

# Mass Spectrometry of Proteins (Biomolecules)

**Proteomics team:**

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Institute of Organic Chemistry and Biochemistry AS CR, v.v.i.

# Request for MS Proteomic Analysis

## New reQuest (MS)



Please note that detailed description of your sample and requested analysis for good results. Provide us with as many details as possible.

### REQUEST DESCRIPTION

Sample Name

Required field! Specify your sample name.

User's Private Note

This note will not be visible for an operator.

Request Type:  Small Molecule Analysis  Proteomic Analysis

Specify your analysis type. (If JavaScript is enabled in your browser, this form will be modified based on your selection. Otherwise all the fields are visible.)

### ANALYSIS DESCRIPTION

Requested Analysis

Required field! Specify the analysis you request.

Results Data Output:  Summary Report  Detailed Report

Select a format for your results. (Please specify requirements for detailed report in Additional Information.)

Sample Return Requested

Check if you want to return remaining sample material.

## IOCB reQuest - Login

E-mail



.....



Login

[NOT REGISTERED?](#) [FORGOT YOUR PASSWORD?](#)

### SAMPLE CHARACTERISTICS

Molecular Formula / Expected Mass

 / 

Specify expected molecular formula (e.g. C34H32O4N4Fe) and monoisotopic mass

Sample Form:  Liquid  Solid  Gel

Specify your sample form. (Please provide a gel image in the section below if applicable.)

Quantity or Concentration

Specify your sample quantity for solid samples (e.g. 10 mg) or concentration for liquid samples (e.g. 10 mg/ml).

Concentration Based On

Please specify the procedure used to measure the concentration (e.g. BCA, Lowry or Bradford).

Buffer Composition and Suspected Impurities

Required field! Specify your sample buffer and suspected impurities (e.g. urea, phosphate, etc.).

Storage and Special Handling

Refrigerator  Freezer  Light Sensitive  Moisture Sensitive

Specify your sample storage and handling requirements.

### PROTEIN / PEPTIDE SEARCH

Protein or Peptide Sequence

Specify protein or peptide sequence.

Modifications

Specify known or suspected modifications (e.g. carbamidomethyl (C), oxidation (M) etc.).

Taxonomy

Specify taxonomy of your sample origin.

Database

Specify database to be used for search.

### STRUCTURE OR GEL IMAGE

Image File  Soubor nevybrán.

Please upload the structure or gel image file (jpg, png or gif) and it will be added to the printed form automatically.

### ADDITIONAL INFORMATION

Additional Notes

# Biomolecules

Soft ionization techniques necessary

MALDI

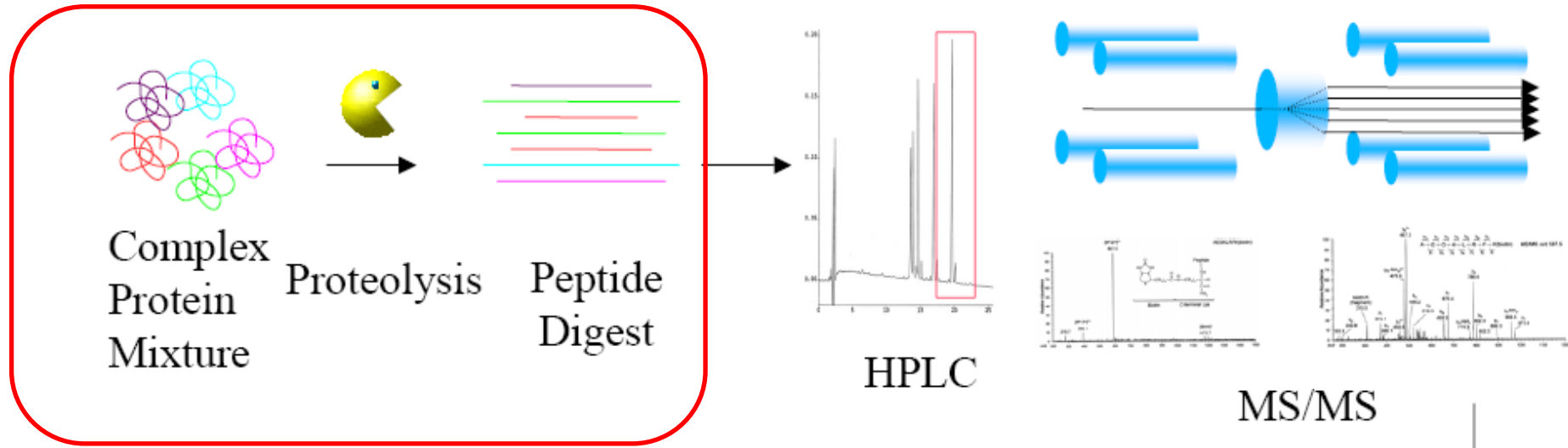
ESI

## Proteins

- **Identification** – follow this presentation
- **Characterisation** - different presentation
- **Quantification** - different presentation

# Identification of proteins by Tandem MS

Sample preparation



Protein Database

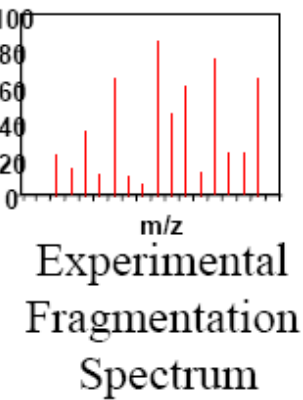
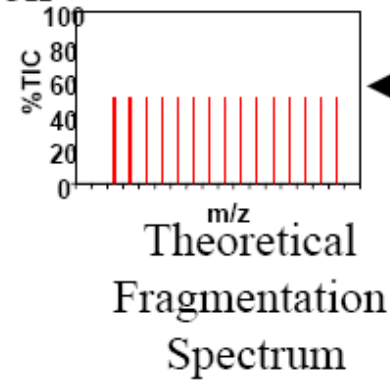
MRNSYRFLASSL  
 SVVVSLLIPED  
 VCEKIIGNEVT  
 PHSRPYMLLSL  
 DRKTCAGALIA  
 KDVLTAHCNLS  
 NKRSQVILGAHS  
 ITTYEPTKQIML  
 VKKEFPYPCYDP  
 ATREGDLKLLQE

*In Silico*  
Digestion

LASSLSVVVSLLCEK  
 IIGNEVTPHSR  
**PYMLLSLDR**  
 TICAGALIAK  
 DWVLTAHCNLSNKR  
 ITTYEPTK  
 QIMLVK  
 EFPYPCYDPATR  
 EGDLLK

*In Silico*  
Fragmentation

P YMLLSLDR  
 PYM VLLSLDR  
 PYMV LLSLDR  
 PYMVL LSLDR  
 PYMVLL SLDR  
 PYMVLLS LDR  
 PYMVLLSL DR  
 PYVLLSLD MR  
 PYMVLLSLD R



# Sample preparation

*First question to be asked:*

*Is my Sample Buffer compatible with*

- Enzymatic digestion ?
- LC separation ?
- nanospray (the most intolerant step – no detergent, very low salt concentration) ?

