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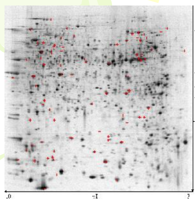
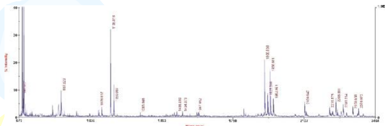
Proteomics provides information on:

- -protein expression levels
- -subcellular distribution
- -modifications
- -interactions

Proteomics has a key role in cell signalling studies

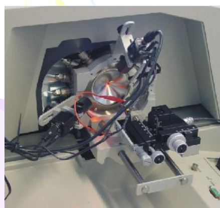




THE PROTEOME

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)

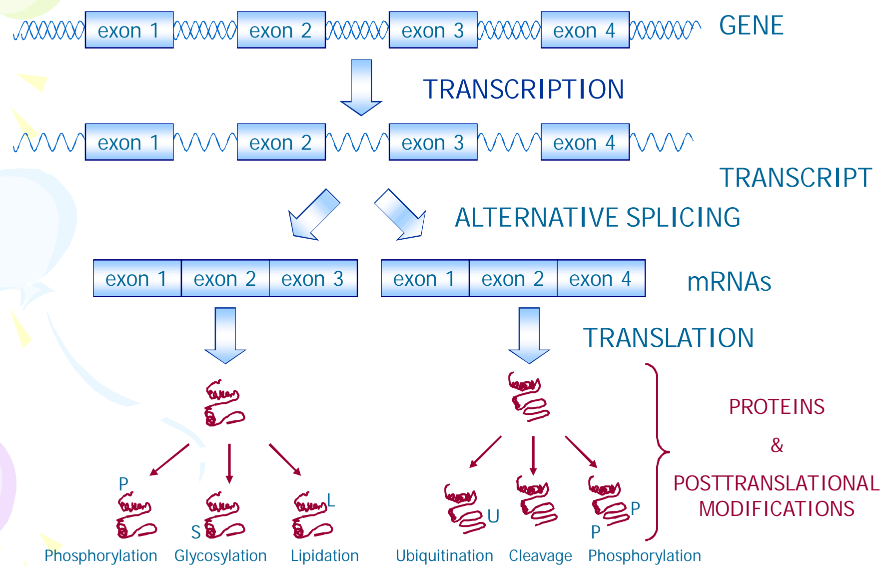


Methods in proteomics

Protein and peptide separation: electrophoresis (SDS-PAGE and 2-DE) and different forms of chromatography

Protein identification and characterisation: mass spectrometry

Complexity of proteome



(Figure adapted from Peng & Gygi 2001)

Proteomics levels

Expression proteomics

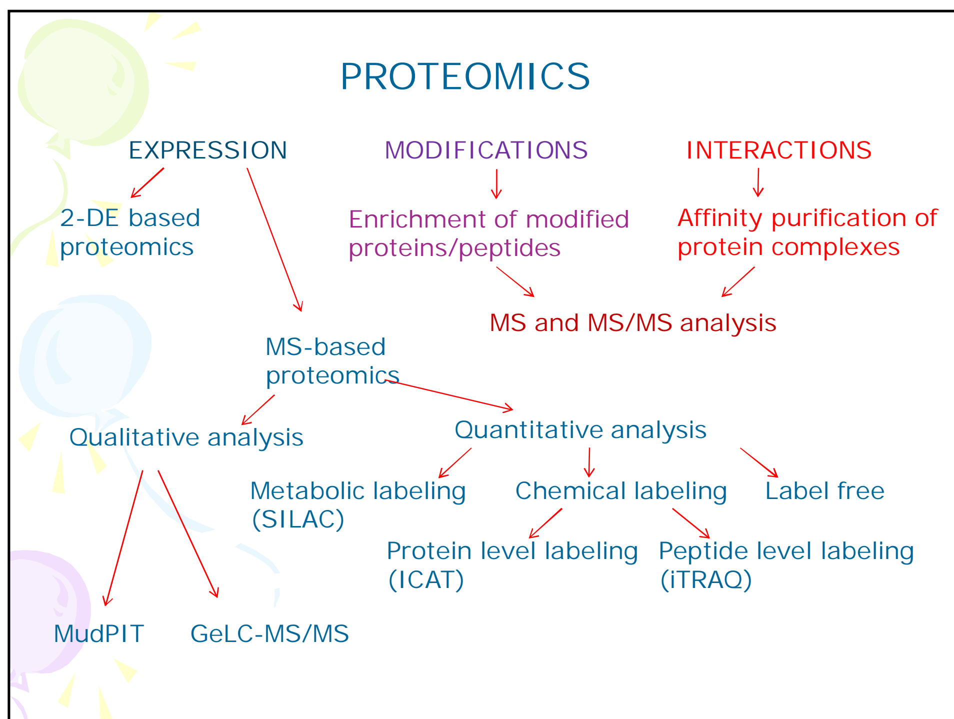
Which gene products are expressed, when and how much

PTM-omics, "Modificomics"

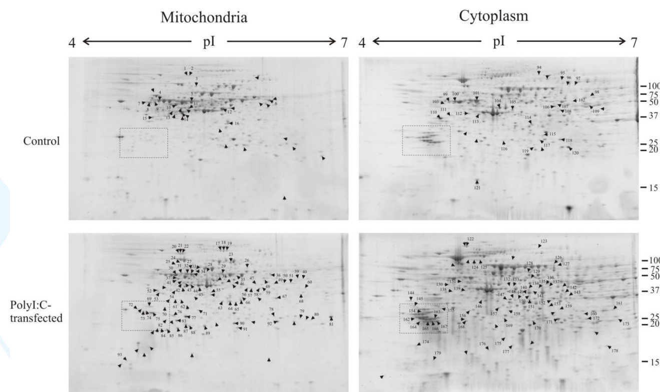
Which variants are present of each protein, when and how much

Cell map proteomics, "Interactomics"

Who interacts, when and where



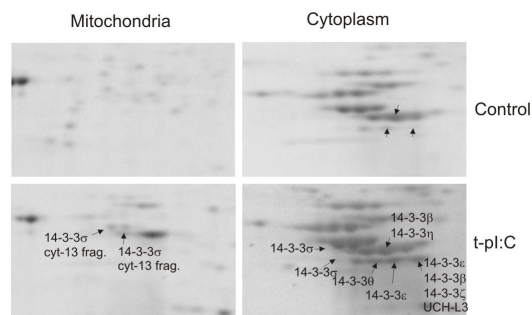
Two-dimensional electrophoresis based proteomics: Viral dsRNA induced differences in keratinocytes

