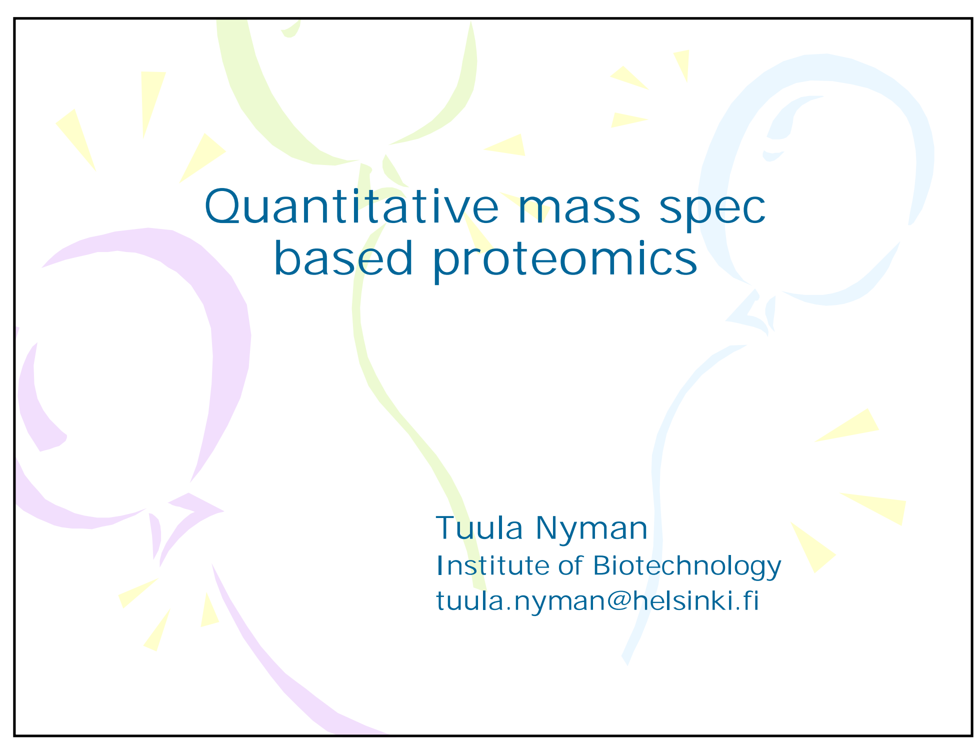
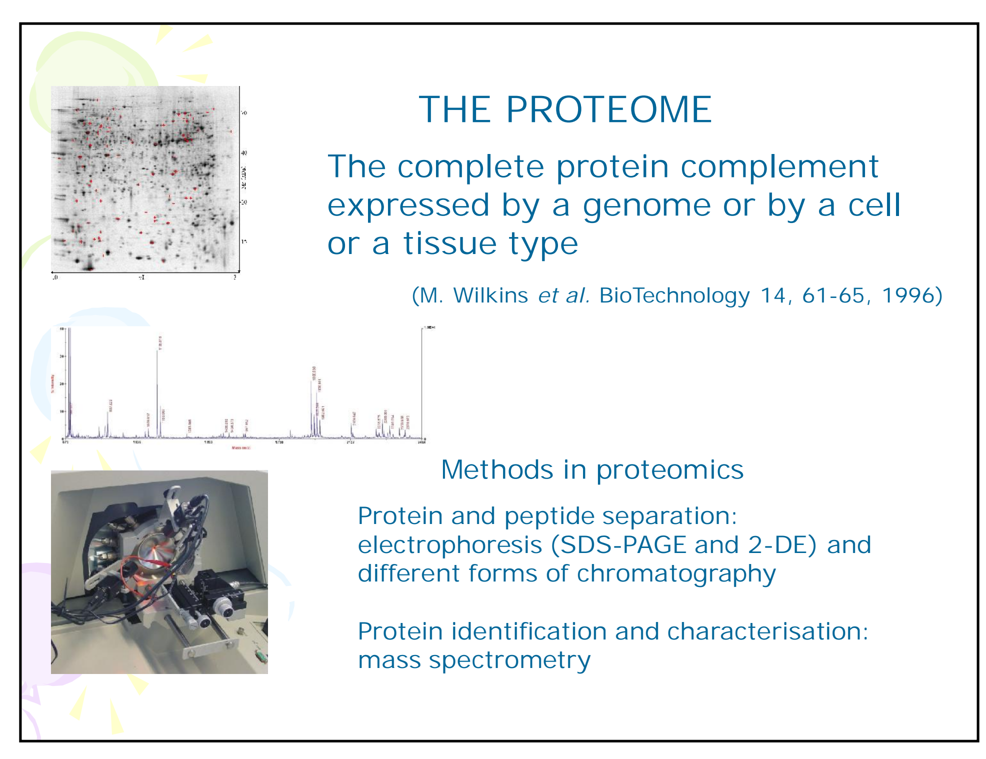

Quantitative mass spec
based proteomics
Tuula Nyman
Institute of Biotechnology
tuula.nyman@helsinki.fi


THE PROTEOME
The complete protein complement expressed by a genome or by a cell or a tissue type
(M. Wilkins et al. BioTechnology 14, 61-65, 1996)
Methods in proteomics
Protein and peptide separation: electrophoresis (SDS-PAGE and 2-DE) and different forms of chromatography
Protein identification and characterisation: mass spectrometry

Proteomics levels

Expression proteomics

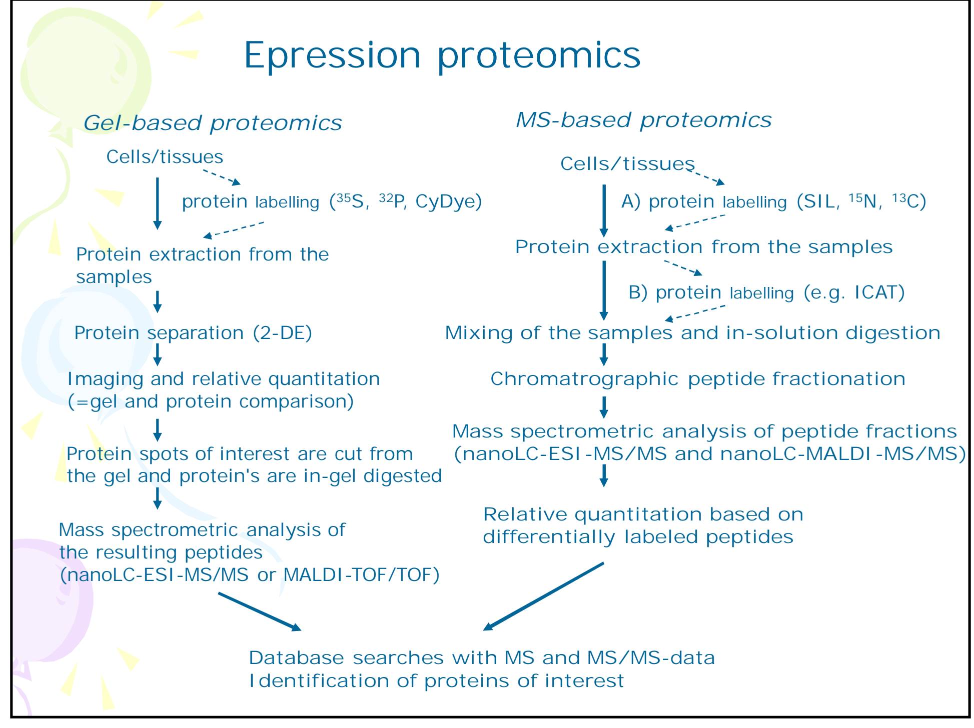
Which gene products are expressed, when and how much

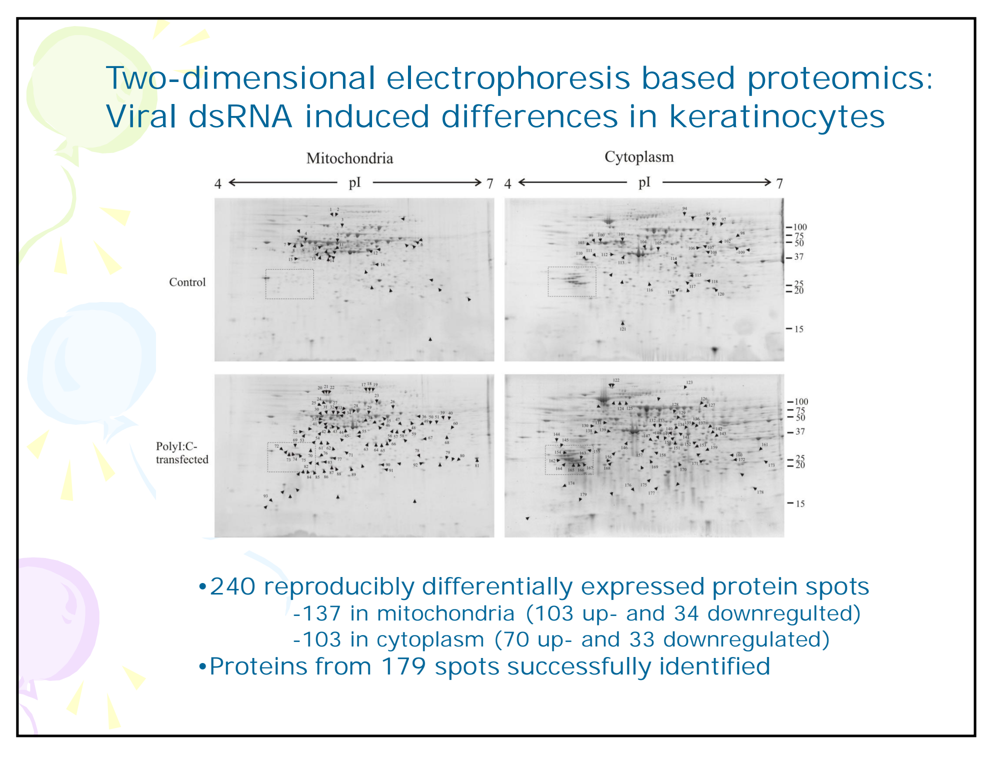
PTM-omics, "Modificomics"

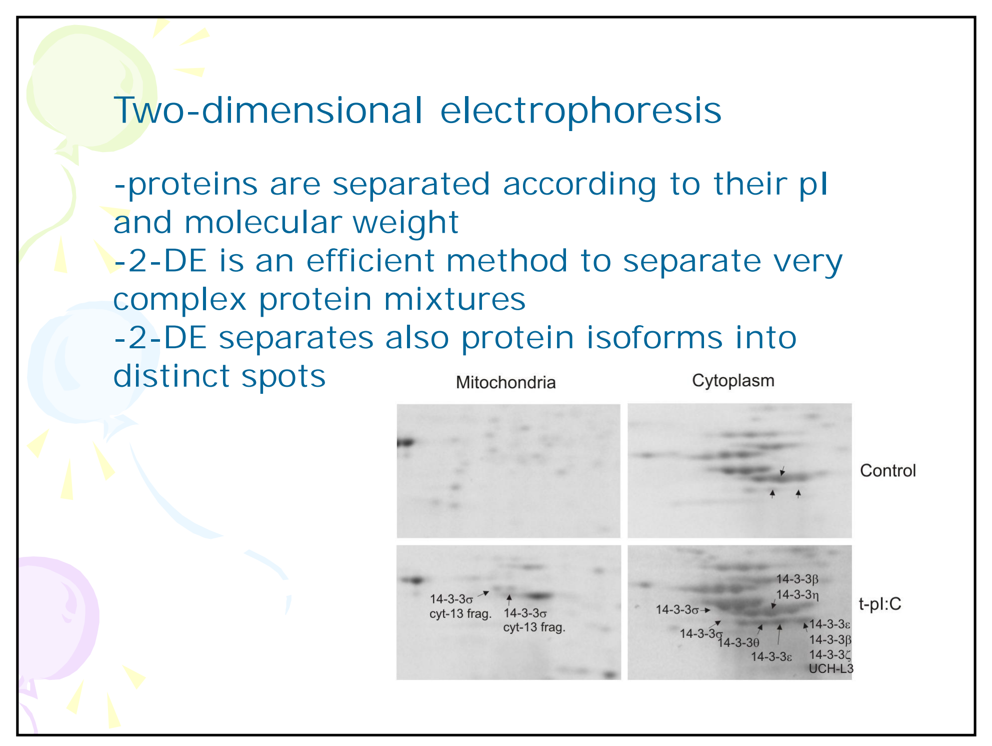
Which variants are present of each protein, when and how much

Cell map proteomics, "Interactomics"

Who interacts, when and where




Two-dimensional electrophoresis based proteomics:
Viral dsRNA induced differences in keratinocytes
Mitochondria
Cytoplasm
pI
Control
PolyI:C-transfected
•240 reproducibly differentially expressed protein spots
-137 in mitochondria (103 up- and 34 downregulted)
-103 in cytoplasm (70 up- and 33 downregulated)
•Proteins from 179 spots successfully identified


Two-dimensional electrophoresis
-proteins are separated according to their pI and molecular weight
-2-DE is an efficient method to separate very complex protein mixtures
-2-DE separates also protein isoforms into distinct spots
Mitochondria
Cytoplasm
Control
t-pI:C
14-3-3σ cyt-13 frag.
14-3-3σ cyt-13 frag.
14-3-3β
14-3-3η
14-3-3σ
14-3-3ε
14-3-3β
14-3-3ζ
UCH-L3

2-DE is a good separation method but...