Answer: **YES**



Proceed with general digestion procedure

Answer: **NO**



Can we find a way how to make it work?

# General Digestion Procedure

- Dissolve in basic pH buffer (ammonium bicarbonate pH 8)
- Reduction of SS by **Dithiothreitol**
- Alkylation by **Iodoacetamide**
- Add **Trypsin** in 1:50 – 1:200 ratio (enzyme:protein)
- 10 hours digestion (overnight)
- evaporation
- Dissolve peptides in 0,1% Formic acid in water
- Ready to be analysed by LC-MS/MS

Similar procedure applied to **gel band** with the exception of **destaining** the gel, working with **limited volume of buffer** and **extraction** of the peptides after digestion

# When modification needed?

- My sample contains too much salt
  - desalt the sample prior or after digestion by C18 (C4) SPE column
- My sample contains detergent
  - **do not bring it!**
    - or we can try to apply*
      - detergent removal spin column
      - on-filter digestion with the washing steps before – eFASP
- I am interested not only in soluble proteins – e.g. membrane proteome, deep coverage of proteins
  - consider on filter digestion with adding of detergent on purpose - eFASP

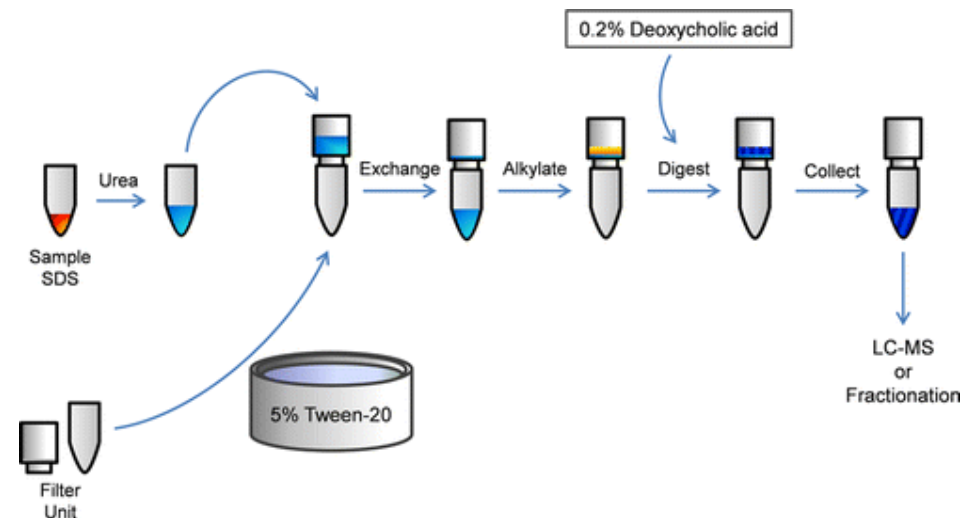
I need higher coverage of my protein  
or  
tryptic peptides does not work for me

- Digest with different protease than trypsin
  - Chymotrypsin, AspN, GluC, LysC, Pepsin, ArgC
- Or chemicaly
  - CNBr – cleave at Met



# Different digestion conditions

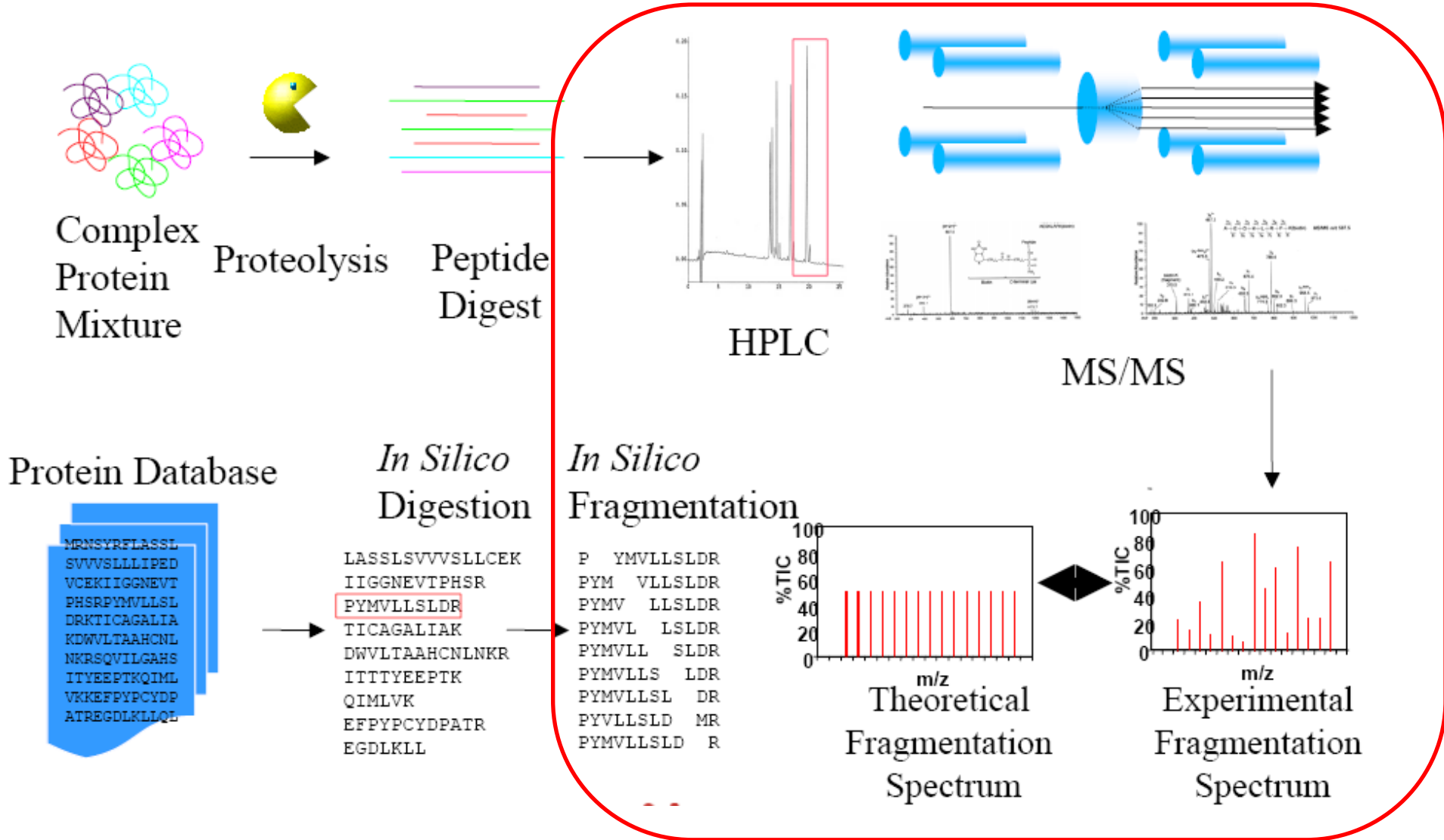
- Digestion in presence of **detergent**
  - Rapigest – acid labile detergent
  - deoxycholic acid, sodium deoxycholate – can be removed by extraction by ethyl acetate after digestion
- **FASP - Filter Aided Sample Preparation procedure**
  - Start with 4% SDS buffer, dissolve your proteins and place it on 10kDa filter unit
  - Wash 3 times with 8M Urea, 3 times with Ammonium bicarbonate, filter
- **eFASP – enhanced Filter Aided Sample Preparation procedure**
  - Similar to FASP with addition of deoxycholic acid for digest and removal by ethyl acetate

