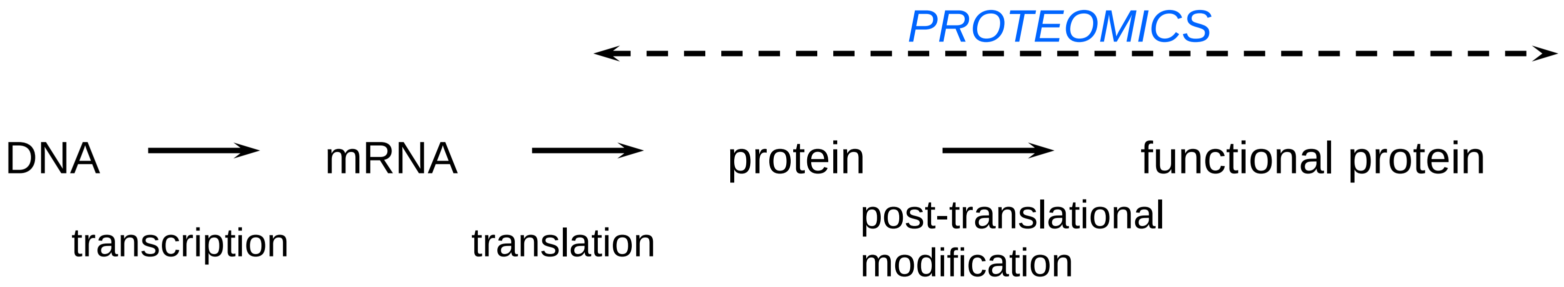
DEPARTMENTS OF CHEMISTRY AND BIOCHEMISTRY

GRADUATE COURSE IN MASS SPECTROMETRY: LECTURE 6

Proteomics

Shabaz Mohammed

November 17, 2016



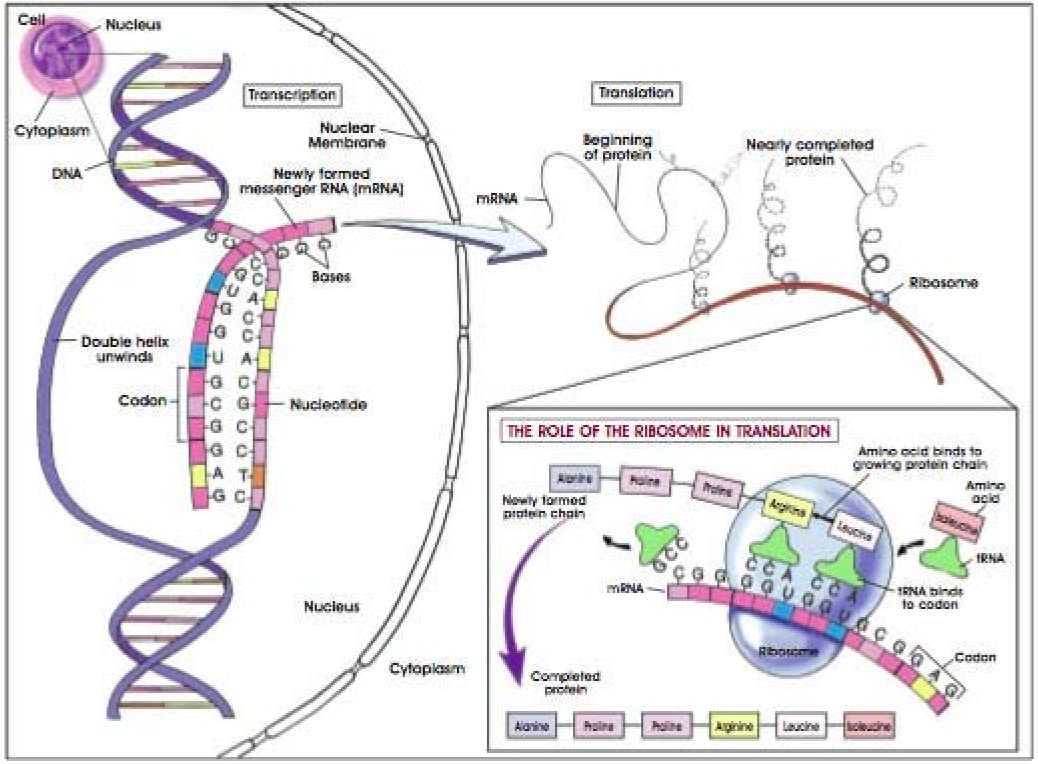
PROTEOMICS

DNA → mRNA → protein → functional protein