-certain protein classes, e.g. very big or small proteins and proteins with extreme pI:s are absent or underrepresented in 2-DE gels

-a lot of manual lab work

MS-based proteomics

-possibility to study all protein classes

-for quantificatication the proteins/peptides are usually labeled with stable isotopes

-also label-free approaches

-most steps can be automated

Stable isotope labeling

-Protein samples are labeled with different stable isotopes, after labeling the proteins/peptides in different samples are still chemically (almost) identical but have a mass difference

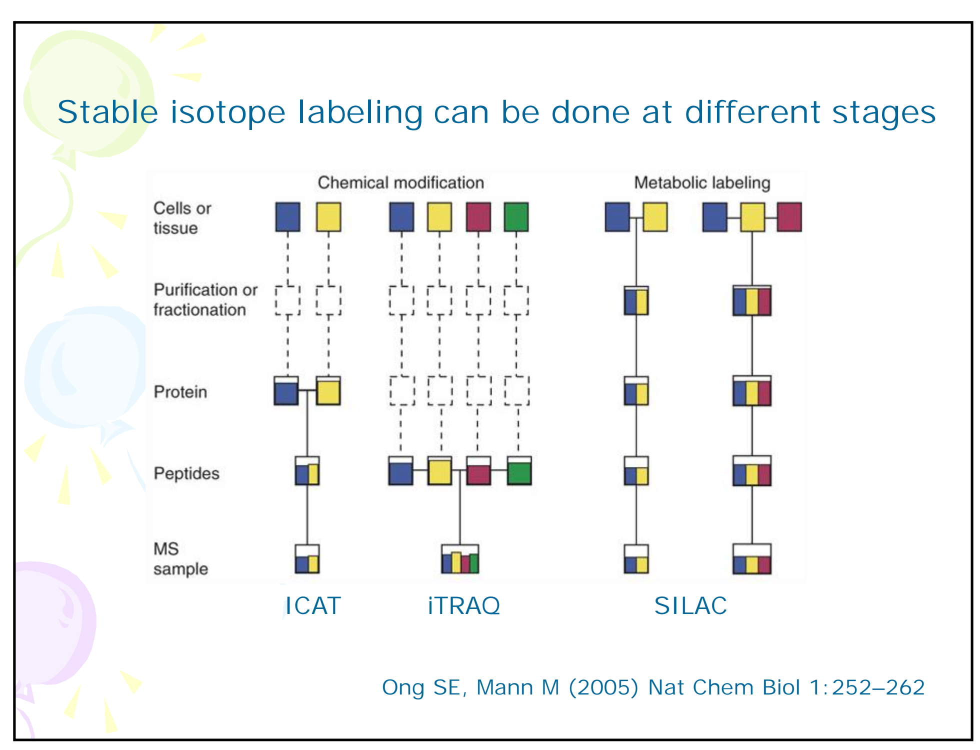
-Samples are mixed after labeling and relative protein quantification between the samples is done based on MS or MS/MS data

Stable isotope labeling

Isotope Coded Affinity Tags (ICAT),
Steve Gygi et al, Nature Biotechnology 1999

Stable isotope labeling by amino acids
in cell culture (SILAC),
Shao-En Ong et al, Mol Cell Proteomics 2002

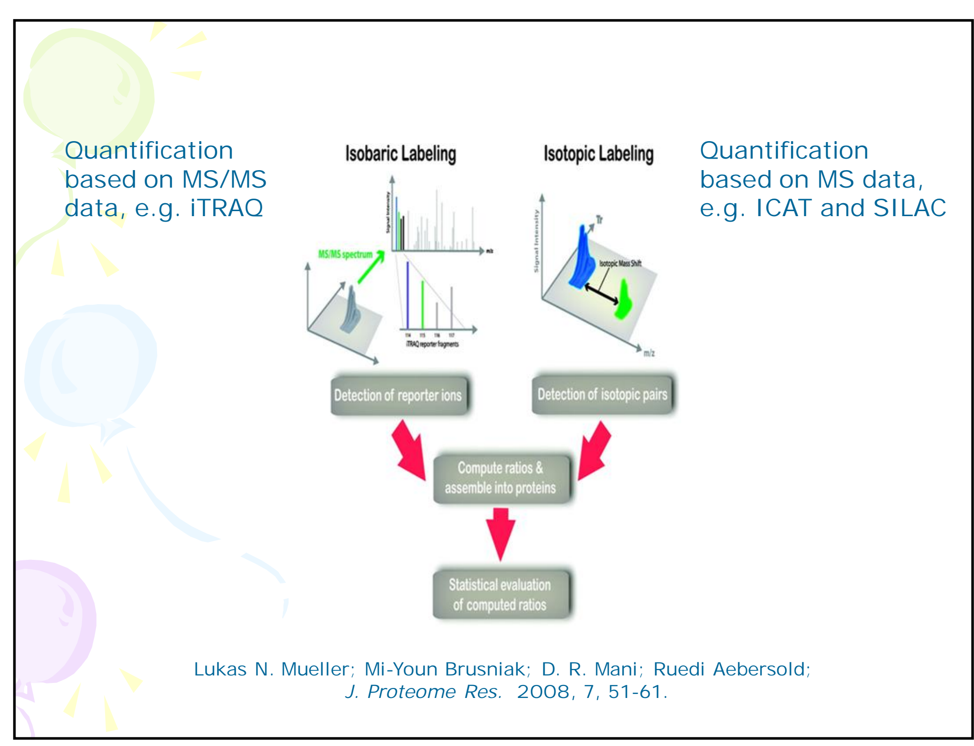
Isotope tagged relative and absolute
quantitation (iTRAQ),
Ross PL et al, Mol Cell Proteomics 2004



Stable isotope labeling

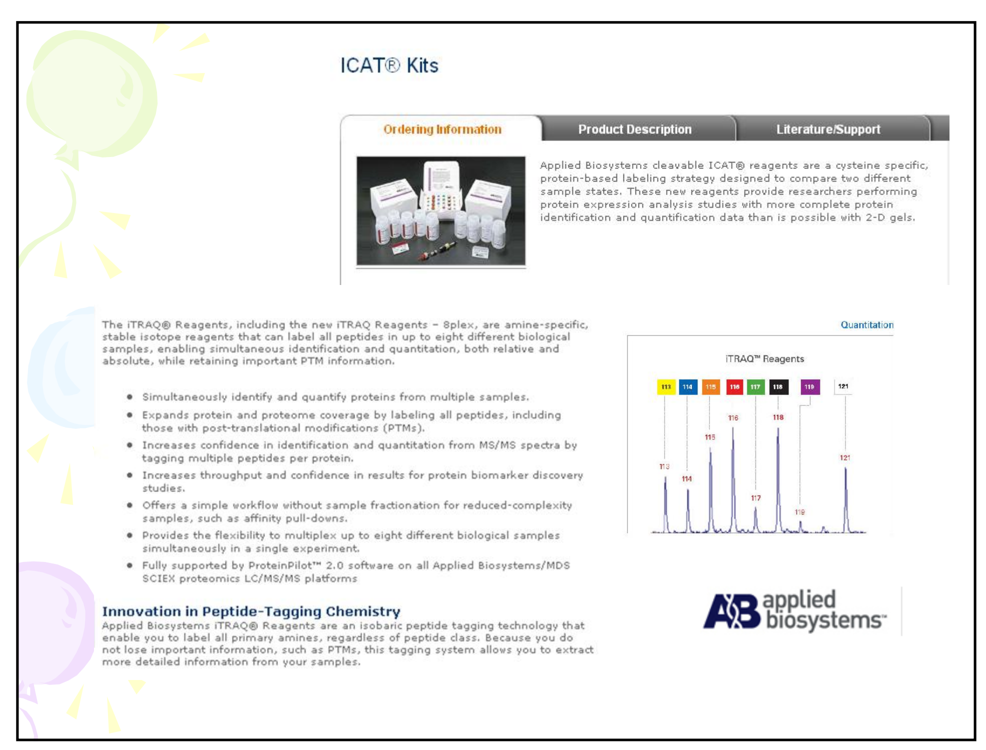
Reporter: Quantitation based on the relative intensities of fragment peaks at fixed m/z values within an MS/MS spectrum.
For example, iTRAQ and Tandem Mass Tags

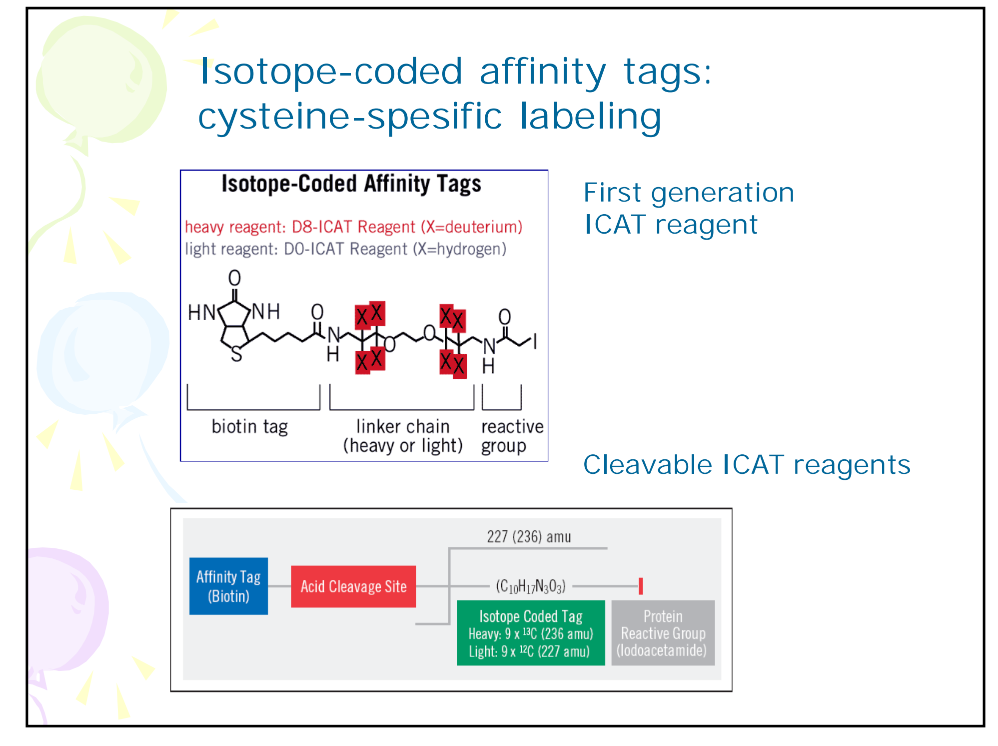
Precursor: Quantitation based on the relative intensities of extracted ion chromatograms (XICs) for precursors within a single data set.
The most widely used approach, which can be used with any chemistry that creates a precursor mass shift.
For example, 18O, AQUA, ICAT, ICPL, Metabolic, SILAC, etc.