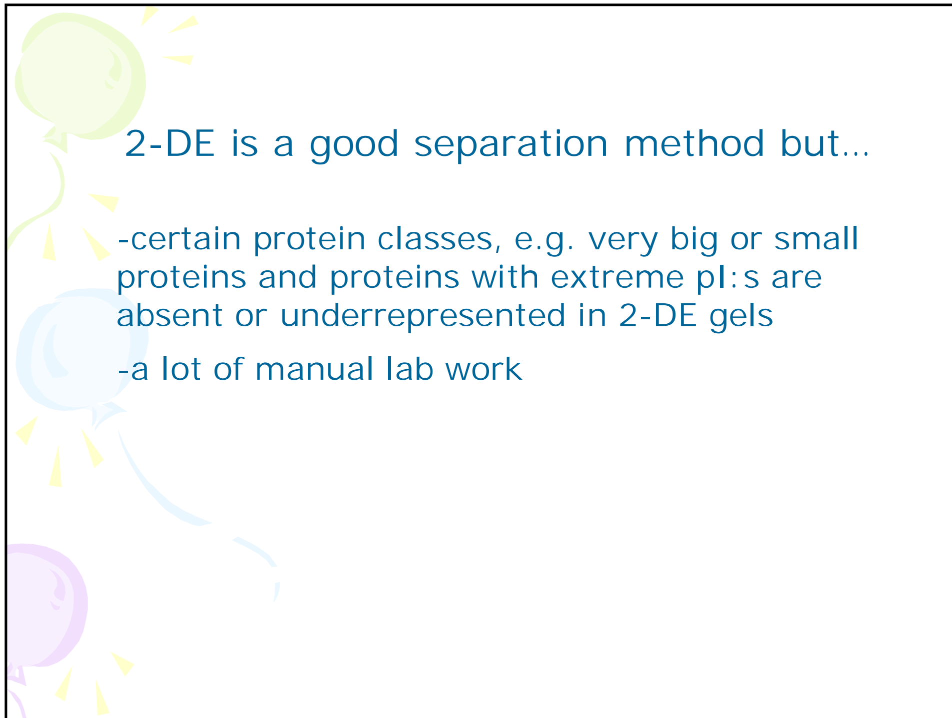


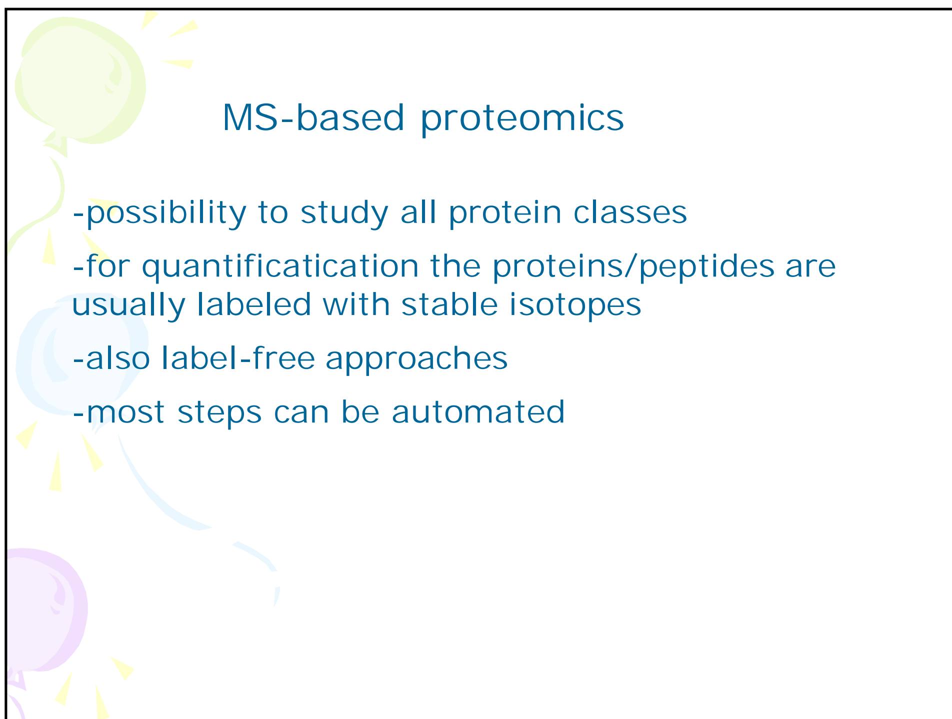
- 240 reproducibly differentially expressed protein spots
  - -137 in mitochondria (103 up- and 34 downregulted)
  - -103 in cytoplasm (70 up- and 33 downregulated)
- Proteins from 179 spots successfully identified

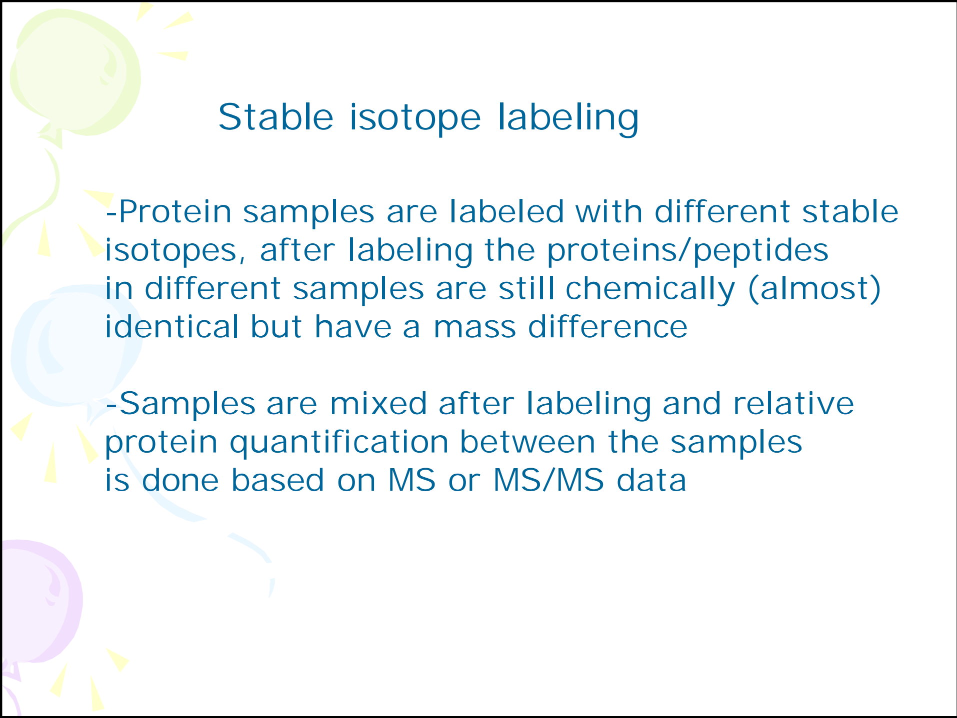
Two-dimensional electrophoresis

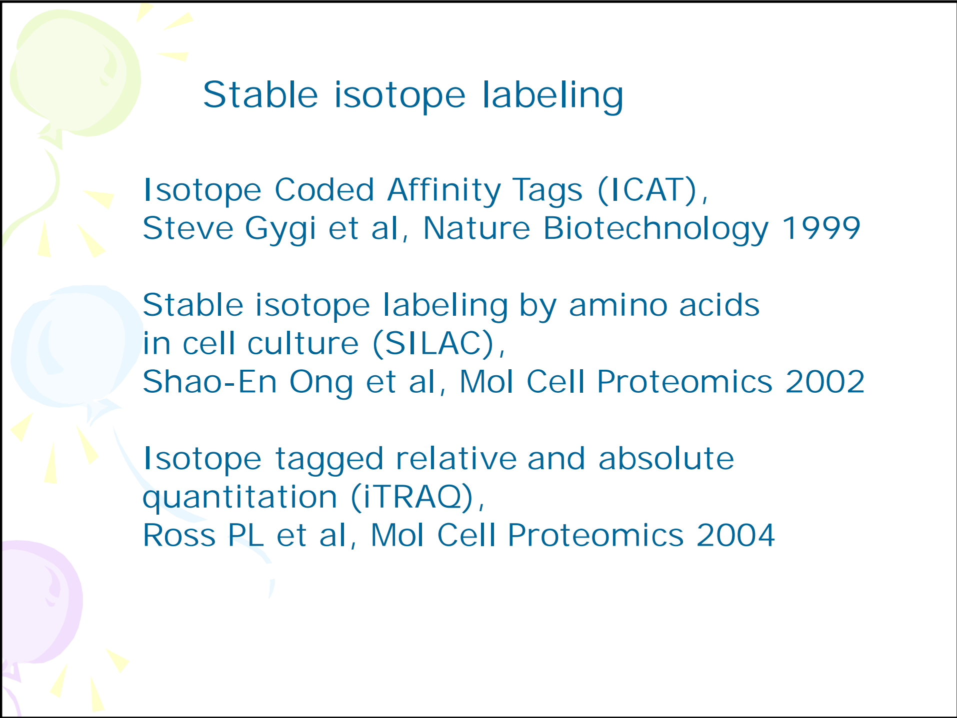
-proteins are separated according to their pI and molecular weight
-2-DE is an efficient method to separate very complex protein mixtures
-2-DE separates also protein isoforms into distinct spots