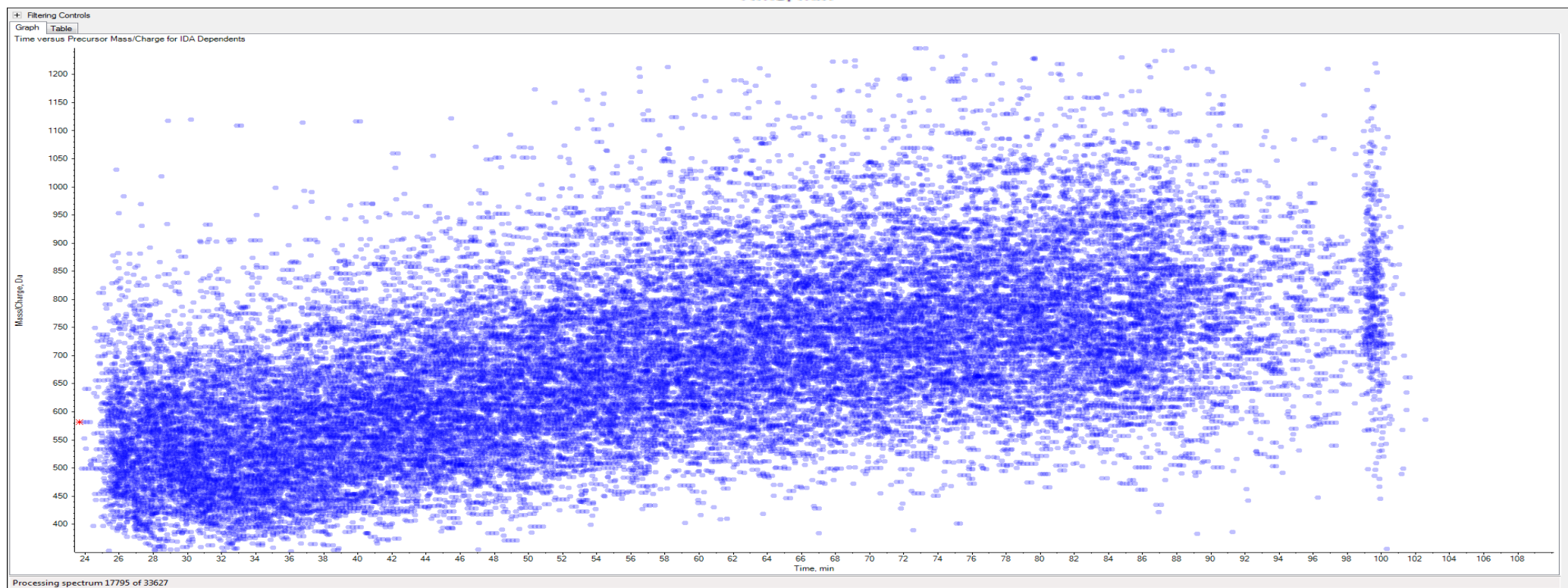
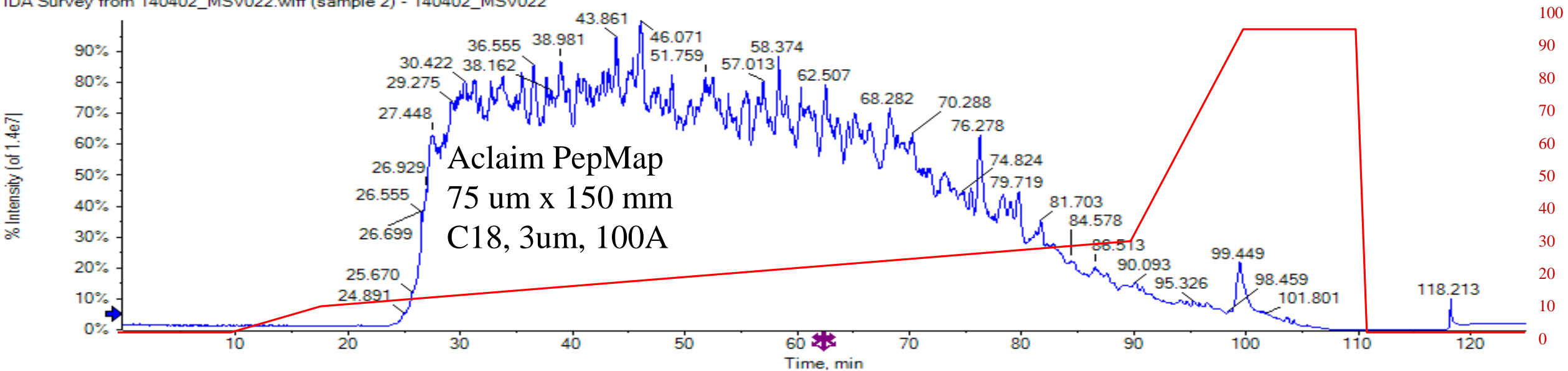


# Ideal digestion conditions of proteins

- **Unfold** the protein/proteins of interest
- Completely **denatured** protein to be digested yet do not affect the protease itself
- Use the **minimum amount of protease** to protein to prevent partial digestion - autoproteolysis
- **Specific cleavage** - cleave the proteins after every lysine and arginine (valid for trypsin only) – if not miscleavages occur

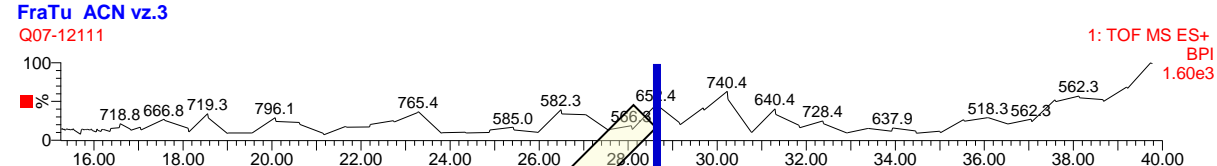
# Identification of protein by Tandem MS