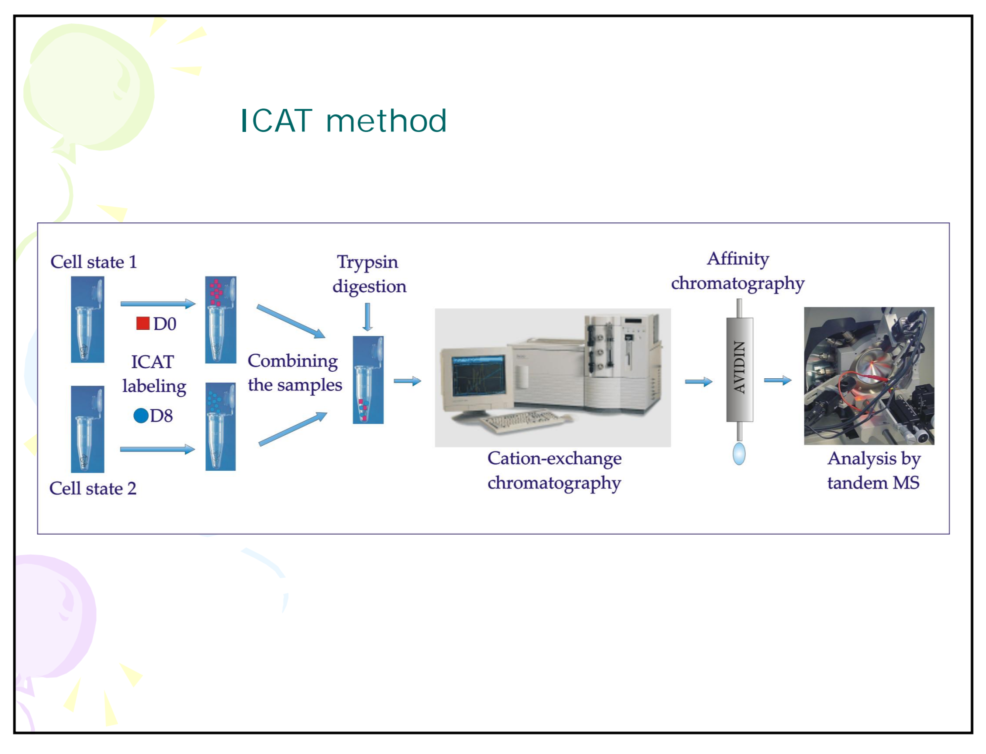


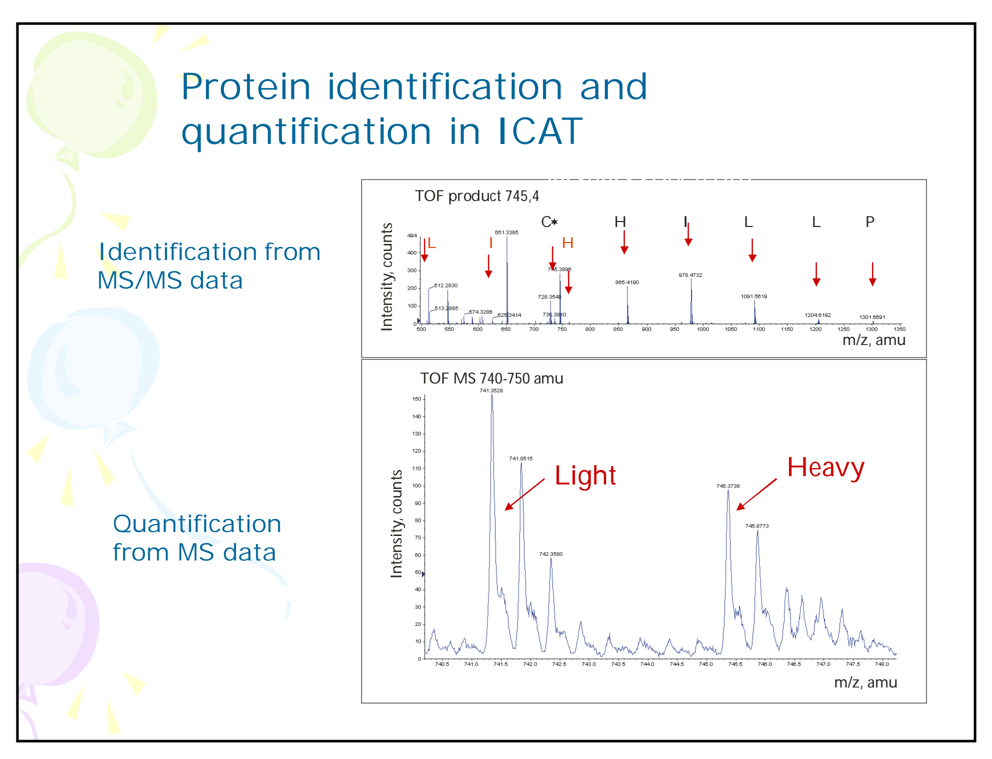
Quantification based on MS/MS data, e.g. iTRAQ
Isobaric Labeling
MS/MS spectrum
Isotopic Labeling
Isotopic Mass Shift
Quantification based on MS data, e.g. ICAT and SILAC
Detection of reporter ions
Detection of isotopic pairs
Compute ratios & assemble into proteins
Statistical evaluation of computed ratios
Lukas N. Mueller; Mi-Youn Brusniak; D. R. Mani; Ruedi Aebersold;
J. Proteome Res. 2008, 7, 51-61.

SILAC Protein Quantitation Kits
DMEM and RPMI kits and reagents for stable isotope labeling using amino acids in cell culture (SILAC).
Stable isotope labeling with amino acids in cell culture (SILAC) is a powerful method to identify and quantify relative differential changes in complex protein samples. The SILAC Method uses in vivo metabolic incorporation of "heavy" 13C- or 15N-labeled amino acids into proteins followed by mass spectrometry (MS) analysis for accelerated comprehensive identification, characterization and quantitation of proteins.
SILAC Highlights:
Efficient – 100% label incorporation into proteins of living cells
Reproducible – eliminates intra-experimental variability caused by differential sample preparation
Flexible – media deficient in both L - lysine and L - arginine, allowing for more complete proteome coverage through dual amino acid isotope labeling
Compatible – label proteins expressed in a wide variety of mammalian cell lines adapted to grow in DMEM or RPMI 1640 medium, including HeLa, 293T, COS7, U2OS, A549, A431, HepG2, NIH 3T3, Jurkat and others
Thermo SCIENTIFIC
Pierce Protein Research Products
Metabolic Labeling - SILAC™ Kits
Metabolic Labeling - SILAC™ Kits
SILAC™ Membrane Protein ID and Quantitation Kit Includes:
Heavy SILAC™ Stable Isotopic [13C6]-L-Lysine (*Lys)
Light SILAC™ Amino Acids
(2) Custom Membrane Lysis Buffers
Defined Media
GIBCO™ brand SILAC™ D-MEM
or
GIBCO™ brand SILAC™ RPMI 1640
Fetal Bovine Serum, Dialyzed
SILAC™ Phosphoprotein ID and Quantitation Kit Includes:
Heavy SILAC™ Stable Isotopic [13C6]-L-Lysine (*Lys)
Light SILAC™ Amino Acids
Invitrogen PiMAC™ (metal affinity resin)
Defined Media
GIBCO™ brand SILAC™ D-MEM
or
GIBCO™ brand SILAC™ RPMI 1640
Fetal Bovine Serum, Dialyzed
invitrogen™


ICAT® Kits
Ordering Information
Product Description
Literature/Support
Applied Biosystems cleavable ICAT® reagents are a cysteine specific, protein-based labeling strategy designed to compare two different sample states. These new reagents provide researchers performing protein expression analysis studies with more complete protein identification and quantification data than is possible with 2-D gels.
The iTRAQ® Reagents, including the new iTRAQ Reagents – 8plex, are amine-specific, stable isotope reagents that can label all peptides in up to eight different biological samples, enabling simultaneous identification and quantitation, both relative and absolute, while retaining important PTM information.
Simultaneously identify and quantify proteins from multiple samples.
Expands protein and proteome coverage by labeling all peptides, including those with post-translational modifications (PTMs).
Increases confidence in identification and quantitation from MS/MS spectra by tagging multiple peptides per protein.
Increases throughput and confidence in results for protein biomarker discovery studies.
Offers a simple workflow without sample fractionation for reduced-complexity samples, such as affinity pull-downs.
Provides the flexibility to multiplex up to eight different biological samples simultaneously in a single experiment.
Fully supported by ProteinPilot™ 2.0 software on all Applied Biosystems/MDS SCIEX proteomics LC/MS/MS platforms
Innovation in Peptide-Tagging Chemistry
Applied Biosystems iTRAQ® Reagents are an isobaric peptide tagging technology that enable you to label all primary amines, regardless of peptide class. Because you do not lose important information, such as PTMs, this tagging system allows you to extract more detailed information from your samples.
Quantitation
iTRAQ™ Reagents
applied biosystems

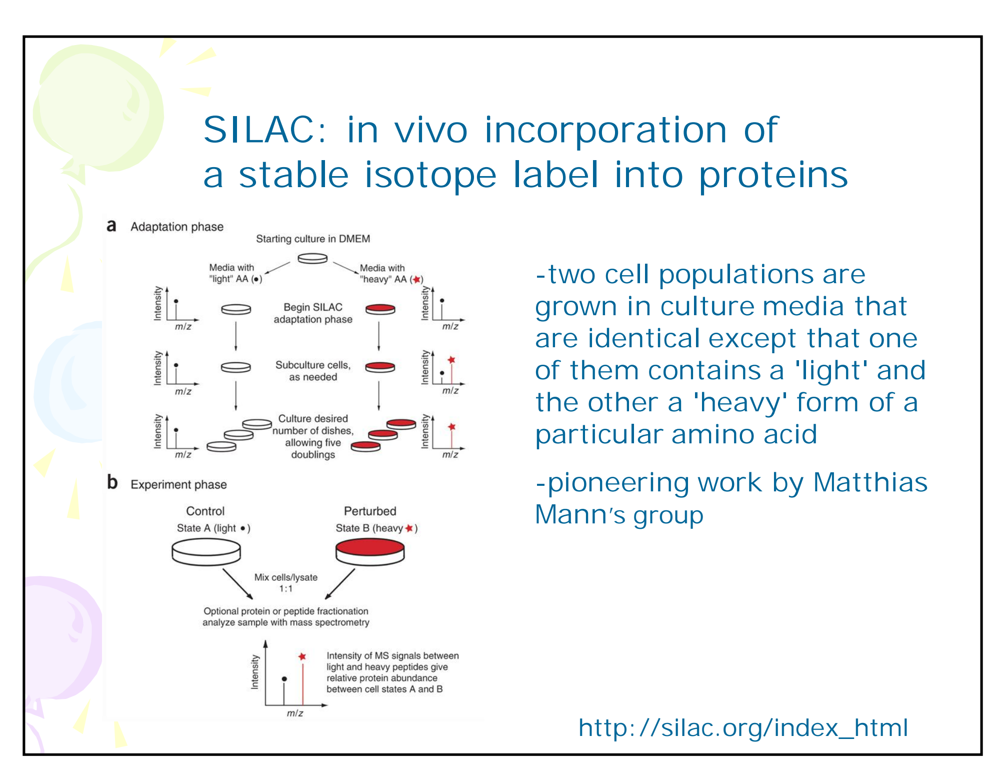

Isotope-coded affinity tags:
cysteine-spesific labeling
Isotope-Coded Affinity Tags
heavy reagent: D8-ICAT Reagent (X=deuterium)
light reagent: D0-ICAT Reagent (X=hydrogen)
biotin tag
linker chain (heavy or light)
reactive group
First generation ICAT reagent
Cleavable ICAT reagents
227 (236) amu
Affinity Tag (Biotin)
Acid Cleavage Site
(C10H17N3O3)
Isotope Coded Tag
Heavy: 9 x 13C (236 amu)
Light: 9 x 12C (227 amu)
Protein Reactive Group (Iodoacetamide)


ICAT method
Cell state 1
D0
ICAT
labeling
D8
Cell state 2
Trypsin
digestion
Combining
the samples
Cation-exchange
chromatography
Affinity
chromatography
AVIDIN
Analysis by
tandem MS


Protein identification and
quantification in ICAT
Identification from
MS/MS data
Quantification
from MS data
TOF product 745,4
C* H I L L P
L I H
Intensity, counts
m/z, amu
TOF MS 740-750 amu
Light
Heavy
Intensity, counts
m/z, amu

The ICAT technique is cysteine specific

-Simplifies mixtures into cysteine containing peptides only
-Complexity down to around 10-20%
-Cysteine in 80-90% of all proteins only.
-Incomplete proteome coverage
-Loss of PTM information
-Only pair-wise comparison possible

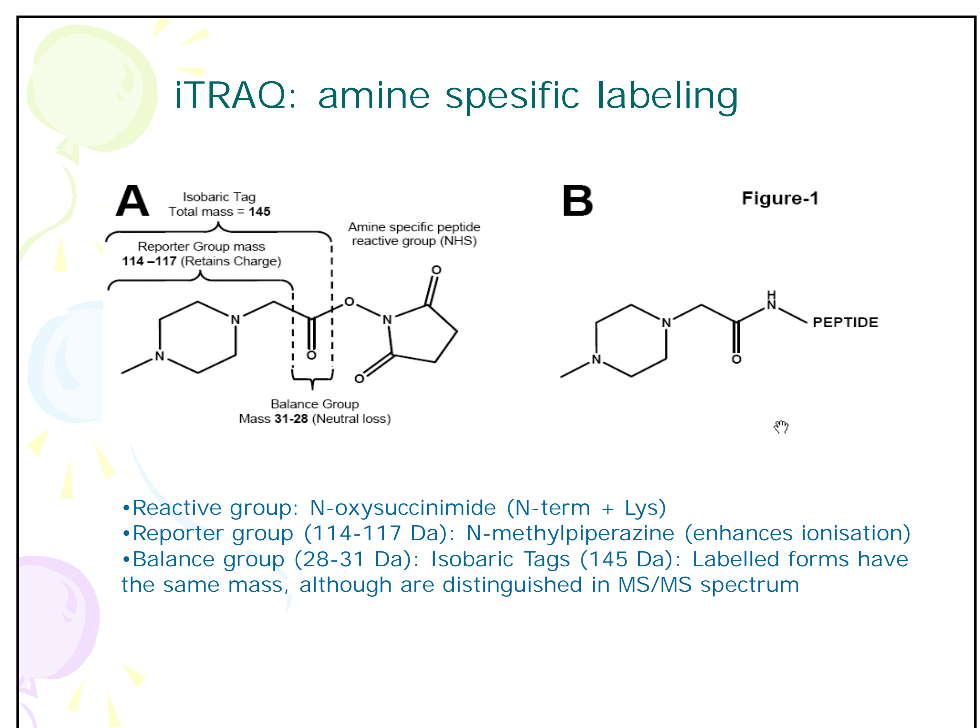


SILAC Amino Acids

A variety of amino acids are suitable in SILAC and the use of arginine, leucine, lysine, serine, methionine and tyrosine have already been described in literature by several groups. The use of an essential amino acid that does not metabolize to a different amino acid is most desirable in order to avoid a mixture of labelled amino acid products (See discussion on arginine conversion).
Ideally, the amino acid used in SILAC should be able to introduce a large enough mass difference from the unlabeled peptide so that the two peak clusters can be easily distinguished.