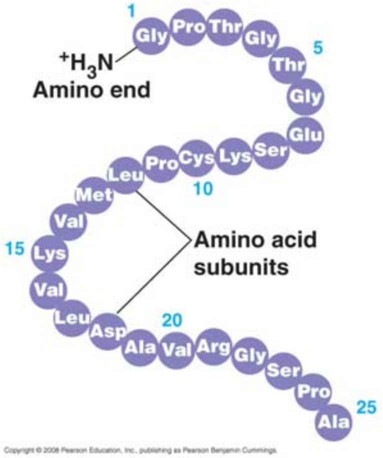
transcription

translation

post-translational modification



Primary



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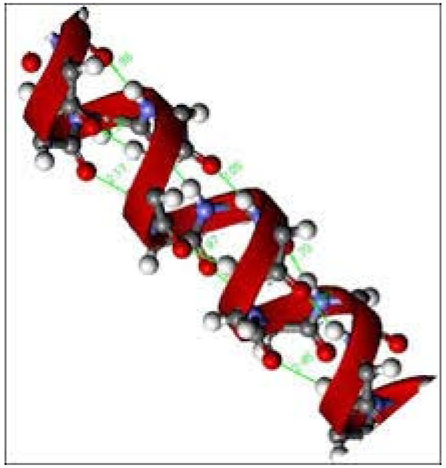
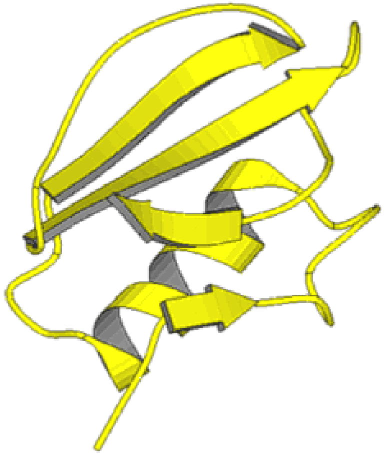
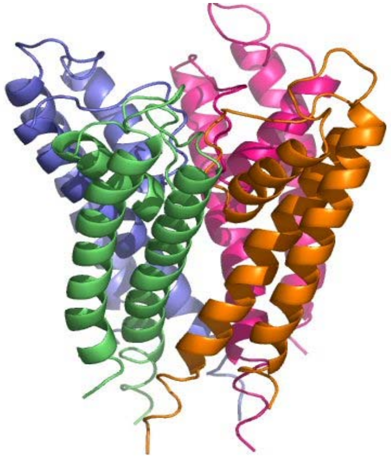
Secondary