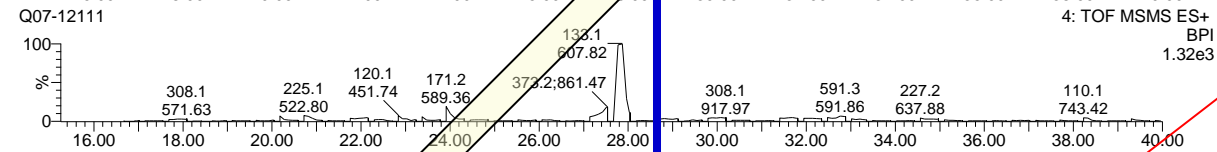


# Data Dependent Analysis DDA

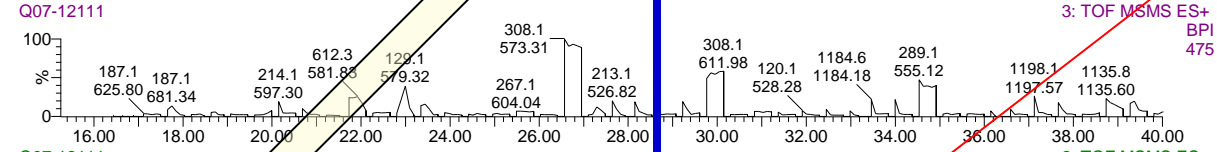
MS



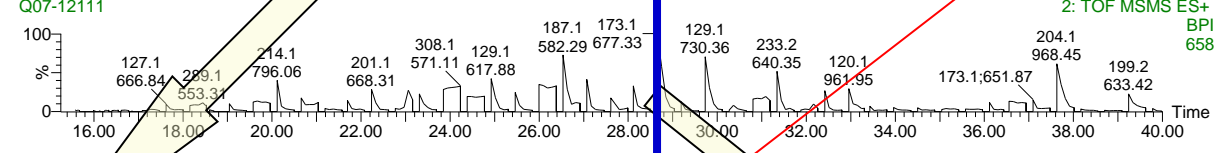
MSMS 1



MSMS 2



MSMS 3

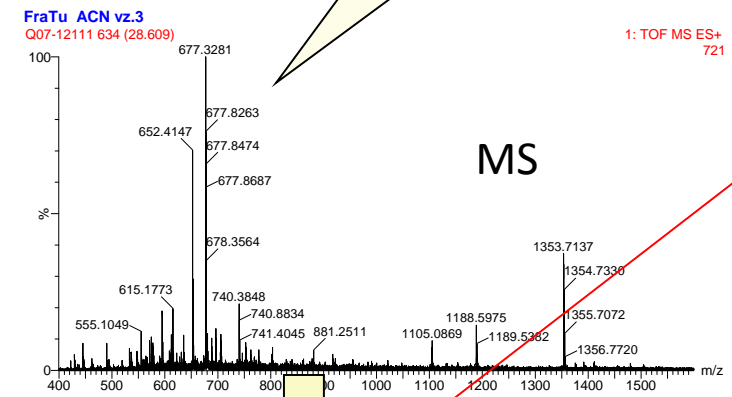


## Smoothing and deisotoping

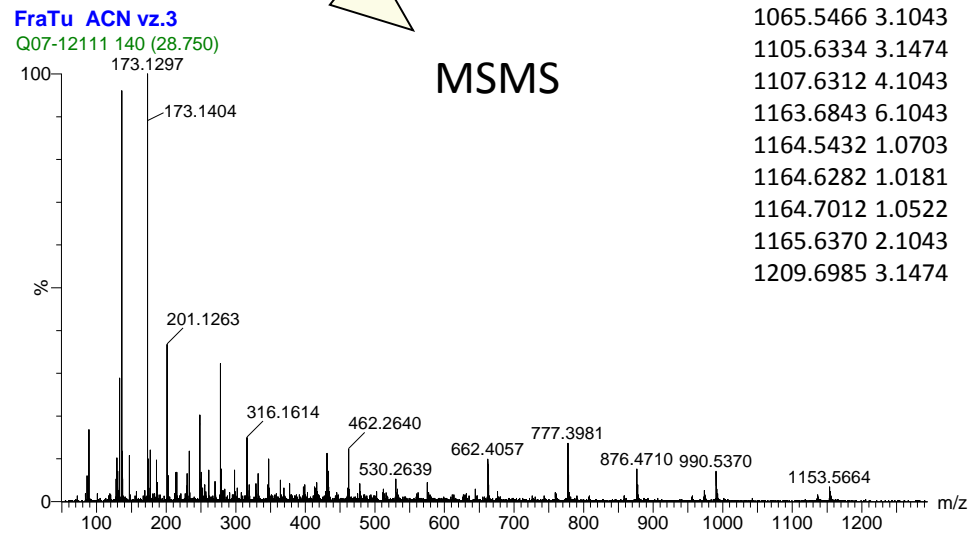
677.3281, 721, 2

- 70.0332 1.0794
- 70.0671 8.1202
- 72.0876 11.3469
- 73.5504 2.1043
- 74.0656 3.1383
- 83.0607 2.1134
- 84.0828 25.2925
- 85.0589 1.0522
- 85.0898 3.0612
- 86.1002 48.8639
- 96.0747 4.1043
- 98.0287 3.1927
- 98.0612 2.2358
- 98.1019 5.9909
- ...
- 283.2370 2.1927
- 284.1254 11.0181
- 284.1629 37.8798
- 284.2126 9.7188
- 285.0384 4.7891
- ..
- 1065.5466 3.1043
- 1105.6334 3.1474
- 1107.6312 4.1043
- 1163.6843 6.1043
- 1164.5432 1.0703
- 1164.6282 1.0181
- 1164.7012 1.0522
- 1165.6370 2.1043
- 1209.6985 3.1474