	Leucine D3	Arginine 13C6
Label	3 Deuteriums	6 Carbon-13s
Mass difference	+ 3 Da	+ 6 Da
Co-elution in LC (C18)	No	Yes
% of tryptic peptides (IPI_Human)	70%	50%
No. of labels/peptide	Variable	One
Location of label	Variable	C-termini
Cost	X	15X

Prefractionation: protein separation by SDS-PAGE



iTRAQ workflow

Isolated protein pellets
↓
Protein reduction, alkylation, and in-solution digestion
↓
iTRAQ labelling of the peptides
↓
Pooling of the labelled samples
↓
SCX fractionation of the peptides
↓
LC-MS/MS analysis for the SCX fractions
↓
Protein ID and quantification based on MS/MS data

iTRAQ/ Sample preparation

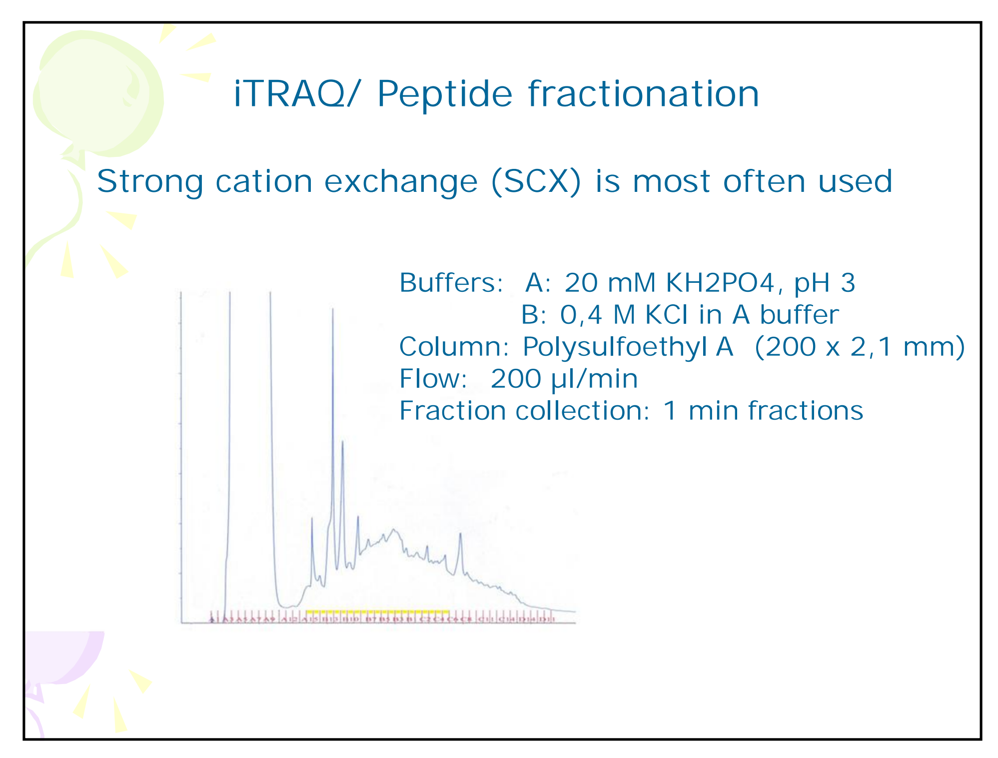
-protein pellet is the preferred starting material (e.g. 2D Clean Up Kit)

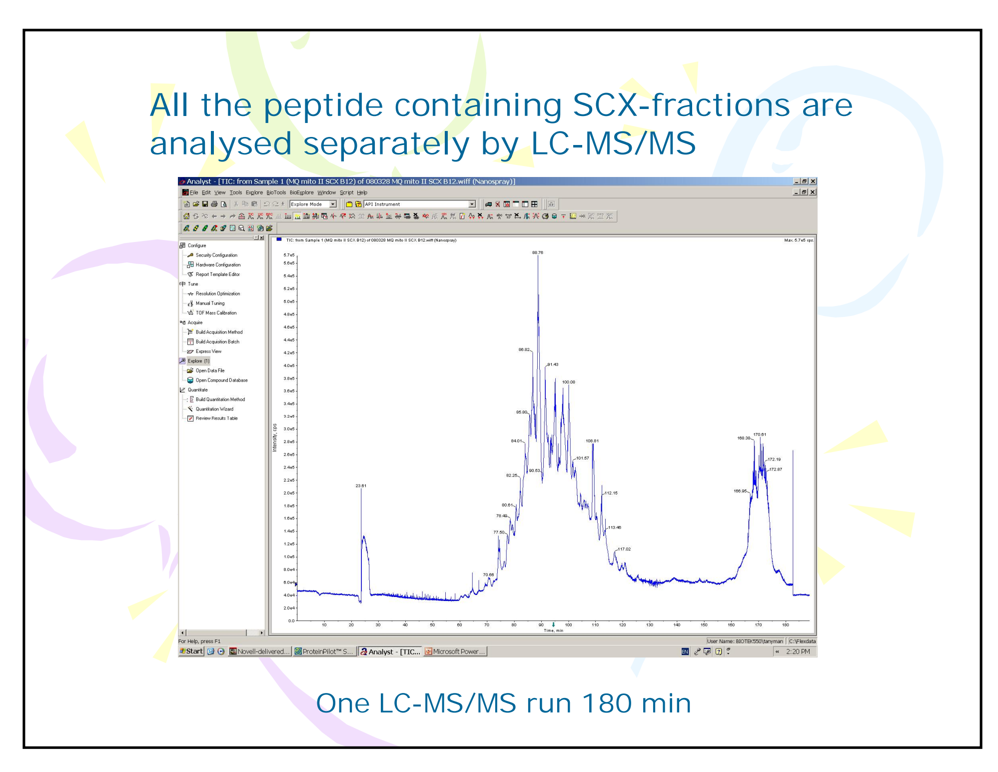
-protein pellet is dissolved in SDS-containing buffer to ensure that proteins are in solution

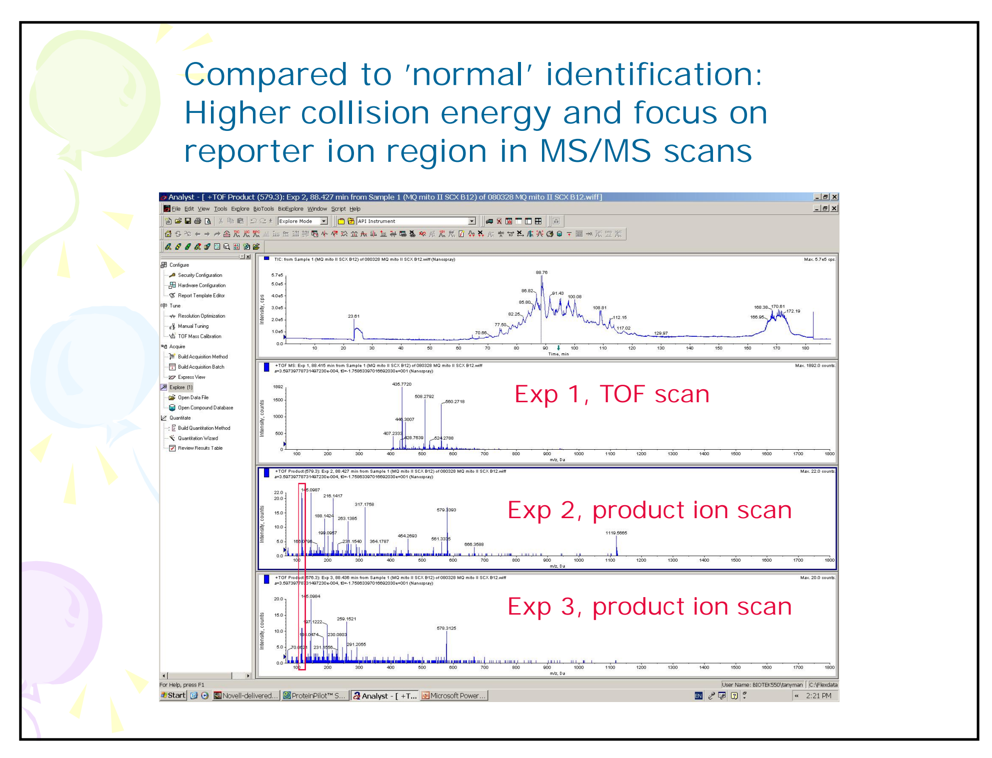
-disulphide bond reduction and Cys alkylation

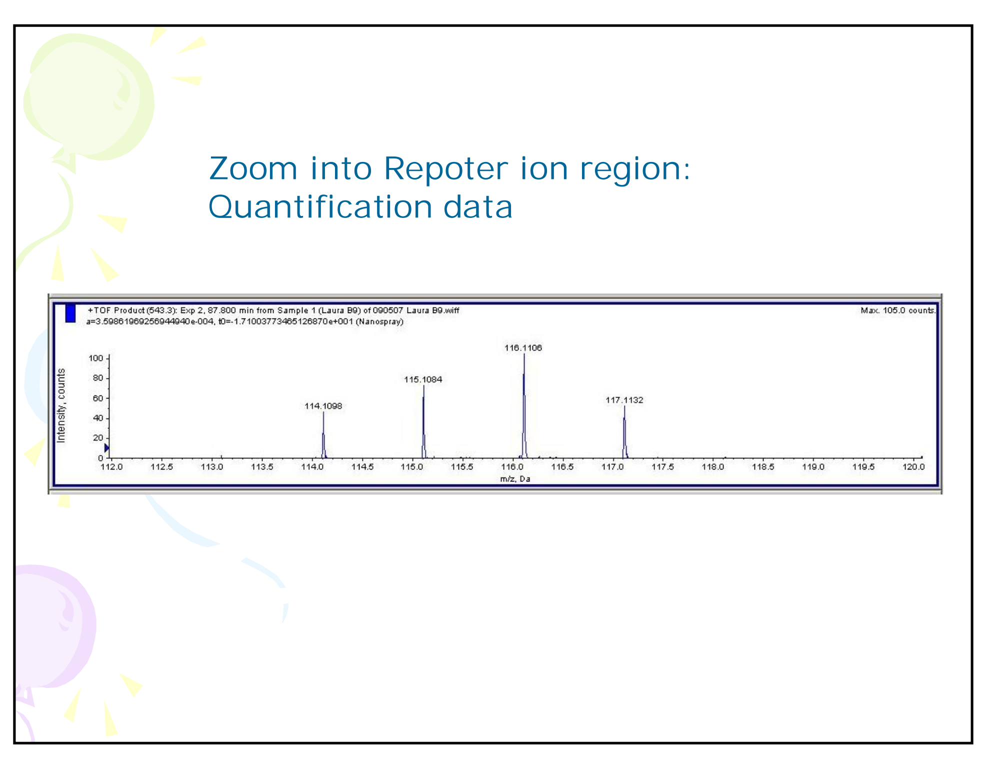
-protein digestion with trypsin o/n (use exess of trypsin because of the SDS in the buffer)