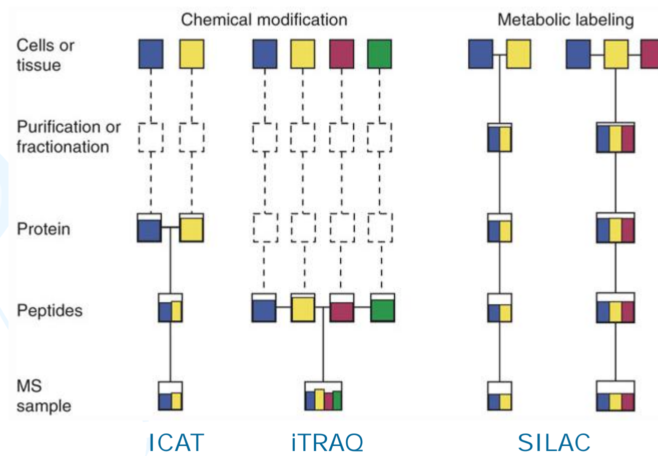

2-DE is a good separation method but...
-certain protein classes, e.g. very big or small proteins and proteins with extreme pI:s are absent or underrepresented in 2-DE gels
-a lot of manual lab work


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 can be done at different stages



ICAT iTRAQ SILAC

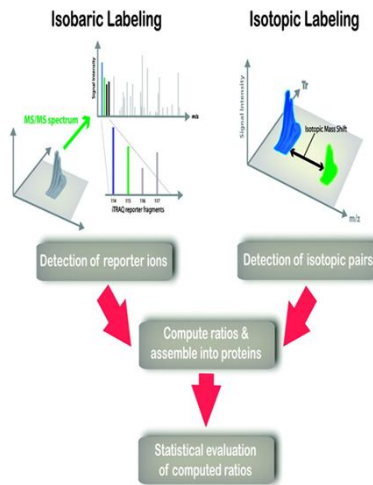
Ong SE, Mann M (2005) Nat Chem Biol 1:252–262

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 (TMT)

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 And TMT



Quantification based on MS data, e.g. ICAT and SILAC

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" ^{13}C- or ^{15}N-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™

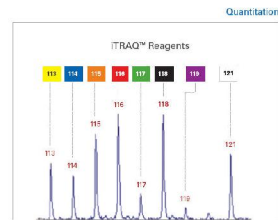
ICAT® Kits

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



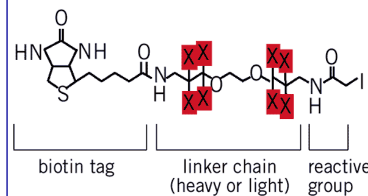
Innovation in Peptide-Tagging Chemistry

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.

Isotope-coded affinity tags: cysteine-spesific labeling

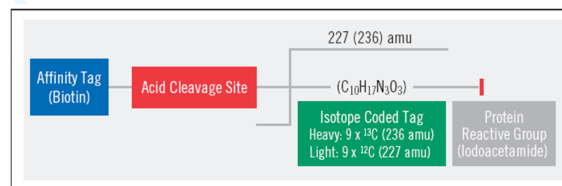
Isotope-Coded Affinity Tags

heavy reagent: D8-ICAT Reagent (X=deuterium)
light reagent: D0-ICAT Reagent (X=hydrogen)

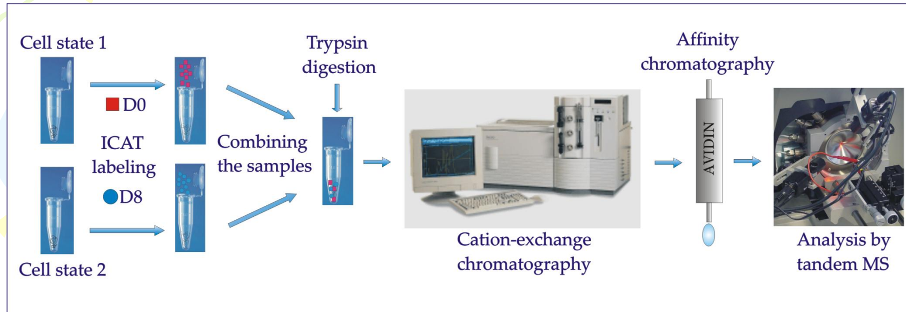


First generation ICAT reagent

Cleavable ICAT reagents

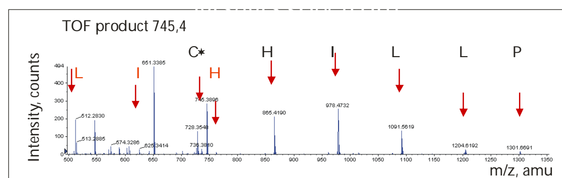


ICAT method

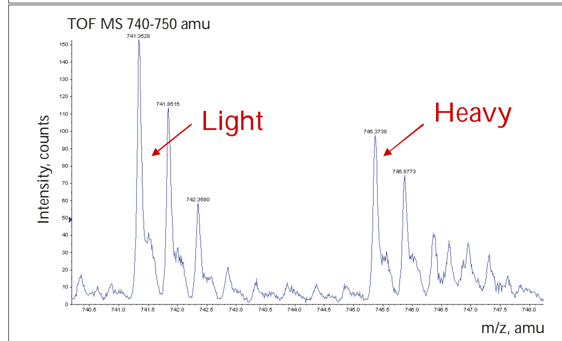


Protein identification and quantification in ICAT

Identification from MS/MS data



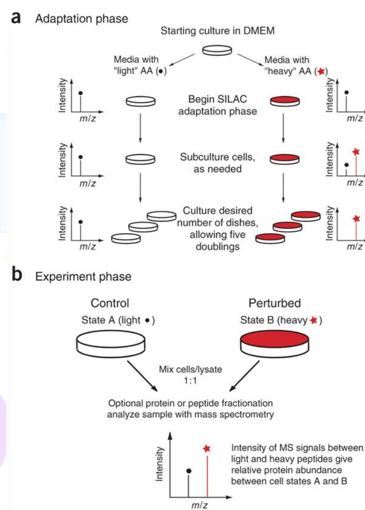
Quantification from MS data



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: in vivo incorporation of a stable isotope label into proteins



-two cell populations are grown in culture media that are identical except that one of them contains a 'light' and the other a 'heavy' form of a particular amino acid

-pioneering work by Matthias Mann's group

http://silac.org/index_html

SILAC Amino Acids

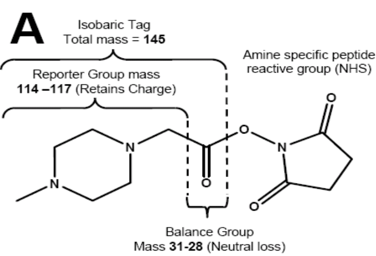
A variety of amino acids are suitable in SILAC and the use of arginine, leucine, lysine, serine, methionine and tyrosine have already been described in literature by several groups. The use of an essential amino acid that does not metabolize to a different amino acid is most desirable in order to avoid 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

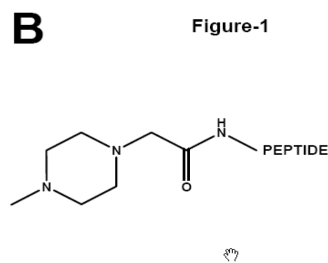
Prefractionation: protein separation by SDS-PAGE

iTRAQ: amine spesific labeling

A



B **Figure-1**



- Reactive group: N-oxysuccinimide (N-term + Lys)
- Reporter group (114-117 Da): N-methylpiperazine (enhances ionisation)
- Balance group (28-31 Da): Isobaric Tags (145 Da): Labelled forms have the same mass, although are distinguished in MS/MS spectrum