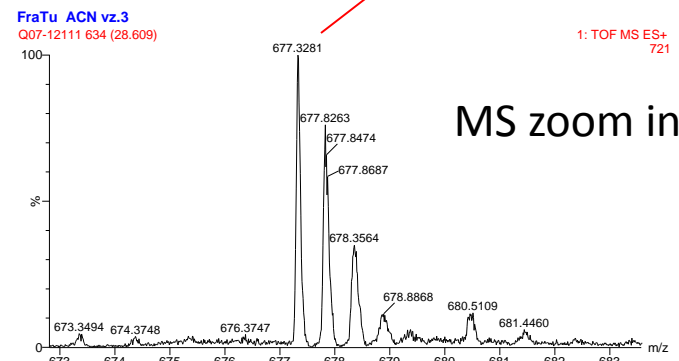
MS



MSMS

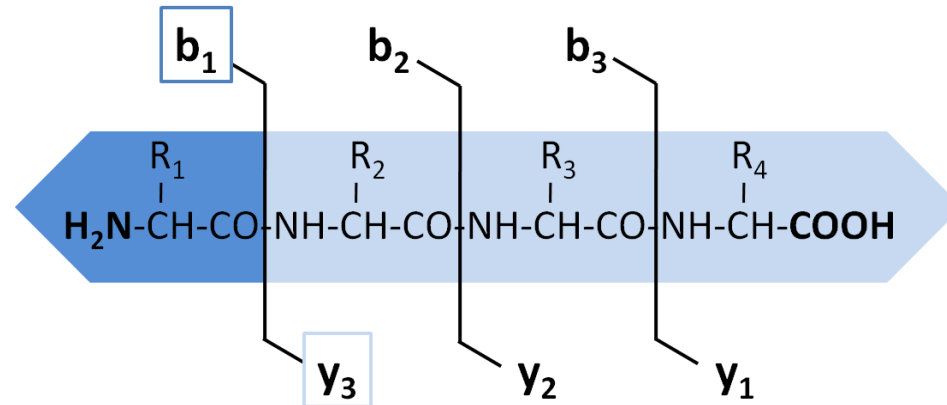


MS zoom in



# Peptide fragmentation during CID

- Preferential breakage



# MSMS scoring function

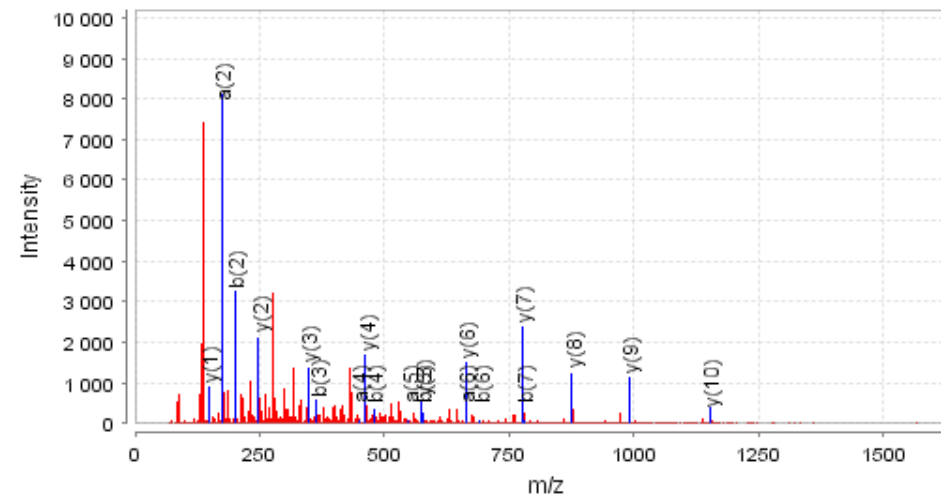
The comparison of theoretical and experimental MS/MS spectra is performed by **scoring function** and the score (ideally complemented by **p-value**) is used to recognize the correct peptide from the database. Reliable **peptide identification** can be then considered for **protein identification**.

Value Types:  Percentage of most intense peaks: 70<x<100 50<x<70 30<x<50 10<x<30 <10

	1	2	3	4	5	6	7	8	9	10	11	12
a		-0.006		-0.018	-0.026	-0.059						
a-H2O		-0.006	-0.006	0.025	0.024							
a-NH3				-0.029	-0.028	-0.041						
b		-0.008	-0.014	-0.019	-0.025	-0.027	-0.006					
b-H2O		-0.007	-0.016	-0.018	-0.011							
b-NH3				-0.032	-0.032	-0.04			-0.112			
	S	L	Y	N	V	D	S	I	D	V	T	K
y			-0.013	-0.033	-0.034	-0.026	-0.026	-0.023	-0.017	-0.012	-0.008	-0.006
y-H2O					-0.018	-0.039	0.029		-0.012	-0.01	-0.008	
y-NH3			-0.044	-0.047								
	12	11	10	9	8	7	6	5	4	3	2	1

Match of an experimental spectrum with a peptide sequence

- Intense peaks should match
- As many as possible peaks should match
- Series of contiguous matches



# MSMS scoring function

**Score** - dependent on the search engine

**E-value** - statistical expectation value

- expected number of peptides with score better or equal than observed score under the assumption that peptides are matching the experimental spectrum by **random chance**
- Invariant under different scoring methods, clearer interpretation of goodness of match
  
- Correlation function – **Sequest**
- Shared fragment count and dot blot
  - **MASCOT, TANDEM, OMSSA, MaxQuant**
- Empirically observed rules – **SpectrumMill**
- Statistically derived fragmentation frequencies – **Phenyx**
- sequence tag algorithm, allowing the degree of implication by an MS/MS spectrum of each region of a database to be determined on a continuum – **Paragon (Protein Pilot)**

Neither the best match nor a high search score (or low E-value) are reliable indicators of true match – ***discriminating true from false is critical step***

*False discovery rate (FDR) analysis*



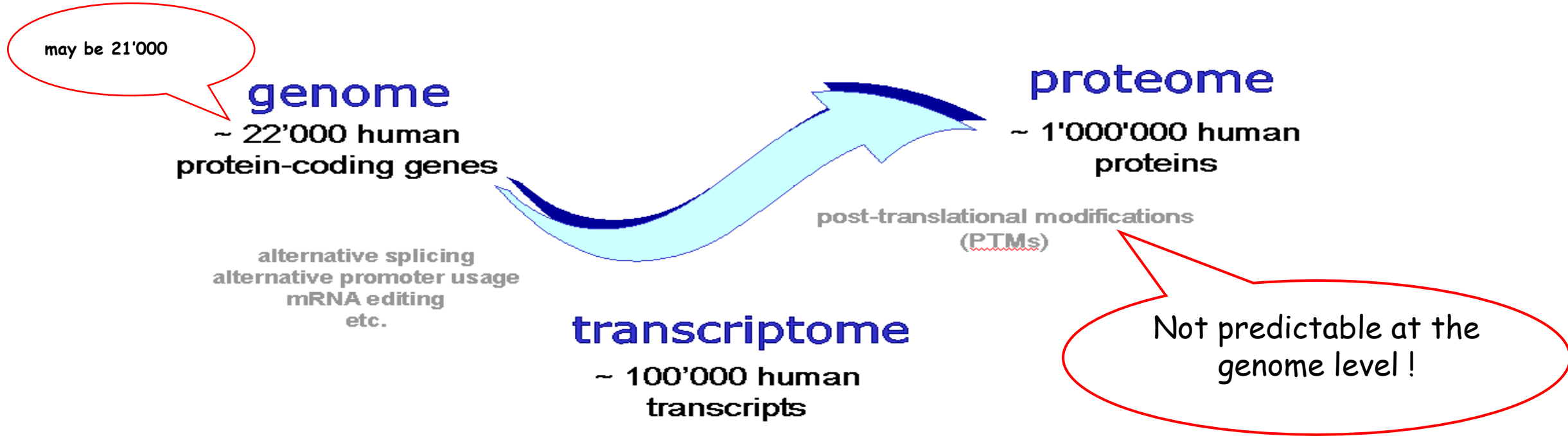
# Modifications

- Fixed modification
  - Cystein alkylation by Iodoacetamide - +57mu
- Variable
  - Met oxidation
  - Pyroglutamate (Nterminal Q,E)
  - Deamidation (N,Q)
  - Phosphorylation (STY)
  - etc.

