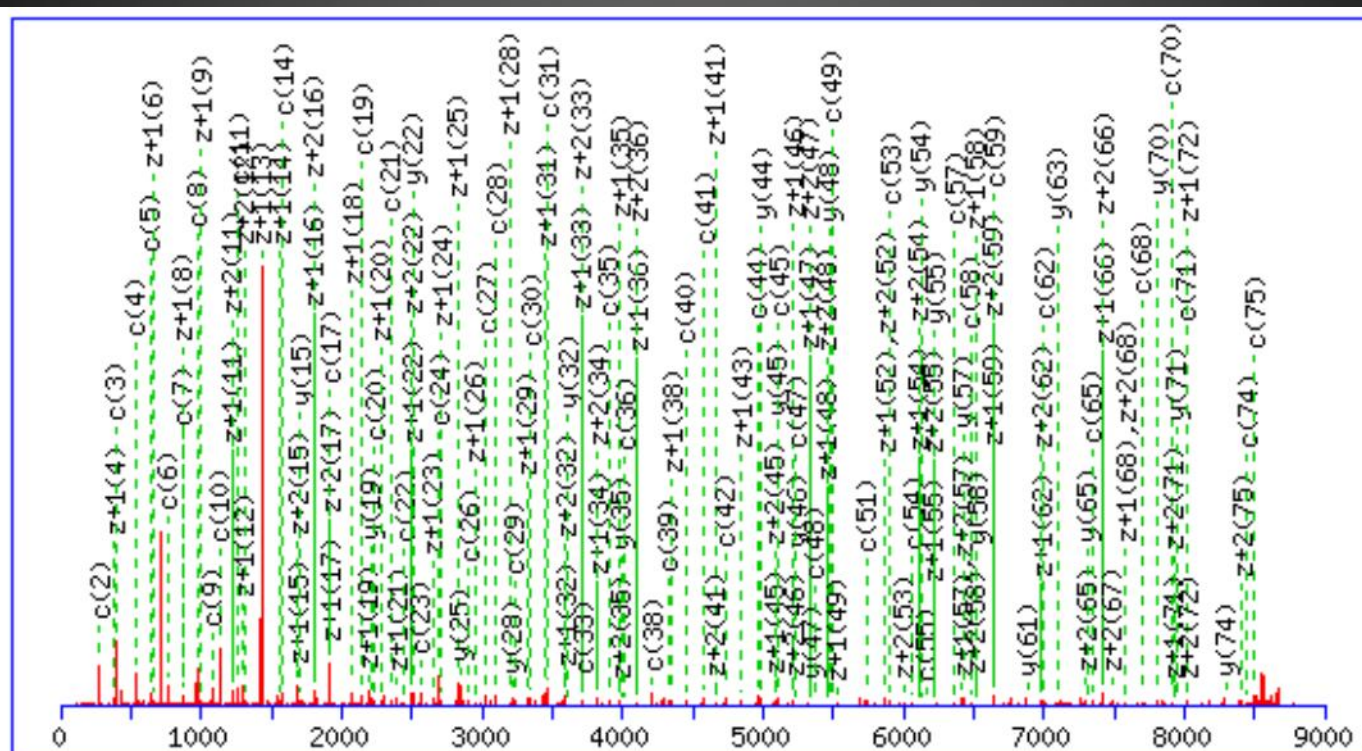
Mass: 8560

Score: 181

Queries matched: 1

Ubiquitin - Bos taurus (Bovine)

Check to include this hit in error tolerant search or archive report



Monoisotopic mass of neutral peptide Mr(calc): 8559.6167

Ions Score: 181 Expect: 6.6e-14

Matches (Bold Red): 151/300 fragment ions using 1893 most intense peaks