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

-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

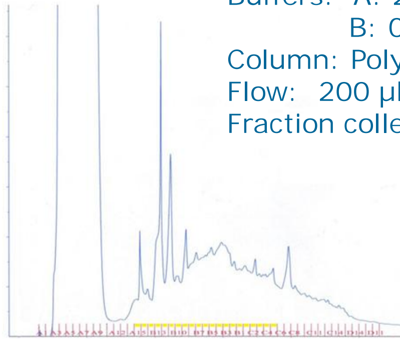
-disulphide bond reduction and Cys alkylation

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

-iTRAQ labeling after digestion

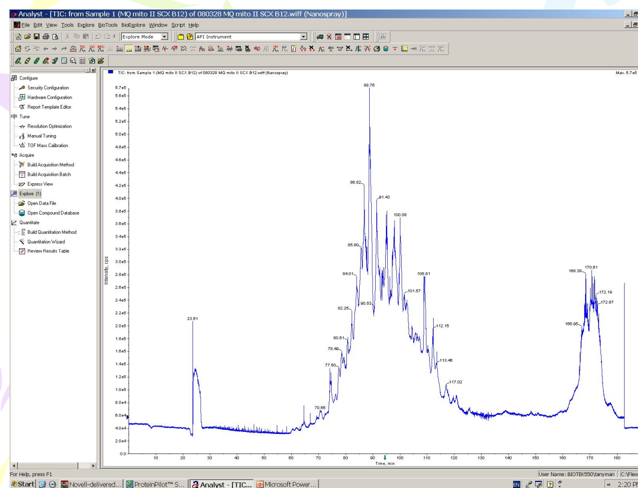
iTRAQ/ Peptide fractionation

Strong cation exchange (SCX) is most often used



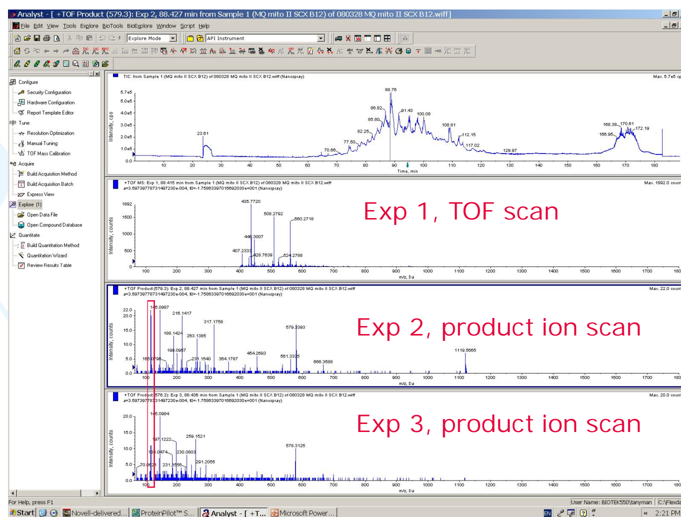
Buffers: A: 20 mM KH2PO4, pH 3
B: 0,4 M KCl in A buffer
Column: Polysulfoethyl A (200 x 2,1 mm)
Flow: 200 µl/min
Fraction collection: 1 min fractions

All the peptide containing SCX-fractions are analysed separately by LC-MS/MS

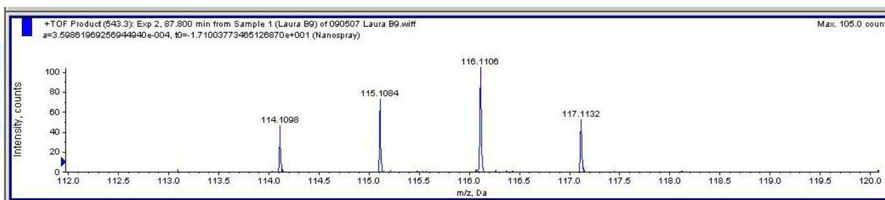


One LC-MS/MS run 180 min

Compared to 'normal' identification: Higher collision energy and focus on reporter ion region in MS/MS scans



Zoom into Repoter ion region: Quantification data



iTRAQ data processing

First check labeling efficiency:
-database search with fixed iTRAQ modifications
-database search without iTRAQ modifications
→ number and quality of IDs in these two?

Quality of reporter ions?
→ manual investigation of MS/MS data

If the labeling is OK and reporter ions are intense enough for quantification proceed with data processing

Protein ID and quantification method

Paragon Method

Paragon Method: iTRAQ SwissProt human Delete

Describe Sample

Sample Type: iTRAQ 4plex (Peptide Labeled)
Cys Alkylation: MMTS
Digestion: Trypsin
Instrument: QSTAR ESI
Special Factors: Phosphorylation emphasis, Gel-based ID, Urea denaturation, Methyl esterification
Species: Homo sapiens

Specify Processing

Quantitate
ID Focus: Biological modifications, Amino acid substitutions, User-defined modifications
Database: uniprot_sprot 080128
Search Effort: Rapid ID, Thorough ID
Detected Protein Threshold [Unused ProtScore (Conf)] >: 1.3 (95.0%)

iTRAQ Isotope Correction Factors

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

Save Save As... Cancel

Applied Biosystems, ProteinPilot 2.0

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

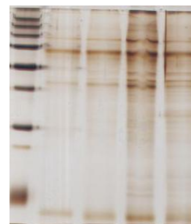
Bias correction: normalise uneven protein amounts in the samples

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

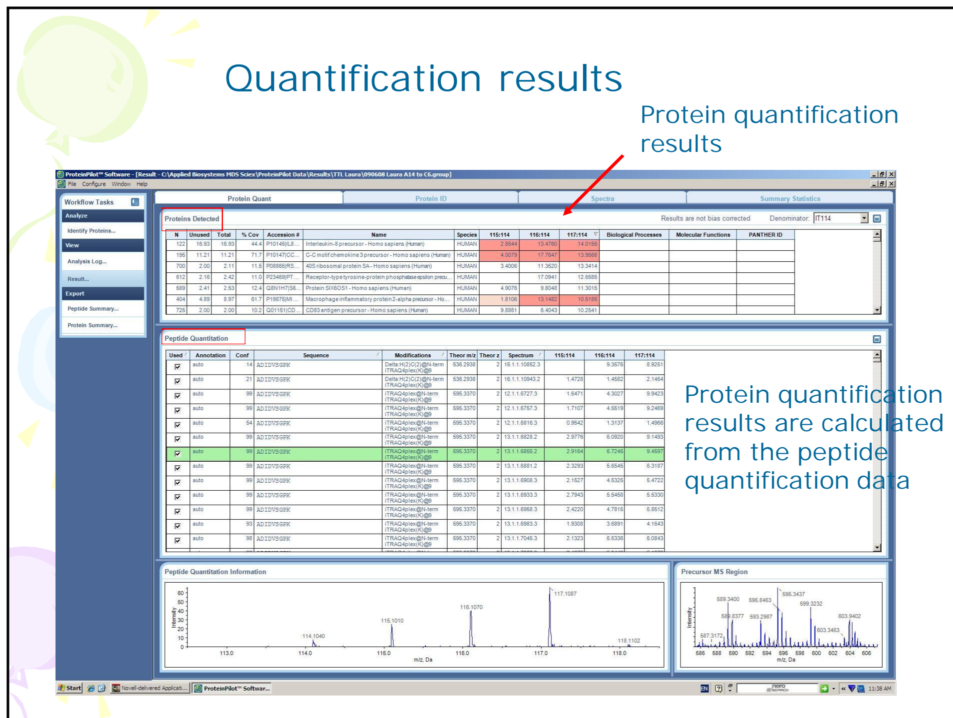
1. Control
2. LPS
3. β-glucan1
4. β-glucan2



1. 2. 3. 4.