The more modification allowed the more time and space taken for search

# Proteome complexity

Example: human



(Jensen O.N., Curr. Opin. Chem. Biol., 2004, 8, 33-41, PMID: 15036154).

# How to understand the results?



Protein ID from gel band  
1D ELFO

ProteinPilot™ Software - [Result - C:\AB SCIE\X\ProteinPilot Data\Data\2013\2013\_November\131114\_gely\131114\_B2.group]

File Configure Window Help

Workflow Tasks

- Identify Proteins
- LC...
- Spot-Based (MS only)...
- Spot-Based (MS and MS/MS)...
- View
- Analysis Log...
- Result...
- Export
- Peptide Summary...
- Protein Summary...
- MGF Peaklist(s)...

Protein ID Spectra Summary Statistics

### Proteins Detected

N	Unused	Total	% Cov	Accessio...	Name	Species	Peptides(96%)	Biological Processes	Molecular Functions	PANTHER ID
1	132.27	132.27	75.0	jb l CaLo...	confirm C terminus, putative OS=Jakub Benysek...	IXOSC	166			
2	57.56	57.56	61.9	cont 000...	pdb 1FNI_A  A Chain A, Crystal Structure Of Por...	Sus scrofa (contaminant)	66			
3	42.05	42.05	46.8	cont 000...	cra hCP181205 1  keratin 10(epidermolytic hyp...	Homo sapiens (contaminant)	25			
4	36.10	36.10	45.8	cont 000...	cra hCP1809934.2  keratin 1(epidermolytic hyp...	Homo sapiens (contaminant)	22			
5	25.86	25.86	20.6	cont 000...	g l 114939 sp P00722 B GAL_EC COLI Beta-galact...	Escherichia coli (contaminant)	15			
6	9.15	9.15	35.9	cont 000...	g l 7441526 p ir A69068 beta-casein variant O'H...	Bos taurus (contaminant)	6			
7	8.00	8.00	26.2	cont 000...	g l 15646 sp P02662 CAS1_BOVIN Alpha-S1-c...	Bos taurus (contaminant)	5			
8	7.01	7.11	14.4	cont 000...	sp P48666 Keratin, type II cytoskeletal 6C (Cyt...	Homo sapiens (contaminant)	4			
9	4.01	4.01	43.6	cont 000...	sp P81605 Dermcidin precursor (Preproteolysi...	Homo sapiens (contaminant)	2			
10	2.25	2.25	17.6	cont 000...	g l 27806963 ref NP_776953.1 casein alpha-S2...	Bos taurus (contaminant)	2			
11	2.00	2.00	25.8	cont 000...	g l 27881412 ref NP_776719.1 casein kappa(Bo...	Bos taurus (contaminant)	1			

### Protein Group 1 - confirm C terminus, putative OS=Jakub Benysek GN=IscW\_ISCW007008 PE=3 SV=1

Proteins in Group			Peptides in Group																
N	Unused	Total	Accessio...	Name	Species	Con...	Conf	Sequence	Modifications	Cleavages	ΔMass	Prec MW	z	Sc	Spectrum	Type			
1	132.27	132.27	jb l CaLo...	confirm C terminus,...	IXOSC	2.00	99	AFTFYNSGVYDFPK			-0.0001	1654.76...	2	23	1.1.1.4914.4	Win...			
						2.00	99	AHGLASDEDYGY		cleaved Y-...	-0.0047	1367.55...	2	16	1.1.1.4685.4	Win...			
						2.00	99	AHGLASDEDYGYLGQDGI		cleaved I-C...	0.0004	1950.85...	3	26	1.1.1.4870.2	Win...			
						2.00	99	AHGLASDEDYGYLGQDGI...	Carbamidomethyl(C)@20		0.0038	2592.12...	4	27	1.1.1.4719.8	Win...			
						2.00	99	ALANVGPVSVSIDAA		cleaved A-...	-0.0003	1382.74...	2	14	1.1.1.4933.3	Win...			
						2.00	99	ALANVGPVSVSDAAVK			-0.0027	1609.90...	2	16	1.1.1.4924.6	Win...			
						2.00	99	APTNALFFPK			-0.0055	1054.57...	2	15	1.1.1.4720.3	Win...			
						2.00	99	ASDEYGYLGQDGI	Carbamidomethyl(C)@16	cleaved L-...	0.0099	2213.92...	3	21	1.1.1.4727.2	Win...			
						2.00	99	AVGYGTLQGEFYWLK		cleaved L-...	-0.0015	1793.93...	2	24	1.1.1.4940.4	Win...			
						2.00	99	AVLPDVTSEFVR		cleaved Q-...	-0.0007	1478.77...	2	13	1.1.1.4990.4	Win...			
						2.00	99	DGYVLI	SQK	cleaved N-...	0.0002	1021.54...	2	15	1.1.1.4708.3	Win...			
						2.00	99	DNKGVASQGTYYELHHH	Carbamidomethyl(C)@4	cleaved H-...	-0.0011	2036.87...	4	19	1.1.1.4578.3	Win...			
						2.00	99	DNKGVASQGTYYELHHH	Carbamidomethyl(C)@4	cleaved H-...	-0.0012	2173.93...	4	19	1.1.1.4524.2	Win...			