Tertiary



Quaternary



Over 200 possible modifications possible to the primary sequence

Dramatic effects possible to structure and ultimately function

Proteomics: definitions

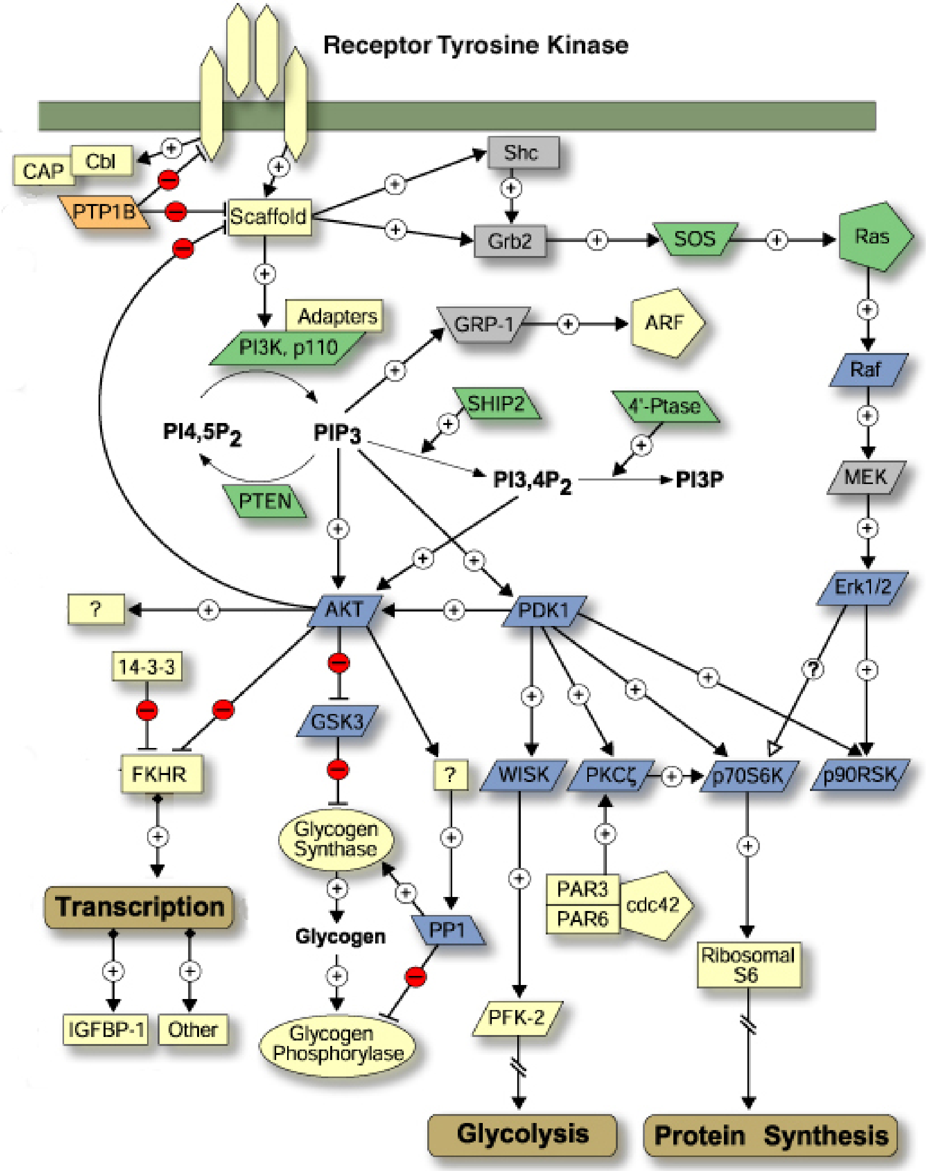
PROTEOME

The total PROTEin complement in a cell, tissue or biological system that is expressed by a genOME

PROTEOMICS (= proteome analysis)

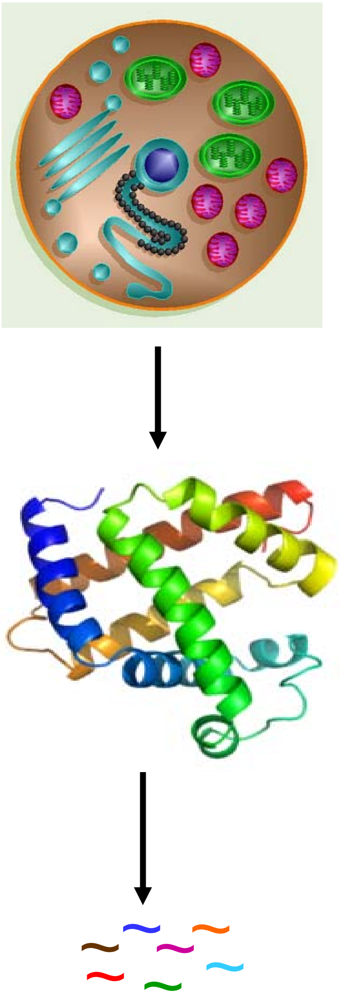
1) Structural proteome analysis: identification of proteins