Quantification results

Protein quantification results



Protein quantification results are calculated from the peptide quantification data

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

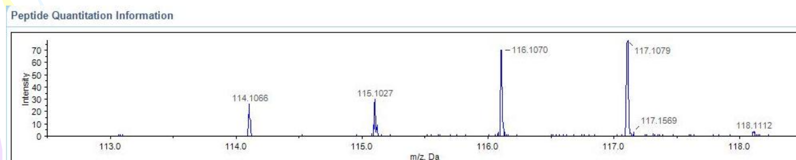
Color	P-value	Ratio
Dark red	< 0.001	> 1
Medium red	0.001 to < 0.01	> 1
Light red	0.01 to < 0.05	> 1
No color	>= 0.05	Any
Light blue	0.01 to < 0.05	< 1
Medium blue	0.001 to < 0.01	< 1
Dark blue	< 0.001	< 1

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

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.

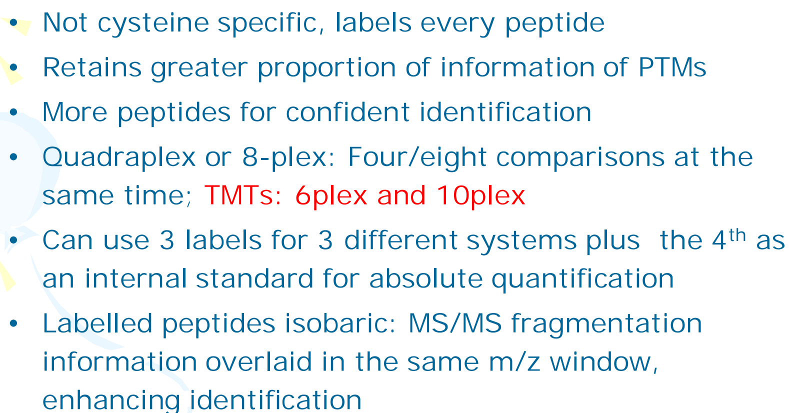
The Pro Group™ Algorithm assigns peptides to one of three types:

- 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.

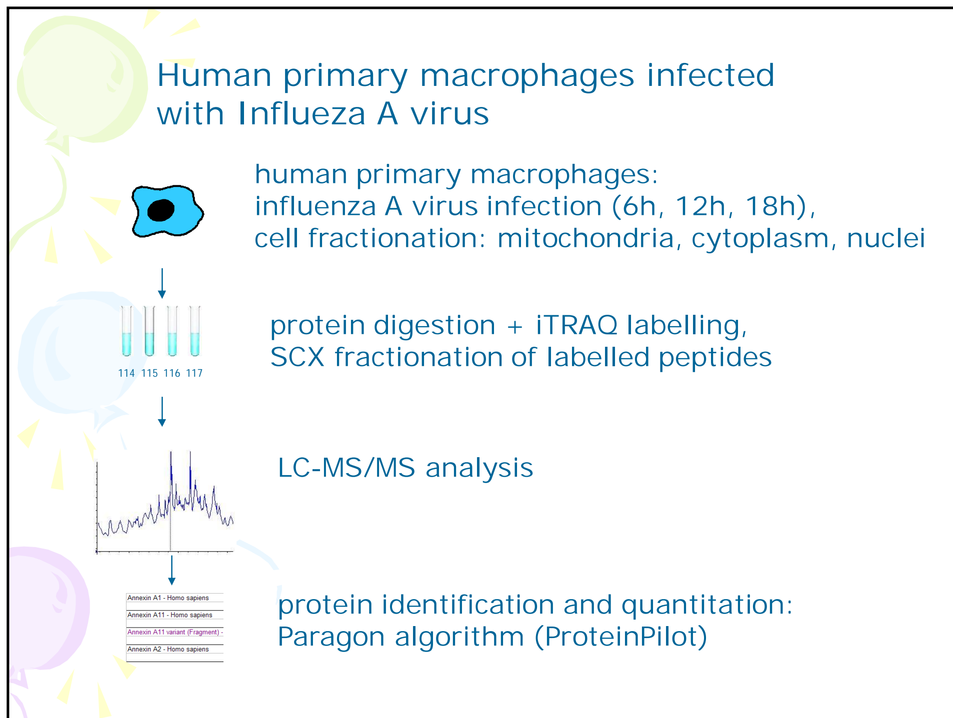
Peptide ratios can be manually included or omitted from average ratio calculation; see Manually Choosing Peptides for the Ratio Calculation.

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; TMTs: 6plex and 10plex
- 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



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

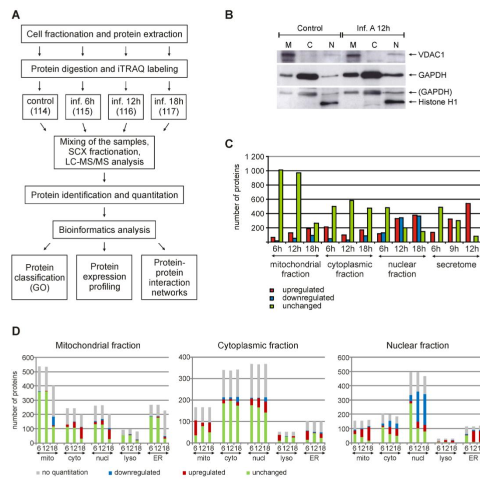
-each iTRAQ sample set was analysed twice with LC-MS/MS to improve the quality of protein identifications and quantifications

-intrument time needed:
3×2×2×20×3h=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

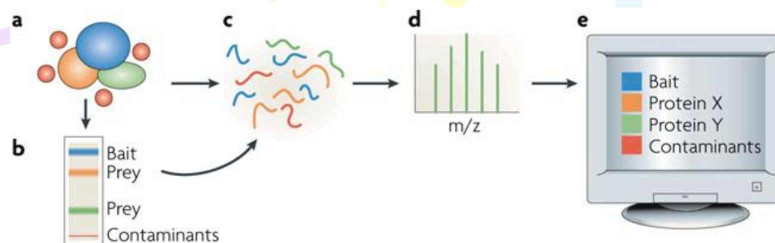
NOTE: This time is markedly reduced with current MS instruments with faster scanning speed!!

Influenza A Virus infection regulates the expression and/or subcellular localization of more than one thousand host proteins at early phases of infection



Lietzén et al, PLoSPathogens 2011

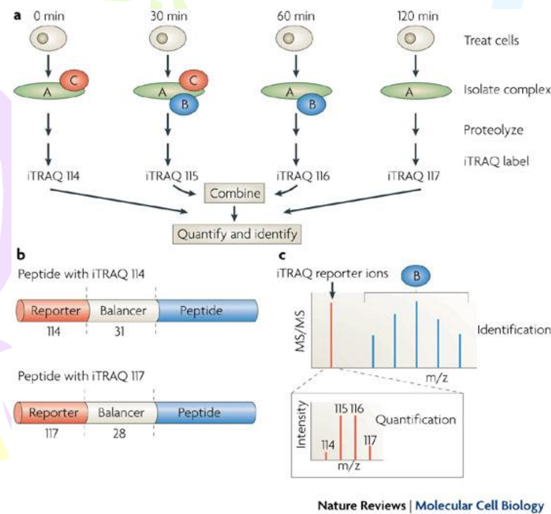
Analysis of protein complexes using mass spectrometry



Nature Reviews | Molecular Cell Biology

8, 645-654 (August 2007)

Isobaric tags to elucidate complex formation dynamics



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

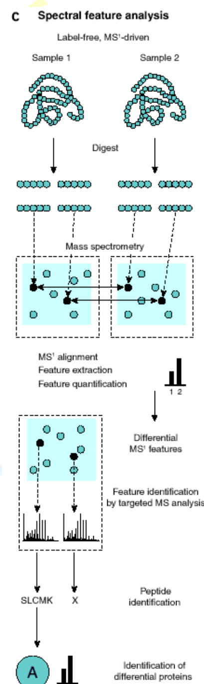
Label-free quantification

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)