### Protein Sequence Coverage - confirm C terminus, putative OS=Jakub Benysek GN=IscW\_ISCW007008 PE=3 SV=1

```
IIISVQLKGGSDLNKIKILLAVVAIFVGLSLGRSQELPWEVDVYKVGVLVLPYAEIREPFTGYFDASQNLNLSRIDYRGMVQTIQVAPDAEGRSEAPYGANVYKLAIMPDKETWQPRICFLVNGTINNTVPLQAVLEPDTSEFVRSQESCWEGTAVDPAERASCEPQLAVTNEHRVSKYTLWVSRDGGRAVPRRYLMMGYN  
TLLGSHFDKYLELYHGFSRKPLPSSVEDITHLINETCRQFPQGAEBHLALHSPMAEFMDGHDAMHMSAFDFKFEHSDRDYGHHTHEHRRRDI FRQNLRFIHSNTRNANRGYIVVNHLDASSEELGVLGRHLHSAEPTNALFFPKDRFSSDLPDYIDWRLYGAVTPEVDQAVCGSCWSFGTVGBLEAALFRKTGRKLRLSBQQGLV  
DCSNQGNNGCDGDEDFRAYEYIRAHGLASDEDYGYLGQDGI
```

Protein Group 1 - confirm C terminus, putative OS=Jakub Benysek GN=IscW\_ISCW007008 PE=3 SV=1

Proteins in Group					
N	Unused	Total	Accessio...	Name	Species
1	132.27	132.27	jb IrcatLo...	confirm C terminus,...	IXOSC

Peptides in Group												
Con...	Conf	Sequence	Modifications	Cleavages	ΔMass	Prec MW	z	Sc	Spectrum	Type		
2.00	99	AFTFYSGVIFYDPK			-0.0001	1654.76...	2	23	1.1.1.4914.4	Win...		
2.00	99	AHGLASDEYDYGAY		cleaved Y-...	-0.0047	1367.55...	2	16	1.1.1.4665.4	Win...		
2.00	99	AHGLASDEYDYGAYLGQDGI		cleaved I-C...	0.0004	1950.85...	3	26	1.1.1.4870.2	Win...		
2.00	99	AHGLASDEYDYGAYLGQDGI...	Carbamidomethyl(C)@20		0.0038	2592.12...	4	27	1.1.1.4719.8	Win...		
2.00	99	ALANVGFVSVS IDAA		cleaved A-...	-0.0003	1382.74...	2	14	1.1.1.4933.3	Win...		
2.00	99	ALANVGFVSVS IDAAVK			-0.0027	1609.90...	2	16	1.1.1.4924.6	Win...		
2.00	99	APTNALPPFK			-0.0055	1054.57...	2	15	1.1.1.4720.3	Win...		
2.00	99	ASDEYDYGAYLGQDGI...	Carbamidomethyl(C)@16	cleaved L-...	0.0099	2213.92...	3	21	1.1.1.4727.2	Win...		
2.00	99	AVGYGT LQGEPLYWLIK		cleaved L-...	-0.0015	1793.93...	2	24	1.1.1.4940.4	Win...		
2.00	99	AVLPDVTSPFEFVR		cleaved Q-...	-0.0007	1478.77...	2	13	1.1.1.4990.4	Win...		
2.00	99	DGYVLI SQK		cleaved N-...	0.0002	1021.54...	2	15	1.1.1.4708.3	Win...		
2.00	99	DNNCGVASQGT YVE LHHH	Carbamidomethyl(C)@4	cleaved H-...	-0.0011	2036.87...	4	19	1.1.1.4578.3	Win...		
2.00	99	DNNCGVASQGT YVE LHHH	Carbamidomethyl(C)@4	cleaved H-...	-0.0012	2173.93...	4	19	1.1.1.4524.2	Win...		

N	Unused	Total	% Cov	Accessio...	Name	Species	Peptides(95%)	Bi
1	132.27	132.27	75.0	jb IrcatLo...	confirm C terminus, putative OS=Jakub Benysek...	IXOSC	166	
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3	42.05	42.05	46.8	cont 000...	cra hCP1812051 keratin 10(epidermolytic hype...	Homo sapiens (contaminant)	25	
4	36.10	36.10	45.8	cont 000...	cra hCP1609934.2 keratin 1(epidermolytic hyp...	Homo sapiens (contaminant)	22	
5	25.86	25.86	20.6	cont 000...	gi 114939 sp P00722 BGAL_ECOLI Beta-galact...	Escherichia coli (contaminant)	15	
6	9.15	9.15	35.9	cont 000...	gi 7441526 pir  A59068 beta-casein variant CnH...	Bos taurus (contaminant)	6	
7	8.00	8.00	26.2	cont 000...	gi 115646 sp P02662 CAS1_BOVIN Alpha-S1-c...	Bos taurus (contaminant)	5	
8	7.01	7.11	14.4	cont 000...	spt P48666 Keratin, typeII cytoskeletal 6C (Cyt...	Homo sapiens (contaminant)	4	
9	4.01	4.01	43.6	cont 000...	spt P81605 Dermcidin precursor (Preproteolysi...	Homo sapiens (contaminant)	2	
10	2.25	2.25	17.6	cont 000...	gi 27806963 ref NP_776953.1 casein alpha-S2...	Bos taurus (contaminant)	2	
11	2.00	2.00	25.8	cont 000...	gi 27881412 ref NP_776719.1 casein kappa[Bo...	Bos taurus (contaminant)	1	

Protein Sequence Coverage - confirm C terminus, putative OS=Jakub Benysek GN=IscW\_ISCW007008 PE=3 SV=1

IISVQLKGGSDLNMKLCILLAVFAVGLSLGRSQLEPEWGDVYKVGVLVLYPAEIREPFTGYFDASQNLRSRIDYYRGMVQTIQVAPDAEGGRSEAPYGANYKIAIMPDKETWQPRRTICFLV  
 TLLGSHFDKRYELIYHGF SRKPLPSSVFDITHLINETCRQFPGPAEHLALHSPMAEFMDGHDAHMHSADFDFKEDHSRDYGHHTHEHRRRDI FRQNLRF IHSTNRRANGYTVENVHLADRSS  
 DCSWNQGNNGCDGGEDFRAYEYIRAHGLASDEYDYGAYLGQDGI CHDTRVNA TVTTIKRNYINVTDKESLQKALANVGFVSVS IDAAVKAFTFYSGVIFYDPKCRNDTDGLDHAVLAVGYTLQ

Color	Peptide Confidence	Included in Percent Coverage
Gray	No match	
Red	> 0 and < 50	% Cov, % Cov (50), and % Cov (95)
Yellow	>= 50 and < 95	% Cov (50), and % Cov (95)
Green	>= 95	% Cov (95)