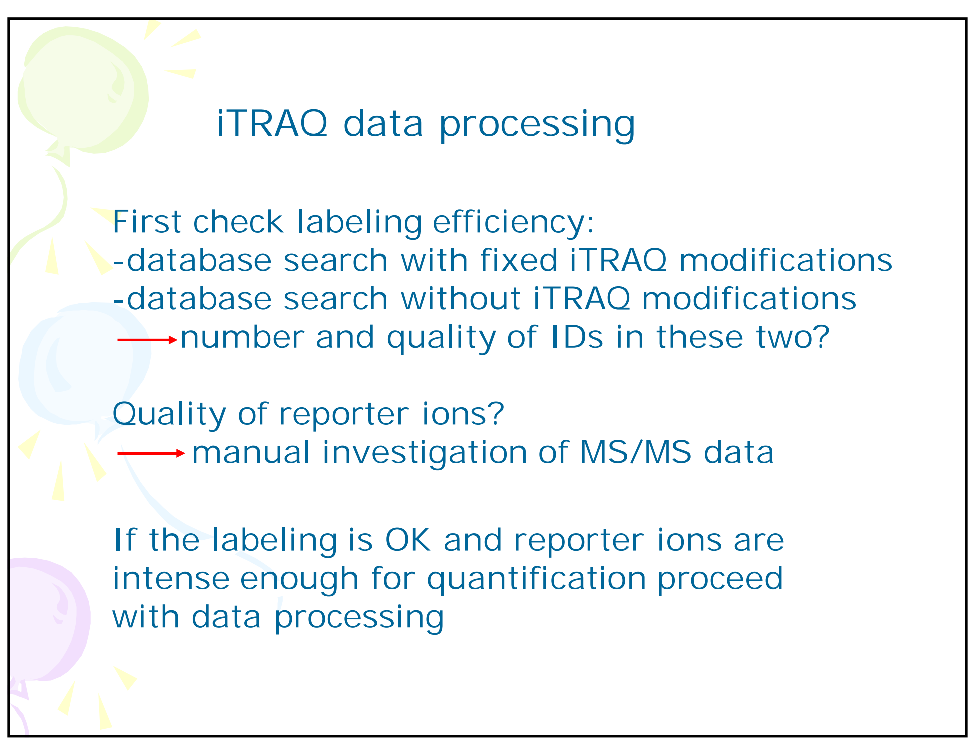
-iTRAQ labeling after digestion

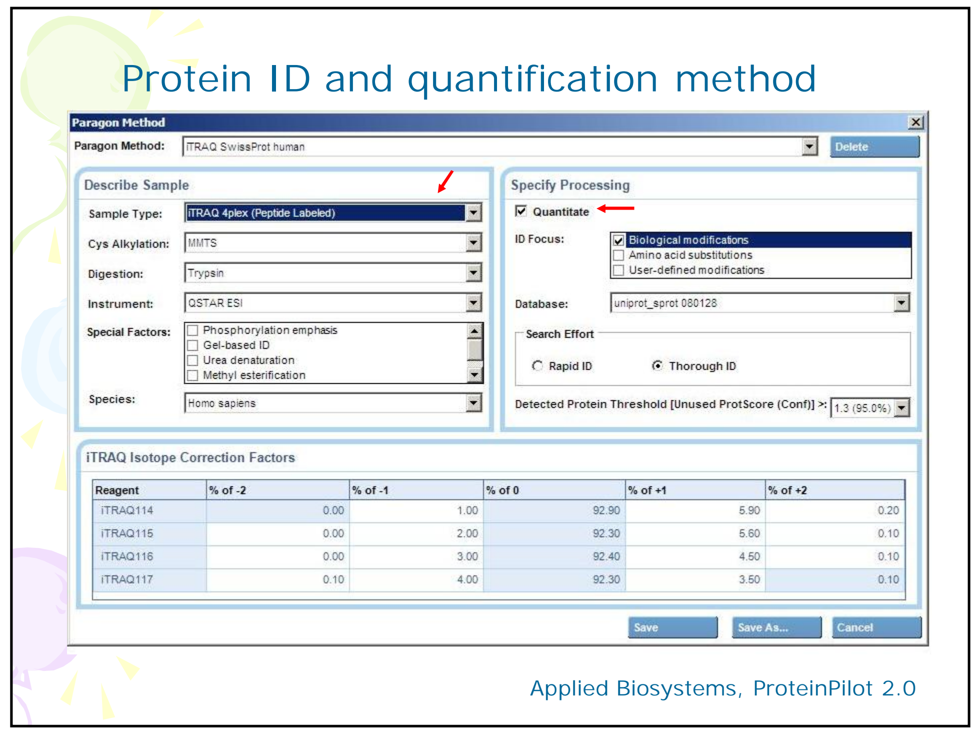

iTRAQ/ Peptide fractionation
Strong cation exchange (SCX) is most often used
Buffers: A: 20 mM KH2PO4, pH 3
B: 0,4 M KCl in A buffer
Column: Polysulfoethyl A (200 x 2,1 mm)
Flow: 200 µl/min
Fraction collection: 1 min fractions


All the peptide containing SCX-fractions are analysed separately by LC-MS/MS
One LC-MS/MS run 180 min

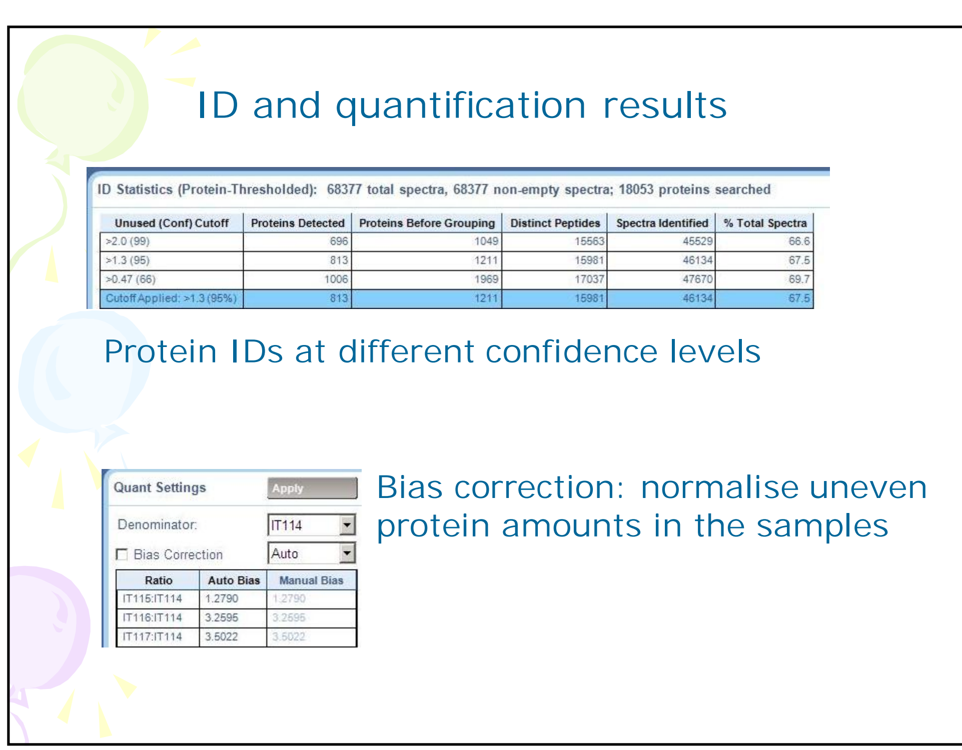

Compared to 'normal' identification:
Higher collision energy and focus on reporter ion region in MS/MS scans
Exp 1, TOF scan
Exp 2, product ion scan
Exp 3, product ion scan

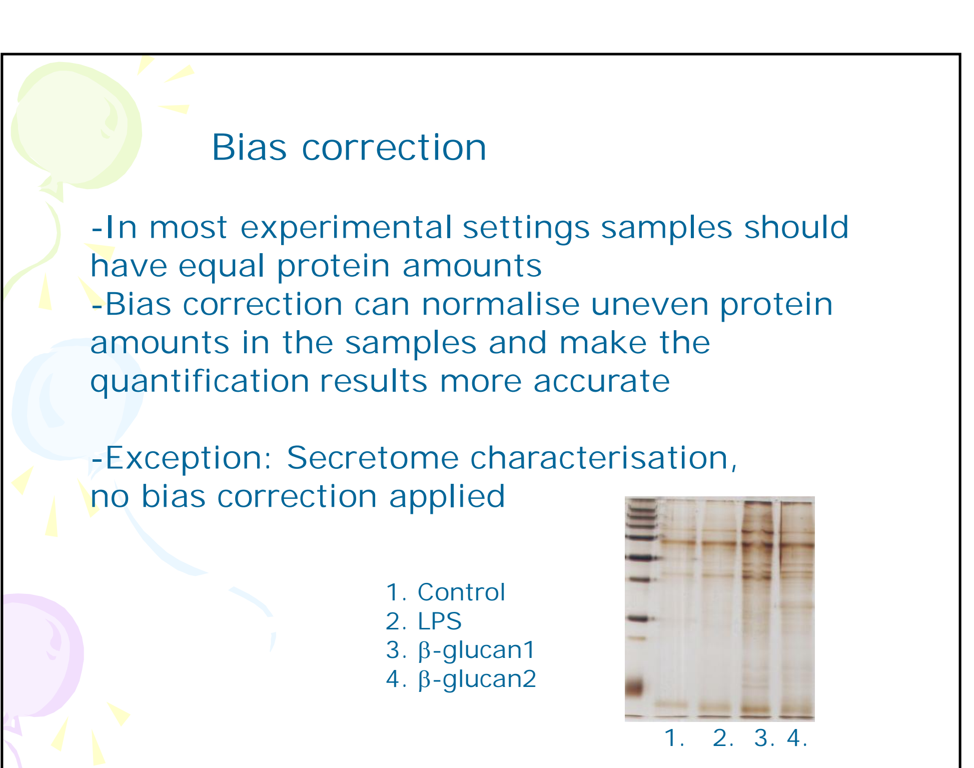

Zoom into Repoter ion region:
Quantification data
+TOF Product (543.3): Exp 2, 87.800 min from Sample 1 (Laura B9) of 090507 Laura B9.wiff
Max. 105.0 counts
114.1098
115.1084
116.1106
117.1132
Intensity, counts
m/z, Da

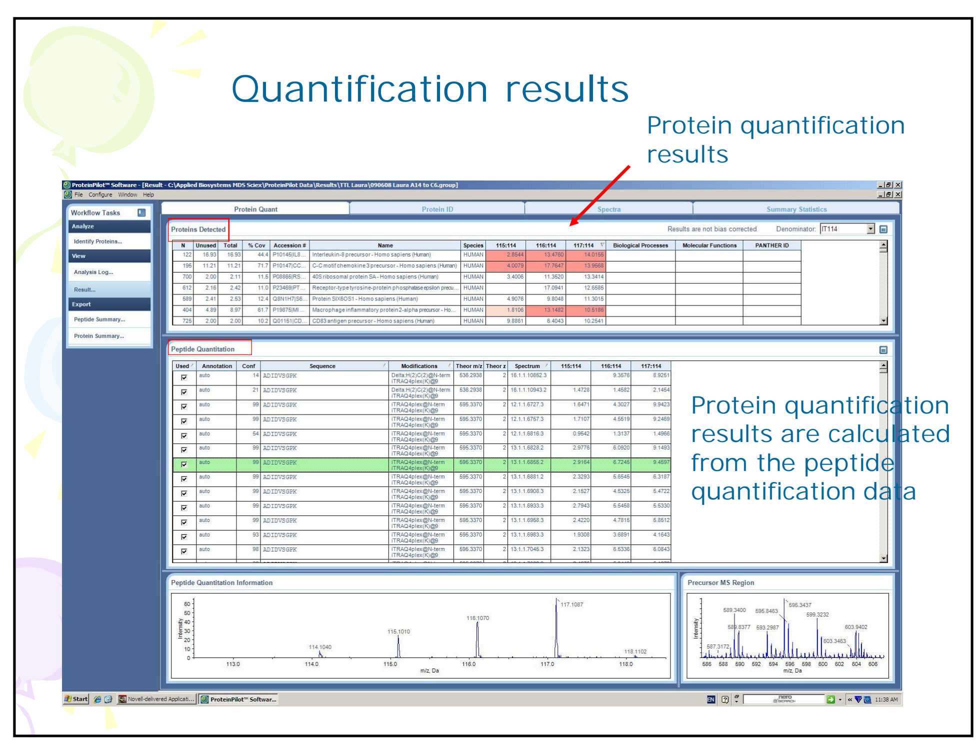




Reagent	% of -2	% of -1	% of 0	% of +1	% of +2
iTRAQ114	0.00	1.00	92.90	5.90	0.20
iTRAQ115	0.00	2.00	92.30	5.60	0.10
iTRAQ116	0.00	3.00	92.40	4.50	0.10
iTRAQ117	0.10	4.00	92.30	3.50	0.10


ID and quantification results
ID Statistics (Protein-Thresholded): 68377 total spectra, 68377 non-empty spectra; 18053 proteins searched
Unused (Conf) Cutoff | Proteins Detected | Proteins Before Grouping | Distinct Peptides | Spectra Identified | % Total Spectra
>2.0 (99) | 696 | 1049 | 15563 | 45529 | 66.6
>1.3 (95) | 813 | 1211 | 15981 | 46134 | 67.5
>0.47 (66) | 1006 | 1969 | 17037 | 47670 | 69.7
Cutoff Applied: >1.3 (95%) | 813 | 1211 | 15981 | 46134 | 67.5
Protein IDs at different confidence levels
Quant Settings
Apply
Denominator:
IT114
Bias Correction
Auto
Ratio | Auto Bias | Manual Bias
IT115:IT114 | 1.2790 | 1.2790
IT116:IT114 | 3.2595 | 3.2595
IT117:IT114 | 3.5022 | 3.5022
Bias correction: normalise uneven protein amounts in the samples


Bias correction
-In most experimental settings samples should have equal protein amounts
-Bias correction can normalise uneven protein amounts in the samples and make the quantification results more accurate
-Exception: Secretome characterisation, no bias correction applied
1. Control
2. LPS
3. β-glucan1
4. β-glucan2
1. 2. 3. 4.