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

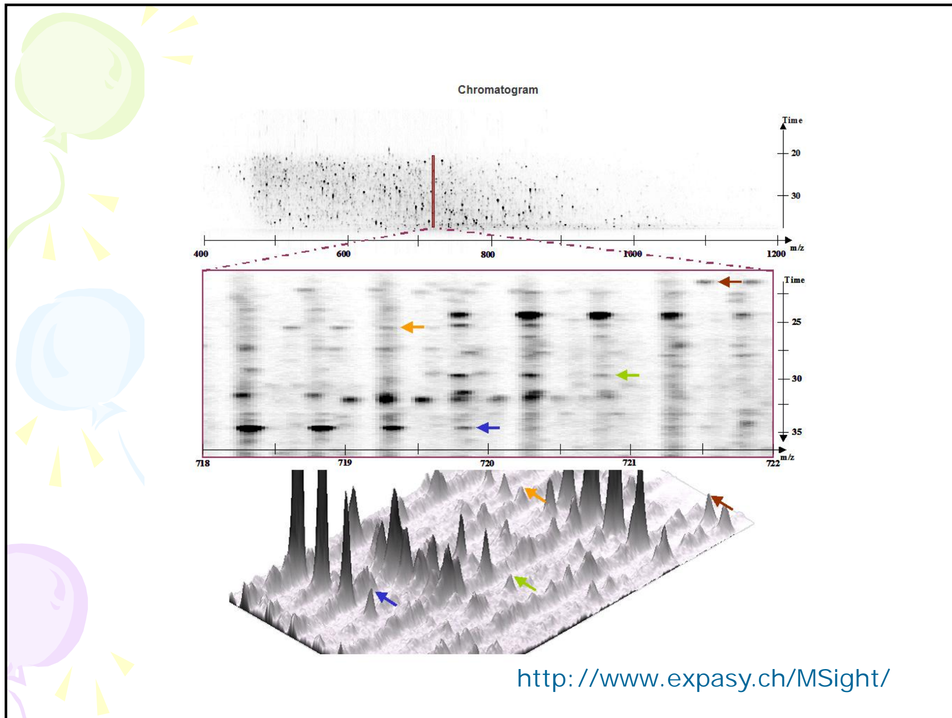
-allows analysis of a large number of spectrum features and allows higher data throughput

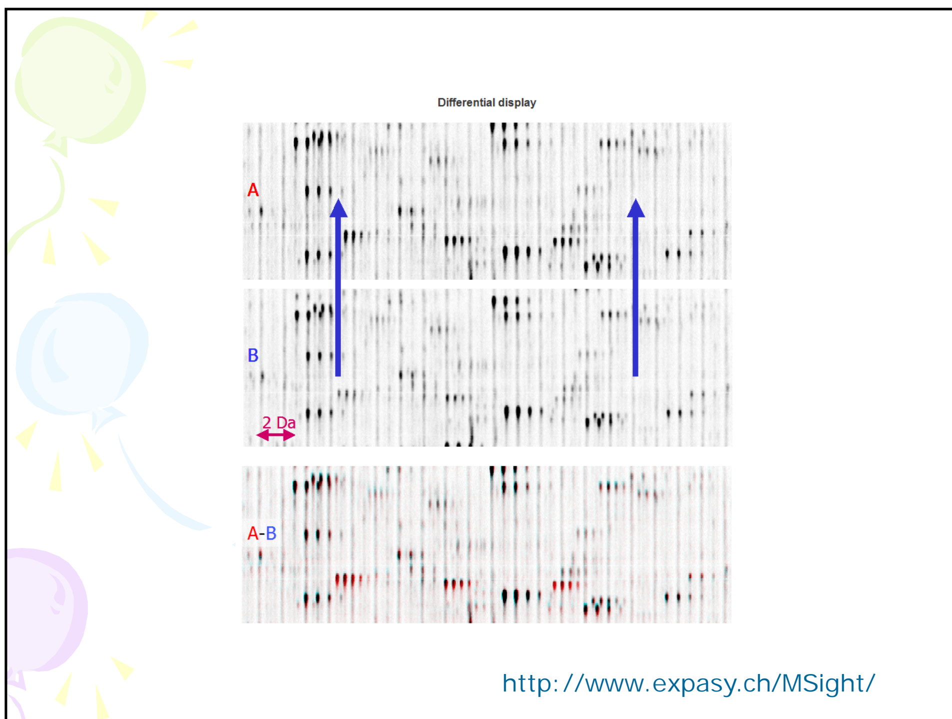
-is compatible with applications that require profiling of multiple biological samples e.g. biomarker discovery

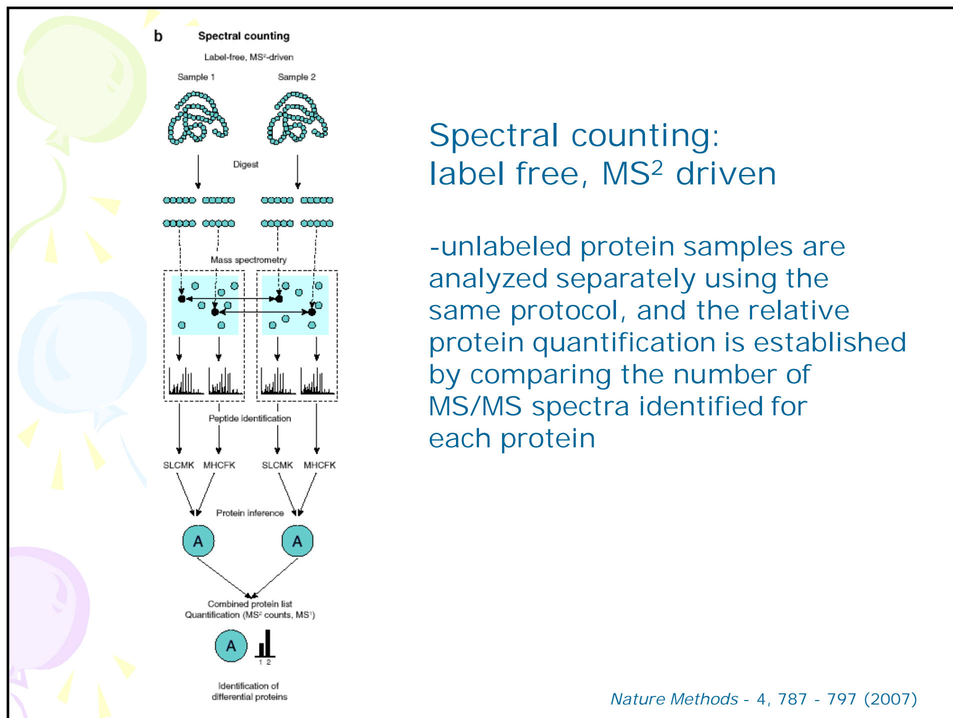
-does not require identification of the peptide sequence corresponding to each observed spectrum feature before quantification