1	N	Unused	Total	%Cov	%Cov(50)	%Cov(95)	Accession	Name	Species	Peptides(95)
2	1	138.47	138.47	65.05	61.08	58.7400019	sp Q28085 CFAH_BOVIN	Complement factor H OS=Bos taurus GN=CFH PE=1 SV=3	BOVIN	82
3	2	115.17	115.17	74.63	72.98	72.9799986	sp P02769 ALBU_BOVIN	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4	BOVIN	60
4	3	72.54	72.54	29.4	29.4	29.3199986	tr F1N3A1 F1N3A1_BOVIN	Thrombospondin-1 OS=Bos taurus GN=THBS1 PE=4 SV=1	BOVIN	40
5	4	57.12	57.12	20.89	20.29	20.2900007	tr G3X7A5 G3X7A5_BOVIN	Complement C3 OS=Bos taurus GN=C3 PE=4 SV=1	BOVIN	30
6	4	0	57.12	20.89	20.29	20.2900007	sp Q2UVX4 CO3_BOVIN	Complement C3 OS=Bos taurus GN=C3 PE=1 SV=2	BOVIN	30
7	5	52.39	52.39	48.58	45.17	42.7599996	sp Q29443 TRFE_BOVIN	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1	BOVIN	27
8	6	50.79	50.79	69.81	69.06	63.0200028	tr V6F9A2 V6F9A2_BOVIN	Apolipoprotein A-I preproprotein OS=Bos taurus GN=APOA1 PE=4 SV=1	BOVIN	26
9	6	0	50.79	69.81	69.06	63.0200028	sp Q29443 TRFE_BOVIN	Apolipoprotein A-I OS=Bos taurus GN=APOA1 PE=1 SV=3	BOVIN	26
10	7	48.69	48.69	43.04	43.04	43.0400014	tr F1N4M7 F1N4M7_BOVIN	Complement factor I OS=Bos taurus GN=CFI PE=2 SV=1	BOVIN	26
11	7	0	48.69	43.04	43.04	43.0400014	tr F1N4M7 F1N4M7_BOVIN	Uncharacterized protein OS=Bos taurus GN=CFI PE=3 SV=2	BOVIN	26
12	8	45.77	45.77	35.98	30.22	29.8299998	tr F1N1I6 F1N1I6_BOVIN	Gelsolin OS=Bos taurus GN=GSN PE=4 SV=1	BOVIN	25
13	9	42.23	42.23	39.66	33.62	32.6400012	tr E1B726 E1B726_BOVIN	Plasminogen OS=Bos taurus GN=PLG PE=3 SV=2	BOVIN	22
14	10	40.46	40.46	75.08	73.19	65.9300029	sp P13384 IBP2_BOVIN	Insulin-like growth factor-binding protein 2 OS=Bos taurus GN=IGFBP2 PE=1 SV=1	BOVIN	24
15	11	39.98	39.98	44.12	40.74	39.4499987	sp P01044 KNG1_BOVIN	Kininogen-1 OS=Bos taurus GN=KNG1 PE=1 SV=1	BOVIN	21
16	12	39.25	39.25	88.97	88.97	88.9699996	sp P02081 HBBF_BOVIN	Hemoglobin fetal subunit beta OS=Bos taurus PE=1 SV=1	BOVIN	23
17	13	36.2	36.2	12.41	11.26	10.8599998	tr E1BH06 E1BH06_BOVIN	Uncharacterized protein OS=Bos taurus GN=C4A PE=4 SV=2	BOVIN	20
18										22

Protein summary

To understand the meaning of the values in each column – see file „Understanding ProteilPilot results“

A																			AA
N	Unused	Total	%Cov(95)	Accessions	Names	Con	Conf	Sequence	Modifications	Cleavages	dMass	Prec MW	Prec m/z	Theor MW	Theor m/z	Theor z	Time	Precursor	PrecursorI
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	AACYERECIPEN	Carbamidomethyl(C)@3	missed R-E	0.0084231	2704.18066	902.4008	2704.1719	902.397949	3	59.7061	262.887	59.7217
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CAAGFEYQQR	Carbamidomethyl(C)@1		-0.0016263	1157.49084	579.7527	1157.4924	579.753479	2	29.6776	614.16	29.7972
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CAAGFEYQQRG	Carbamidomethyl(C)@1	missed R-G	0.0131677	1932.85889	645.2936	1932.8458	645.289246	3	31.7044	2415.8	31.5844
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CAWKPCSPYVIK	Carbamidomethyl(C)@1;	Carbamido	0.0035561	1507.73523	503.5857	1507.7316	503.584473	3	37.5638	1146.02	37.5432
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLEACVISEETMF	Carbamidomethyl(C)@1;	Carbamido	-0.0012348	1596.69324	799.3539	1596.6946	799.354553	2	50.8485	109.317	50.9219
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLSYQLDGSNT	Carbamidomethyl(C)@1;	No Carbam	-0.0056549	2004.92346	1003.469	2004.9285	1003.4715	2	63.9677	303.692	63.9949
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLSYQLDGSNT	Carbamidomethyl(C)@1	missed K-S	0.0039744	2277.08081	760.0342	2277.0769	760.032898	3	50.7217	243.169	50.8243
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLSYQLDGSNT	Carbamidomethyl(C)@1		-0.0023965	1101.48901	551.7518	1101.4913	551.75293	2	33.3058	2543.88	33.4694
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLSYQLDGSNT	Carbamidomethyl(C)@1	missed R-G	-0.0088924	1400.64185	701.3282	1400.6506	701.332642	2	27.8532	68.4562	27.8164
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	CLSYQLDGSNT	Carbamidomethyl(C)@1	missed R-G	-0.0010667	954.42926	478.2219	954.4303	478.222443	2	34.608	335.071	34.487
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	DGWVVPVPR			-0.0053974	924.476501	463.2455	924.48175	463.248138	2	49.0778	1766.07	49.0828
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	DVSCGNPPQVEI	Carbamidomethyl(C)@4		0.0105139	2119.02222	707.348	2119.0117	707.344482	3	40.3115	4269.19	40.2151
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	DVSCGNPPQVEI	Carbamidomethyl(C)@4	missed K-S	0.0218775	2334.16064	584.5474	2334.1387	584.541931	4	33.1735	198.603	33.3243
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	EAFTMIGPR			-0.0006555	1020.50568	511.2601	1020.5062	511.260406	2	44.6736	5102.33	44.3713
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	ECDTNGWTNDI	Carbamidomethyl(C)@2;	Carbamido	0.005699	2148.95142	1075.483	2148.9456	1075.4801	2	73.3895	390.096	73.2872
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	ECSIPEMDPYLN	Carbamidomethyl(C)@2		-0.0017494	1953.85852	977.9365	1953.8601	977.937317	2	67.0893	425.73	67.209
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	ECSIPEMDPYLN	Carbamidomethyl(C)@2	missed R-K	0.0127102	2603.21606	651.8113	2603.2036	651.808167	4	50.0623	203.264	50.1657
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	EIHKEEYAHNEV	Carbamidomethyl(C)@1	missed K-E	0.0117948	2486.14063	622.5424	2486.1284	622.539368	4	30.4653	689.999	30.5798
1	138.47	138.47	58.74	sp Q28085 CFAH	Complement factor H OS	2	99	ENYLIQDAEEIVC	Carbamidomethyl(C)@13		0.0045467	1722.81812	862.4163	1722.8135	862.414001	2	78.3875	320.892	78.4446

Peptide summary