Color Coding in the Protein Quant Tab

The quant ratios in the Proteins Detected table in the Protein Quant tab are color coded to indicate altered expression. Red indicates up-regulation and blue indicates down-regulation.

The intensity of the coloring indicates the certainty of the altered expression. The more certain the up-regulation, the more red the cells; the more certain the down-regulation, the more blue the cells.

Note: The coloring is determined by the p-value (the certainty that the observed change is real), **not** by the size of the ratio; see The P-value in the Quantitation Results.

Color coding in the Protein Quant tab

Color	P-value	Ratio
Dark red	< 0.001	> 1
Medium red	0.001 to < 0.01	> 1
Light red	0.01 to < 0.05	> 1
No color	>= 0.05	Any
Light blue	0.01 to < 0.05	< 1
Medium blue	0.001 to < 0.01	< 1
Dark blue	< 0.001	< 1

The user needs to decide the fold difference for altered expression, usually 1.5 or 2 fold difference is reported

The Protein Quant Tab

This tab – only shown when quantitation is part of the analysis – shows the winner proteins and their quant ratios. It also displays the individual ratio determinations for each peptide and allows you to view spectral evidence for quantitation. The Denominator field allows you to change the control reagent; see Changing the Denominator for Quant Ratios. The tab also shows which type of bias correction, if any, is in use; see Bias Correction in the Pro Group™ Algorithm Results.

PVal (P-value)
A measure of the certainty that the average ratio differs from 1. The smaller the p-value, the more likely any differential expression is real; see The P-value in the Quantitation Results.

EF (Error Factor)
A measure of the error in the average ratio; see Calculating the Error Factor for the Average Ratio.

Name	115:114	PVal 115:114	EF 115:114	116:114	PVal 116:114	EF 116:114	117:114	PVal 117:114	EF 117:114
40S ribosomal protein S28 - Homo sapiens (Human)	0.9200	0.6202	1.5394	2.3823	0.0057	1.5624	12.5071	0.0000	1.3265
60S ribosomal protein L30 - Homo sapiens (Human)	1.4614			2.6219			12.2764		
Mitochondrial antiviral-signaling protein - Homo sapiens (...	1.1039	0.7506	EF > 2	2.1596	0.0424	1.9197	10.4020	0.0486	EF > 2
Adaptin ear-binding coat-associated protein 2 - Homo sap ...	1.0896	0.7519	EF > 2	2.8915	0.0025	1.4294	10.2202	0.0000	1.2043
SEC23-interacting protein - Homo sapiens (Human)	1.9990			4.2065			9.6211		
40S ribosomal protein S20 - Homo sapiens (Human)	1.2080	0.4155	1.8928	2.5844	0.0125	1.7523	9.2413	0.0019	1.9767
HCLS1-binding protein 3 - Homo sapiens (Human)	1.3555			3.2365			9.1618		

Peptide Quantitation table

This table lists all the peptides detected for the protein selected in the Proteins Detected table. Table fields and their definitions are listed below.

Used
When checked, the ratios for this peptide are used in the calculation of the average ratio for the protein ratio (shown in the Proteins Detected table); see Which Peptides Are Used for Quant?. You can choose to omit or include peptide(s) from the calculation; see Manually Choosing Peptides for the Ratio Calculation.

Annotation
Displays how the Used check box status (checked or unchecked) was determined: "auto" when the program sets the status, "manual" when a user sets the status; see Which Peptides Are Used for Quant?.

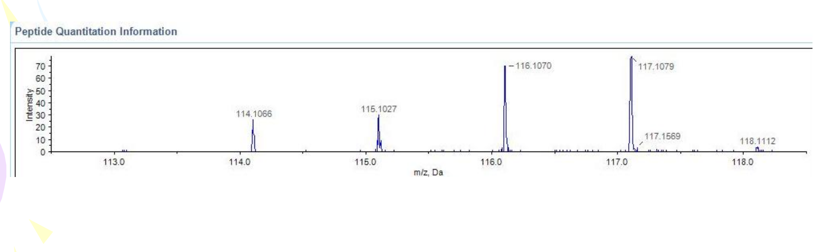
Peptide Quantitation

Used	Annotation	Conf	Sequence	Modifications	Theor m/z	Theor z	Spectrum	115:114	116:114	117:114
☑	auto	4	AALAHSEEVTASQVAATK	iTRAQ4plex@N-term Deamidated(Q)@13 iTRAQ4plex(K)@18	691.7071	3	21.1.1.7338.2	1.3428	3.8268	3.8576
☑	auto	99	AALAHSEEVTASQVAATK	iTRAQ4plex@N-term iTRAQ4plex(K)@18	691.3790	3	1.1.1.5599.2	0.7341	1.8185	5.2124
☑	auto	99	AALAHSEEVTASQVAATK	iTRAQ4plex@N-term iTRAQ4plex(K)@18	691.3790	3	21.1.1.7314.3	1.0669	2.9824	7.3240
☑	auto	5	AEAERAR	iTRAQ4plex@N-term	473.7630	2	26.1.1.3194.2	0.9794	1.2072	0.2793
☑	auto	52	AEEAEAQKR	iTRAQ4plex@N-term iTRAQ4plex(K)@8	440.5768	3	26.1.1.2043.2	0.9542	2.7617	7.9856
☑	auto	98	AEEAEAQKR	iTRAQ4plex@N-term iTRAQ4plex(K)@8	440.5768	3	35.1.1.1777.2	1.0182	3.4002	10.3836
☑	auto	4	AFCGFEDPR	iTRAQ4plex@N-term Methylthio(C)@3	616.2715	2	1.1.1.6097.2	1.3000	2.8912	4.6196
☑	auto	4	AGEVERDLDKADSMIR	No iTRAQ4plex@N-term iTRAQ4plex(K)@10 Dethiomethyl(M)@14	634.3330	3	24.1.1.4011.3	1.2181	2.7908	4.5051

Peptide Quantitation Information pane

This pane displays the data from which the quantitation ratios were determined, for the peptide selected in the Peptide Quantitation table. The data shown depends upon the reagent used for labeling:

- iTRAQ Reagent labeling – The MS/MS spectrum is shown, zoomed into m/z 111.5 to m/z 119.5 for the 4plex reagent and from m/z 110.5 to m/z 122.5 for the 8plex reagent. See Using the Peptide Quantitation Information Pane to Evaluate iTRAQ™ Sample Preparation.
- Cleavable ICAT or SILAC Reagent labeling – The MS spectrum is shown, zoomed into [precursor m/z -32/z] to [precursor m/z + 37/z]. Highlight bars and text labels corresponding to the peaks for the light and heavy forms of the peptide are drawn on the spectrum.



Isoform-Specific Quantitation

The Pro Group™ Algorithm allows for a new type of quantitation analysis: **isoform-specific quantitation**. ProteinPilot™ Software calculates protein ratios using only ratios from the spectra that are **distinct to each protein** (or protein form). This serves to eliminate any masking of changes in expression due to peptides that are shared between proteins.

Consider two different transcripts from the same gene that result in two different splice form variant proteins. A stress on the system may cause a shift in expression in either or both forms. Because these two proteins may have large sections of sequence that are exactly the same, only peptides **distinct to each form** report the true change in expression. If peptides common to both proteins ("shared peptides") are included in the calculations, the ratio will be an averaged ratio that can mask any change in the expression of each form.

When one isoform is present at much higher concentration than the other, the shared peptides report primarily on one form. When isoforms are present in nearly equal amounts, shared peptides will mask any changes in expression to the greatest degree. The Pro Group Algorithm takes the conservative approach and excludes **all** shared peptides.

Which Peptides Are Used for Quant?

Only peptides with a check in the Used column in the Peptide Quantitation table are used in the calculation of the average ratio for the protein. The text in the Annotation column provides more information as to why the peptide is or is not used for quantitation.

The Pro Group™ Algorithm assigns peptides to one of three types:

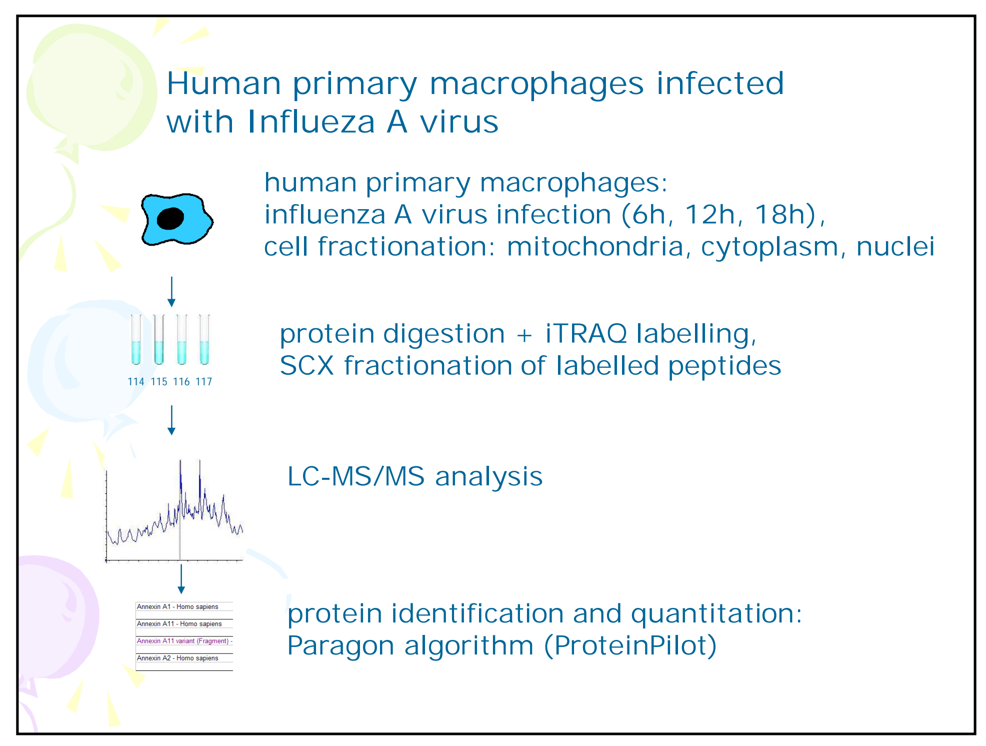
- Peptide is usable for quantitation – At least one ratio is shown, Used is checked, and "auto" is shown in the Annotation column. For iTRAQ™ Reagent-labeled samples, which can have up to seven ratios per peptide, sometimes some of the ratios for a particular peptide are blank. The blank ratios are never used, even when the Used check box is checked.
- Ratio could not be determined or is suspicious in some way – Ratio, Used, and Annotation columns are all blank.
- Ratio can be calculated but peptide is felt to be unusable for quantitation – Ratio is shown, Used is *not* checked, and the Annotation column shows the reason this peptide is deemed unusable.

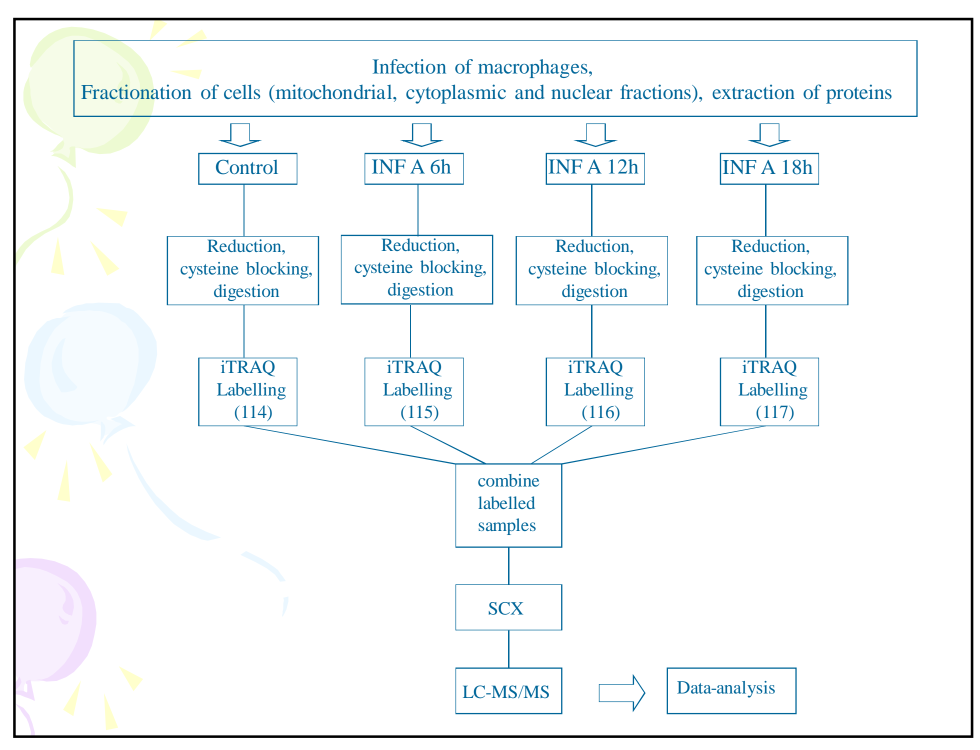
Peptide ratios can be manually included or omitted from average ratio calculation; see Manually Choosing Peptides for the Ratio Calculation.

iTRAQ

Advantages:

- Not cysteine specific, labels every peptide
- Retains greater proportion of information of PTMs
- More peptides for confident identification
- Quadraplex or 8-plex: Four/eight comparisons at the same time
- Can use 3 labels for 3 different systems plus the 4th as an internal standard for absolute quantification
- Labelled peptides isobaric: MS/MS fragmentation information overlaid in the same m/z window, enhancing identification


Human primary macrophages infected
with Influeza A virus
human primary macrophages:
influenza A virus infection (6h, 12h, 18h),
cell fractionation: mitochondria, cytoplasm, nuclei
114 115 116 117
protein digestion + iTRAQ labelling,
SCX fractionation of labelled peptides
LC-MS/MS analysis
protein identification and quantitation:
Paragon algorithm (ProteinPilot)


Infection of macrophages,
Fractionation of cells (mitochondrial, cytoplasmic and nuclear fractions), extraction of proteins
Control
INF A 6h
INF A 12h
INF A 18h
Reduction, cysteine blocking, digestion
iTRAQ Labelling (114)
iTRAQ Labelling (115)
iTRAQ Labelling (116)
iTRAQ Labelling (117)
combine labelled samples
SCX
LC-MS/MS
Data-analysis

-two biological replicates of mitochondrial, cytoplasmic and nuclear cell fractions were analysed

-each iTRAQ sample set was analysed twice with LC-MS/MS to improve the quality of protein identifications and quantifications

-intrument time needed:
3x2x2x20x3h=720h (30 days)

Three different cell fractions
Two biological replicates
Two 'technical' replicates
20 SCX fractions in each replicate
3h lenght of one LC-MS/MS run

NOTE: This time can be reduced with current MS instruments with faster scanning speed!!