-drawback: increased computational complexity

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/

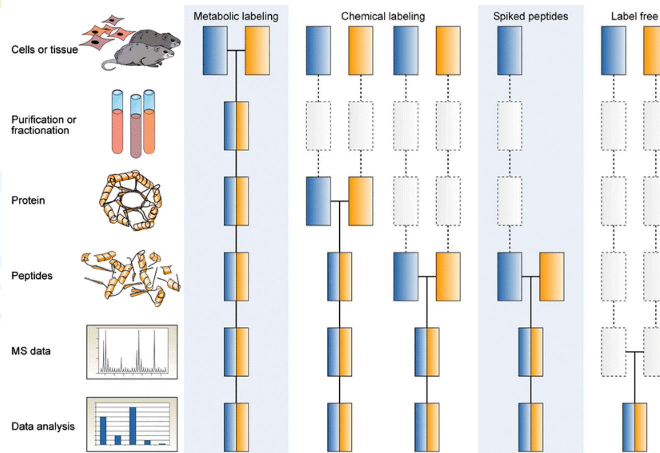


Spectral counting

-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

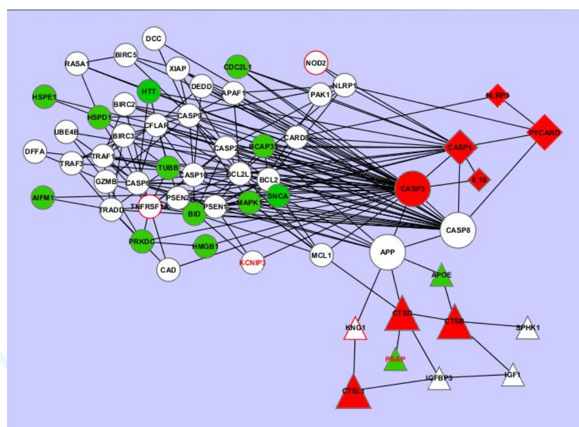
Quantitative MS based proteomics



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

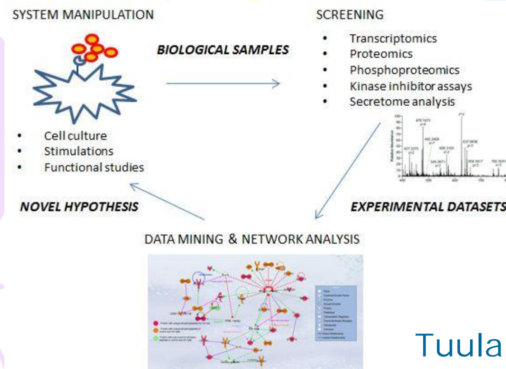
Anal Bioanal Chem (2007) 389:1017–1031

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)

Cell Signalling Networks in Activation of Innate Immunity



Tuula Nyman
Institute of Biotechnology
University of Helsinki
tuula.nyman@helsinki.fi

Activation of innate immunity is the first response to pathogen infection and tissue damage.

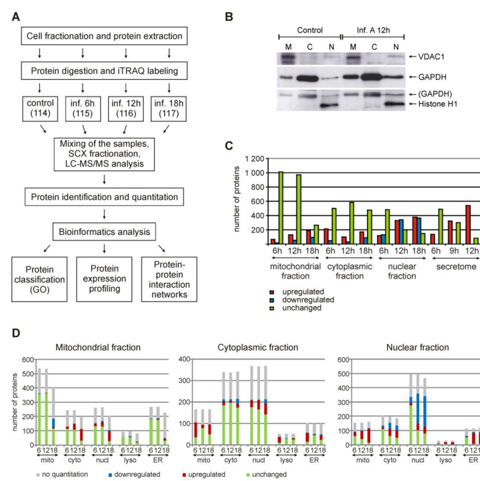
Our research is focused in the detailed characterisation of cell signalling networks involved in the activation of innate immunity.

We use advanced proteomic tools combined with bioinformatics and functional studies.

At present, we study on a proteome-wide level how activation of innate immunity influences global protein secretion and proteins' posttranslational modifications.

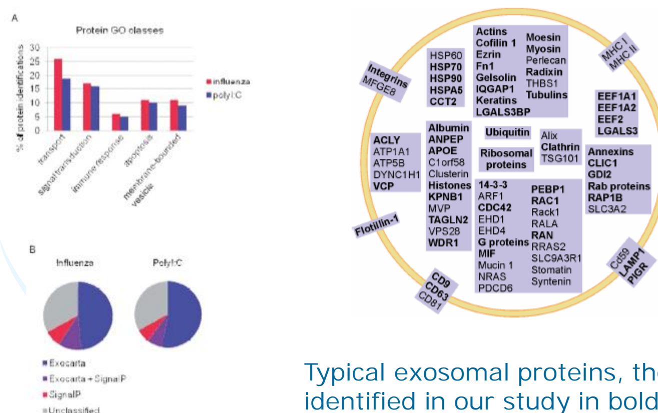
We are also interested in proteomics method development as well as in developing new software tools to enable more efficient and in-depth proteomic data analysis.

Influenza A Virus infection regulates the expression and/or subcellular localization of more than one thousand host proteins at early phases of infection



Lietzén et al, PLoSPathogens 2011

Classification of secretome proteins upon Influenza A virus infection

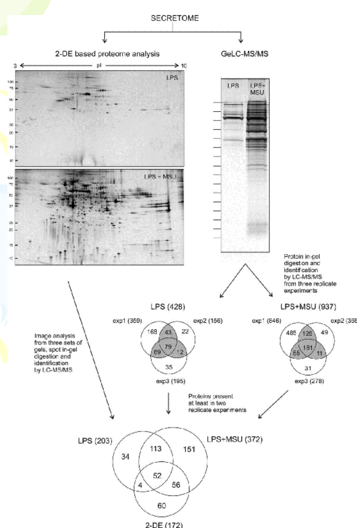


Typical exosomal proteins, the proteins identified in our study in bold

Secretome analysis of human primary macrophages stimulated with MSU

- Monosodium urate (MSU) is an endogenous danger signal that is crystallized from uric acid released from injured cells.
- MSU is known to activate inflammatory response in macrophages but the molecular mechanisms involved have remained uncharacterized.
- Activated macrophages start to secrete proteins to activate immune response and to recruit other immune cells to the site of infection and/or tissue damage.
- Secretome characterization after activation of innate immune system is essential to unravel the details of early phases of defense responses.

Secretome analysis of MSU-stimulated human primary macrophages.



-quantitative two-dimensional gel electrophoresis based proteomics as well as high-throughput qualitative GeLC-MS/MS analysis combined with bioinformatics and functional studies

-priming with bacterial lipopolysaccharide for 21h followed by MSU stimulation for 3 h

Välimäki E et al. Mol Cell Proteomics 2013;12:749-763

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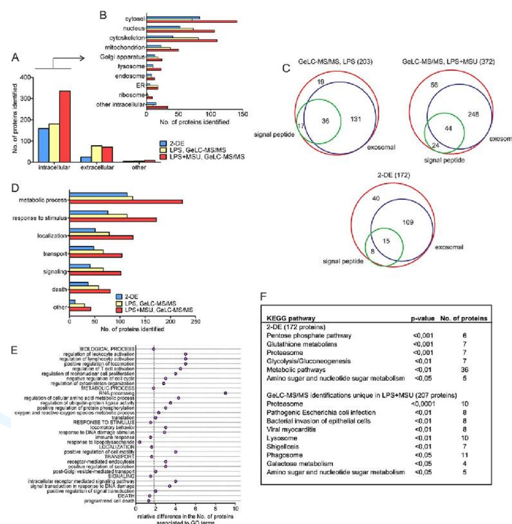
Classification of the secretome proteins.



KEGG pathway	p-value	No. of proteins
2-DE (172 proteins)		
Pentose phosphate pathway	<0,001	6
Glutathione metabolims	<0,001	7
Proteasome	<0,001	7
Glycolysis/Gluconeogenesis	<0,01	7
Metabolic pathways	<0,01	36
Amino sugar and nucleotide sugar metabolism	<0,05	5
GeLC-MS/MS identifications unique in LPS+MSU (207 proteins)		
Proteasome	<0,0001	10
Pathogenic Escherichia coli infection	<0,01	8
Bacterial invasion of epithelial cells	<0,01	8
Viral myocarditis	<0,01	8
Lysosome	<0,01	10
Shigellosis	<0,01	7
Phagosome	<0,05	11
Galactose metabolism	<0,05	4
Amino sugar and nucleotide sugar metabolism	<0,05	5

Välimäki E et al. Mol Cell Proteomics 2013;12:749-763

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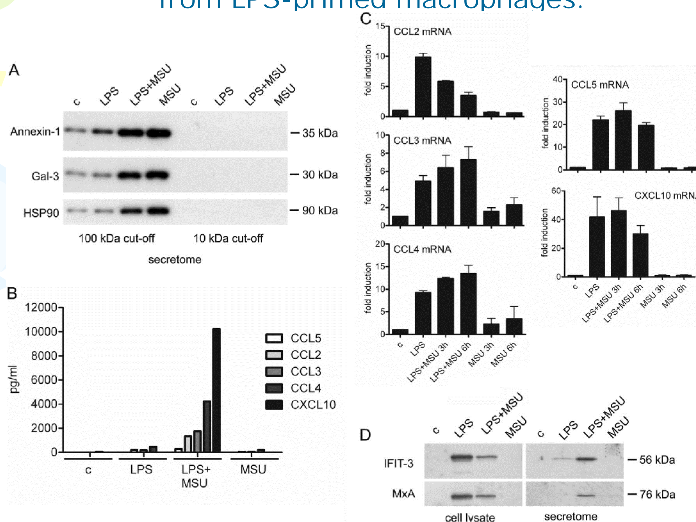
The secretion of danger signal proteins, chemokines and interferon-induced proteins is induced on MSU stimulation from LPS-primed macrophages.