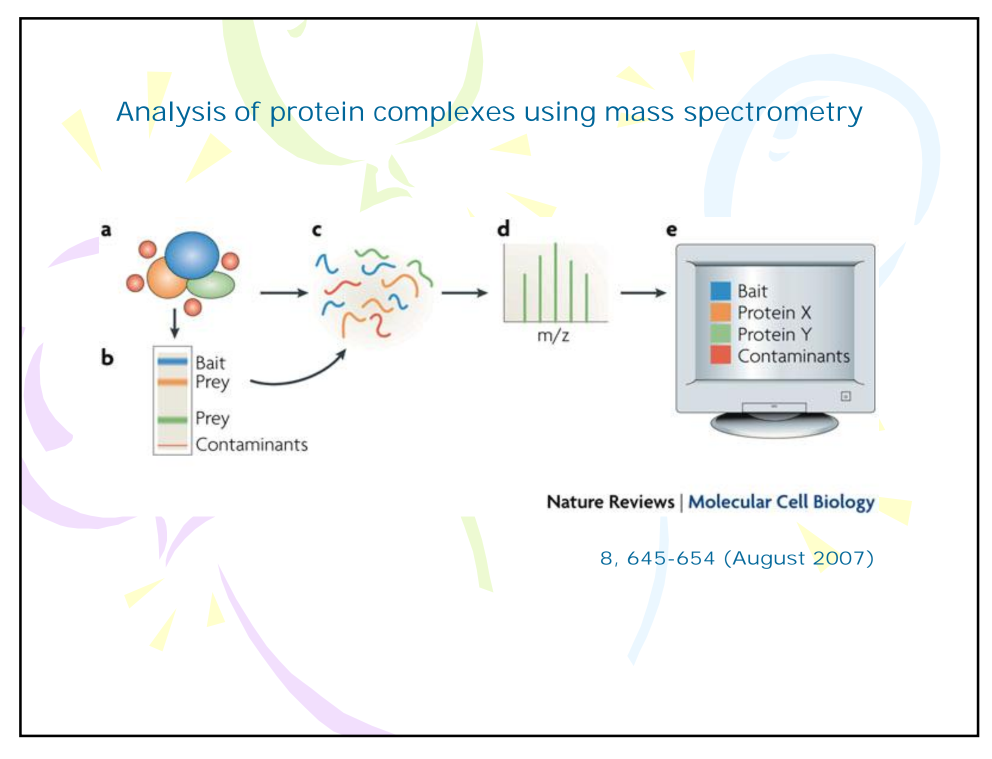
Human primary macrophages infected with Influeza A virus, iTRAQ results

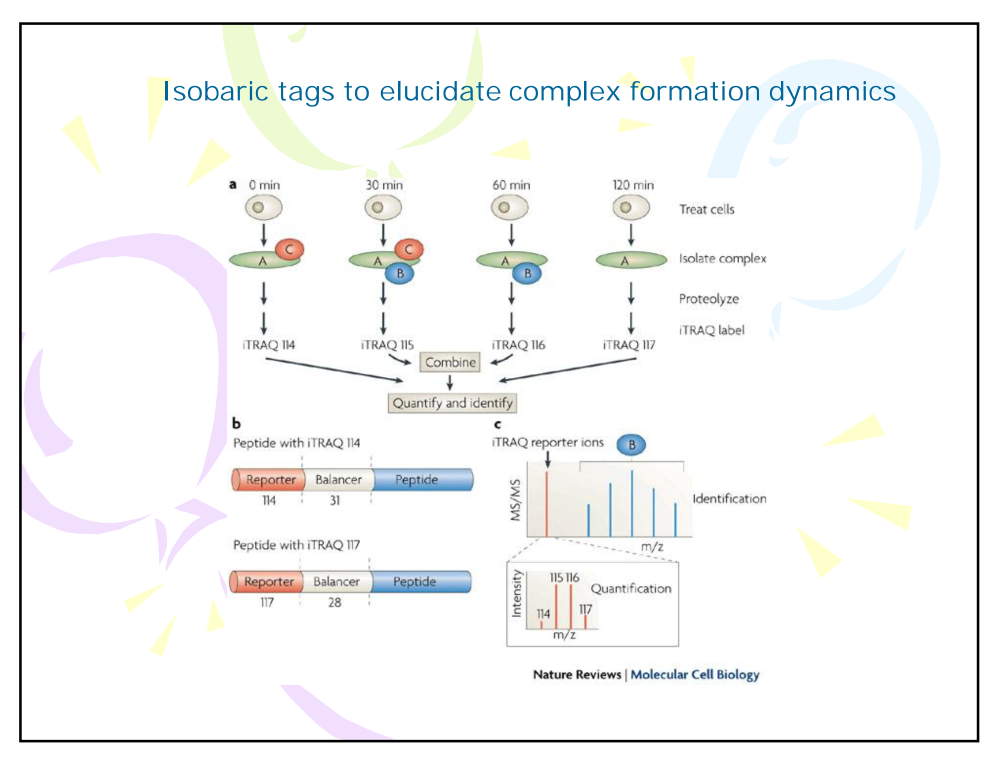
Identified: 3268 proteins
Reliably quantified: 2200 proteins
Differentially regulated: 1321 proteins

		identified + quantitated *	upregulated	downregulated
mitochondrial fraction	6h	1102	66	22
	12h	1160	131	58
	18h	552**	189	97
cytoplasmic fraction	6h	765	220	49
	12h	721	103	30
	18h	746	176	90
nuclear fraction	6h	740	121	136
	12h	882	336	344
	18h	895	376	369

At least 1.5 fold difference

Lietzén *et al*, PLoS Pathogens, 2011


Analysis of protein complexes using mass spectrometry
a
b
c
d
e
Bait
Prey
Prey
Contaminants
m/z
Bait
Protein X
Protein Y
Contaminants
Nature Reviews | Molecular Cell Biology
8, 645-654 (August 2007)


Isobaric tags to elucidate complex formation dynamics
a
0 min
30 min
60 min
120 min
Treat cells
Isolate complex
Proteolyze
iTRAQ label
A
B
C
iTRAQ 114
iTRAQ 115
iTRAQ 116
iTRAQ 117
Combine
Quantify and identify
b
Peptide with iTRAQ 114
Reporter
Balancer
Peptide
114
31
Peptide with iTRAQ 117
Reporter
Balancer
Peptide
117
28
c
iTRAQ reporter ions
MS/MS
Identification
m/z
Intensity
115 116
114
117
Quantification
m/z
Nature Reviews | Molecular Cell Biology

ICAT, SILAC and iTRAQ

ICAT and SILAC:
quantification based on MS data
identification based on MS/MS data

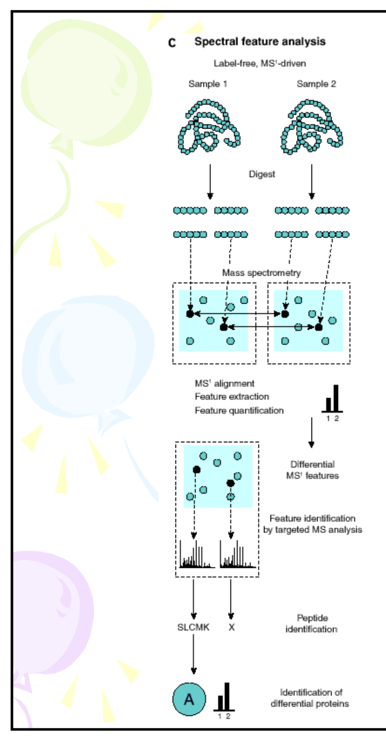
iTRAQ: both ID and quantification based on MS/MS data

ALL produce huge amounts of raw data
→ current bottlenecks are in data analysis and validation of the results

Label-free quantification

Replicate (= Spectral feature analysis)
Label free quantitation based on the relative intensities of extracted ion chromatograms (XICs) for precursors in multiple data sets aligned using mass and elution time.

emPAI (Exponentially Modified Protein Abundance Index)
Label free quantitation for the proteins in a mixture based on protein coverage by the peptide matches in a database search result



Spectral feature analysis: Label-free, MS^1 driven

-the analysis starts with alignment of MS1 data from different samples, extraction of spectral features and their quantification

-spectral features showing differential expression are identified using a targeted MS/MS-based workflow.

Nature Methods - 4, 787 - 797 (2007)

Spectral feature analysis

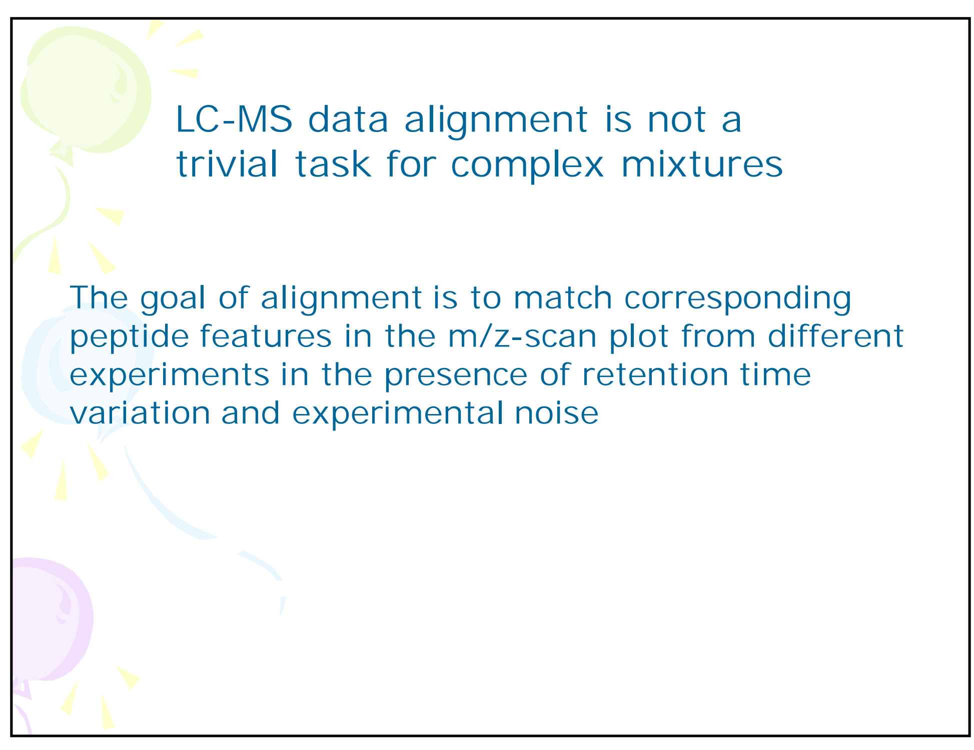
-biological samples are analyzed in separate MS runs and the correspondence between spectral features across the runs is established by means of computational tools

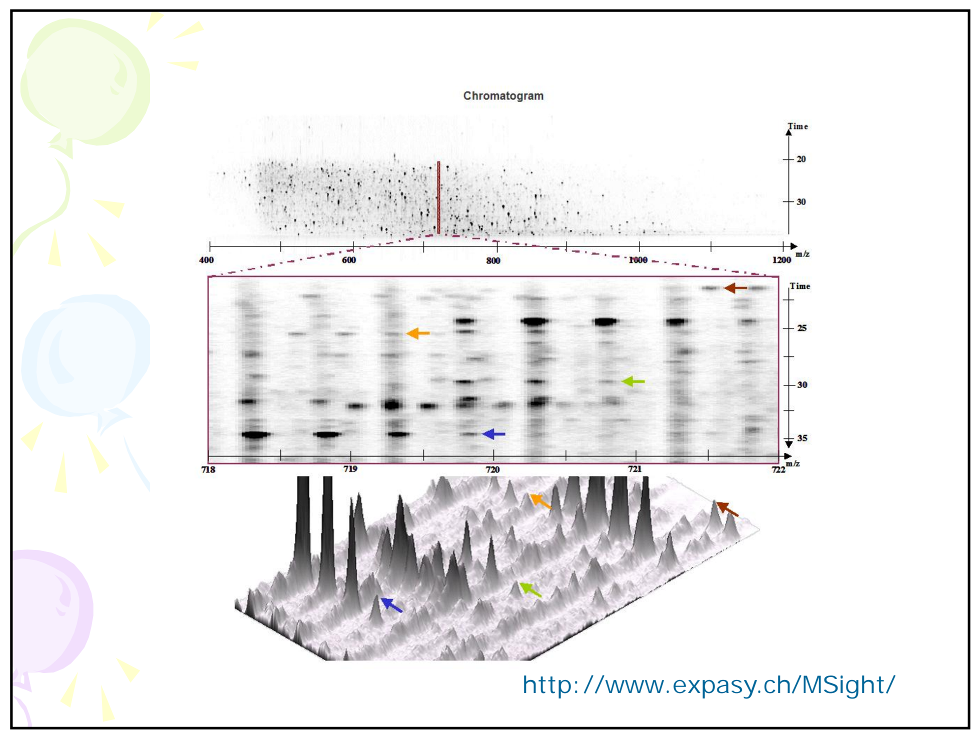
-allows analysis of a large number of spectrum features and allows higher data throughput

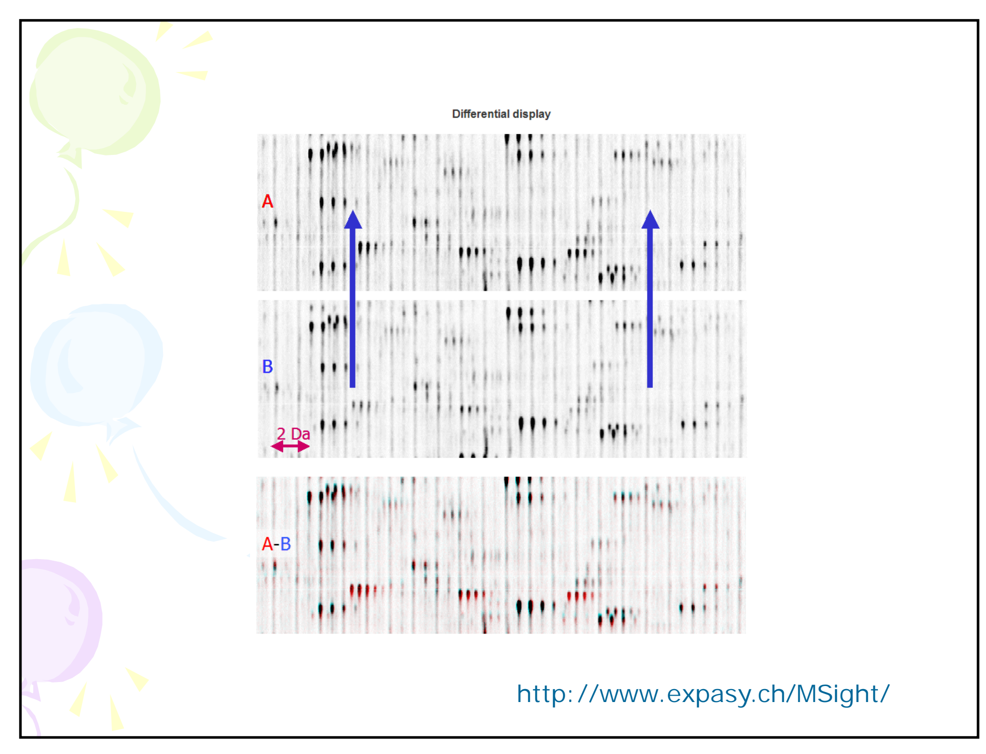
-is compatible with applications that require profiling of multiple biological samples e.g. biomarker discovery

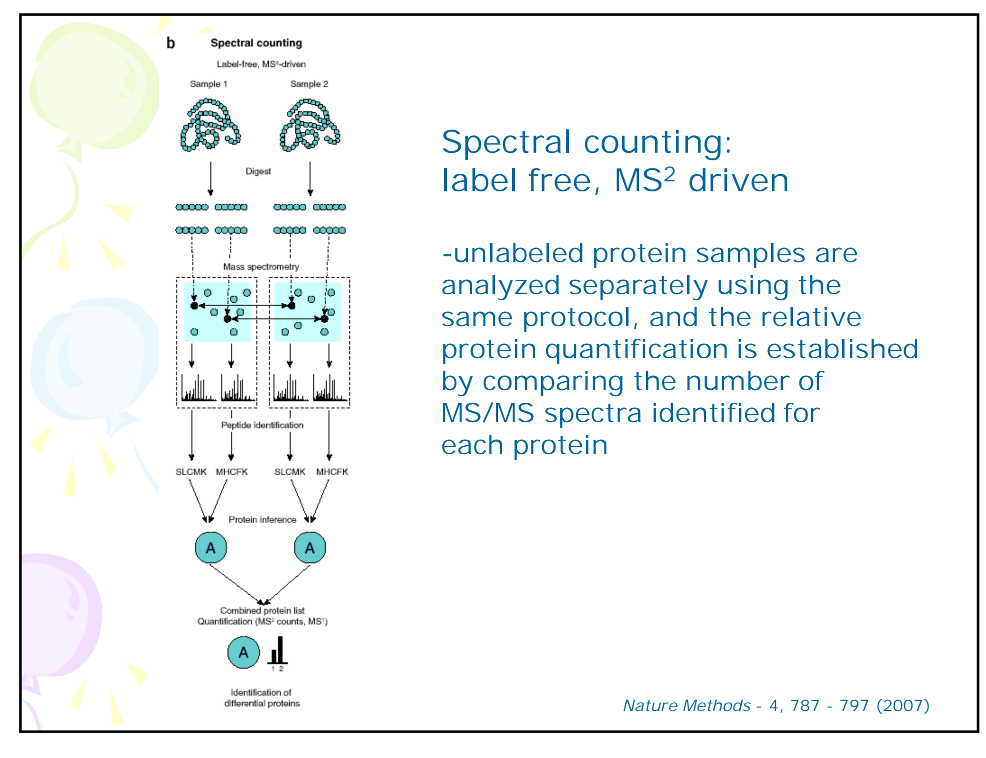
-does not require identification of the peptide sequence corresponding to each observed spectrum feature before quantification

-drawback: increased computational complexity


LC-MS data alignment is not a trivial task for complex mixtures
The goal of alignment is to match corresponding peptide features in the m/z-scan plot from different experiments in the presence of retention time variation and experimental noise


Chromatogram
Time
20
30
400
600
800
1000
1200
m/z
Time
25
30
35
718
719
720
721
722
m/z
http://www.expasy.ch/MSight/


Differential display
A
B
2 Da
A-B
http://www.expasy.ch/MSight/

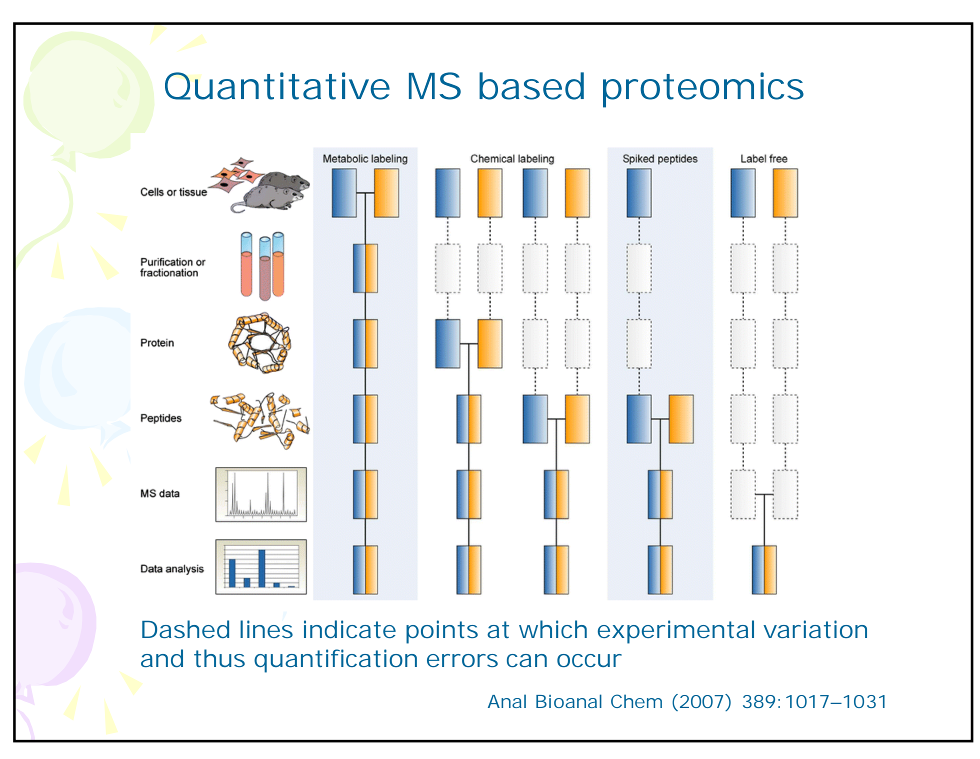

b
Spectral counting
Label-free, MS²-driven
Sample 1
Sample 2
Digest
Mass spectrometry
Peptide identification
SLCMK
MHCFK
SLCMK
MHCFK
Protein inference
A
A
Combined protein list
Quantification (MS² counts, MS¹)
A
Identification of
differential proteins
Spectral counting:
label free, MS² driven
-unlabeled protein samples are
analyzed separately using the
same protocol, and the relative
protein quantification is established
by comparing the number of
MS/MS spectra identified for
each protein
Nature Methods - 4, 787 - 797 (2007)

Spectral counting

-the protein abundance in each sample is estimated from the number of MS/MS spectra identified corresponding to each protein normalized to account for protein length or expected number of tryptic peptides
-as a variation of this strategy, peptide abundance can be determined from the intensity of the corresponding spectrum features

-suffers from inability to quantify low abundance proteins identified from only one or two peptides
-in general is less accurate than the methods based on stable isotope labeling

Quantitative MS based proteomics



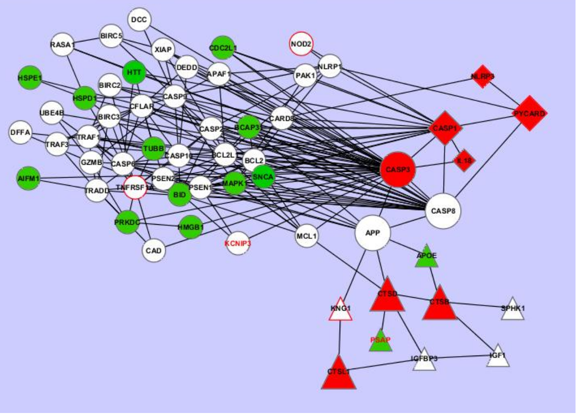
Dashed lines indicate points at which experimental variation and thus quantification errors can occur

Anal Bioanal Chem (2007) 389:1017–1031

Characteristics and applications of quantitative mass spectrometry methods

	Application	Accuracy (process)	Quantitative proteome coverage	Linear dynamic range[a]
Metabolic protein labeling	Complex biochemical workflows	+++	++	1–2 logs
	Comparison of 2–3 states			
	Cell culture systems only			
Chemical protein labeling (MS)	Medium to complex biochemical workflows	+++	++	1–2 logs
	Comparison of 2–3 states			
Chemical peptide labeling (MS)	Medium complexity biochemical workflows	++	++	2 logs
	Comparison of 2–3 states			
Chemical peptide labeling (MS/MS)	Medium complexity biochemical workflows	++	++	2 logs
	Comparison of 2–8 states			
Enzymatic labeling (MS)	Medium complexity biochemical workflows	++	++	1–2 logs
	Comparison of 2 states			
Spiked peptides	Medium complexity biochemical workflows	++	+	2 logs
	Targeted analysis of few proteins			
Label free (ion intensity)	Simple biochemical workflows	+	+++	2–3 logs
	Whole proteome analysis			
	Comparison of multiple states			
Label free (spectrum counting)	Simple biochemical workflows	+	+++	2–3 logs
	Whole proteome analysis			
	Comparison of multiple states			

Bioinformatics tools have a critical role in MS based proteomics data analysis



Pathway analysis done using PINA (http://csbi.ltdk.helsinki.fi/pina/ and Cytoscape (www.cytoscape.org)