Välimäki E et al. Mol Cell Proteomics 2013;12:749-763

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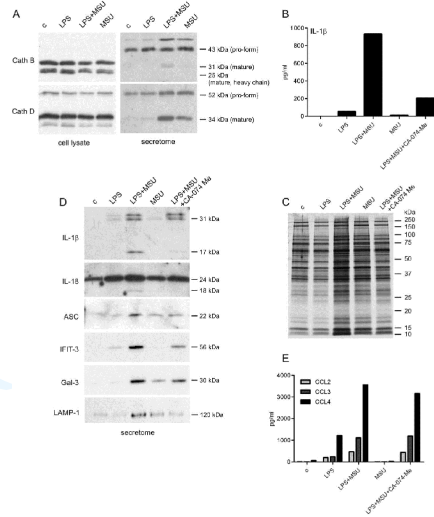
Cathepsin activity is required for unconventional protein secretion on MSU stimulation.



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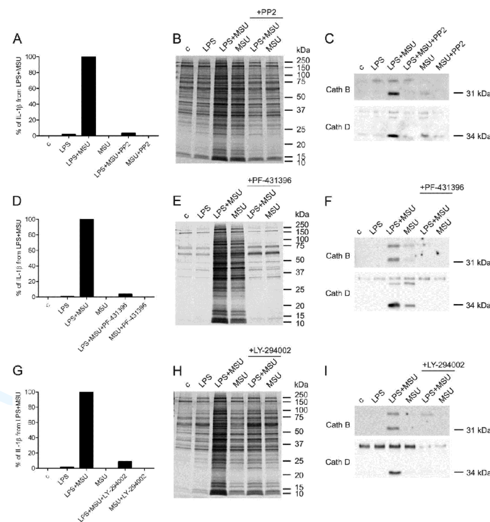
MSU-induced protein secretion is dependent on the activity of Src, Pyk2, and PI3 kinases.



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Schematic model of MSU-induced signaling and protein secretion in human macrophages.

Välimäki E et al. Mol Cell Proteomics 2013;12:749-763

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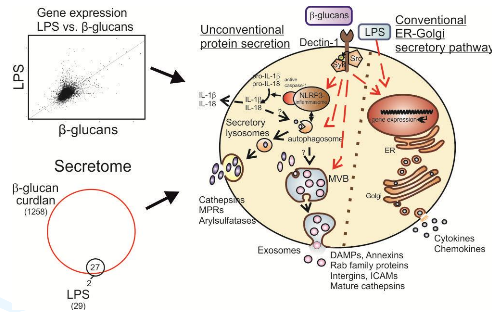
Systems biology approach to provide the first comprehensive characterization of the secretome and associated intracellular signaling pathways involved in activation of dectin-1/Syk in human macrophages

- Dectin-1 is a membrane-bound pattern recognition receptor for β-glucans, which are the main constituents of fungal cell walls.
- Detection of β-glucans by dectin-1 triggers an effective innate immune response.

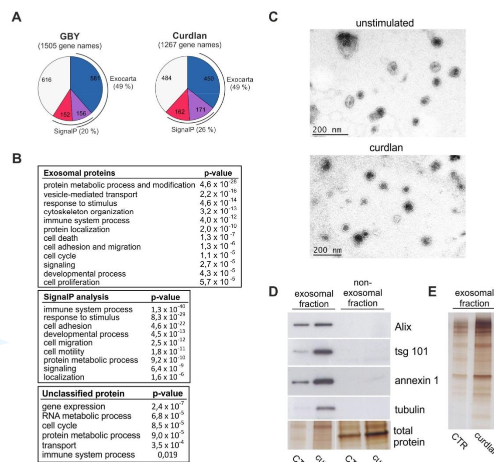
Dectin-1 Pathway Activates Robust Autophagy-Dependent Unconventional Protein Secretion in Human Macrophages



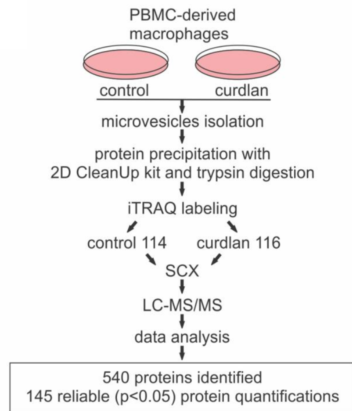
Both β-glucan and LPS stimulation induce significant gene expression changes in macrophages, but only β-glucans activate a robust protein secretion

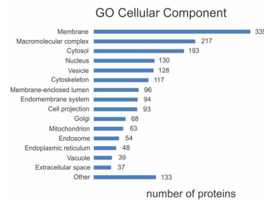
Öhman *et al*, J Immunology 2014

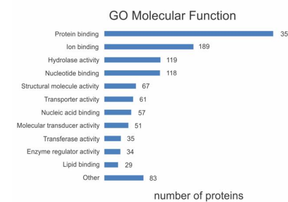
β-glucans activate a robust protein secretion through conventional as well as unconventional, vesicle-mediated protein secretion mechanisms



Quantitative proteomics of extracellular vesicles released from human primary macrophages upon β-glucan stimulation

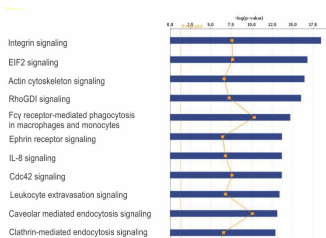




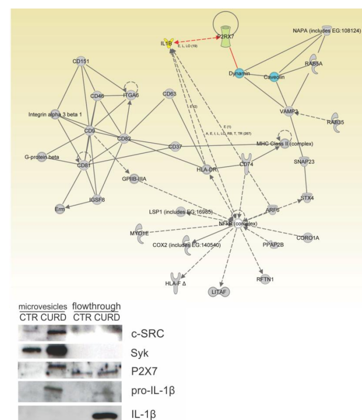


Cyprykn *et al*, J Prot Res 2014

Proteomic study identified 540 distinct proteins from the vesicles including several receptors which have not been identified from vesicles before. Bioinformatics analysis strongly suggests a significant role for these proteins in transfer of biological information.



Networks	Focus molecules	Network score
Cellular movement, Hematological system development and function, Immune cell trafficking	16	15
Cell-to-cell signaling and interaction, Hematological system development and function, Immune cell trafficking	14	14
Cell death and survival, Cellular function and maintenance, Cellular movement	14	12
Cardiovascular system development and function, Organismal development, Cell death and survival	13	11
Cell-to-cell signaling and interaction, Tissue development, Cellular movement	12	10
Cellular movement, Hematological system development and function, Immune cell trafficking	12	10



editorial Journal of proteome research

Can Proteomics Retire the Western Blot?

Here I argue that true integration of proteomics technology into molecular biology laboratories could be a paradigm shift for all of biology and biomedicine

What do we have to do to make this happen? Basically, we have to make quantitative proteome measurements as accessible and convenient as western blots are now.

A kind of Moore's law is in effect in proteomics, drastically enhancing our analytical capabilities every year. Routine and quick analysis of whole proteomes may be closer than we think.

Equally important, proteomics should be incorporated into the education of biologists, so that the next generation of biologists will naturally turn to this powerful technology.

Matthias Mann
Max Planck Institute of Biochemistry (Germany)