2) Functional proteome analysis: determine biochemical and biological characteristics of (a family of) proteins



Adapted from Nature AFCS Gateway

Complexity



cellular genome
~25 000 genes

whole cell lysate
~100 000 proteins

tryptic whole cell digest
~1 000 000 peptides
Not taking PTMs in account

Sample is of ridiculous complexity

Sensitivity and Dynamic Range

One copy/cell corresponds to:

$$\frac{1}{6.023 \cdot 10^{23}} = 1.66 \cdot 10^{-24} \text{ moles}$$

Þ For a net Detection Limit of 1 femtomole (i.e. 10^{-15} moles)

$6 \cdot 10^{8}$ cells are required to detect a single copy

Analyte of interest is potentially of low abundance!

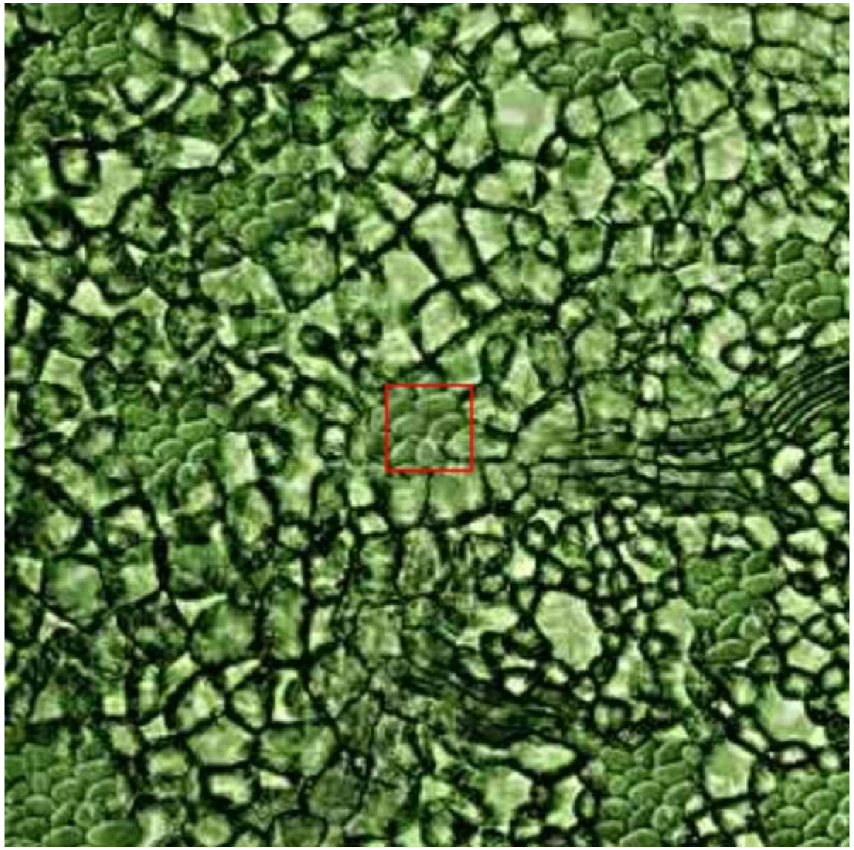
Dynamic range: 12 orders of magnitude

Dynamic range: 12 orders of magnitude

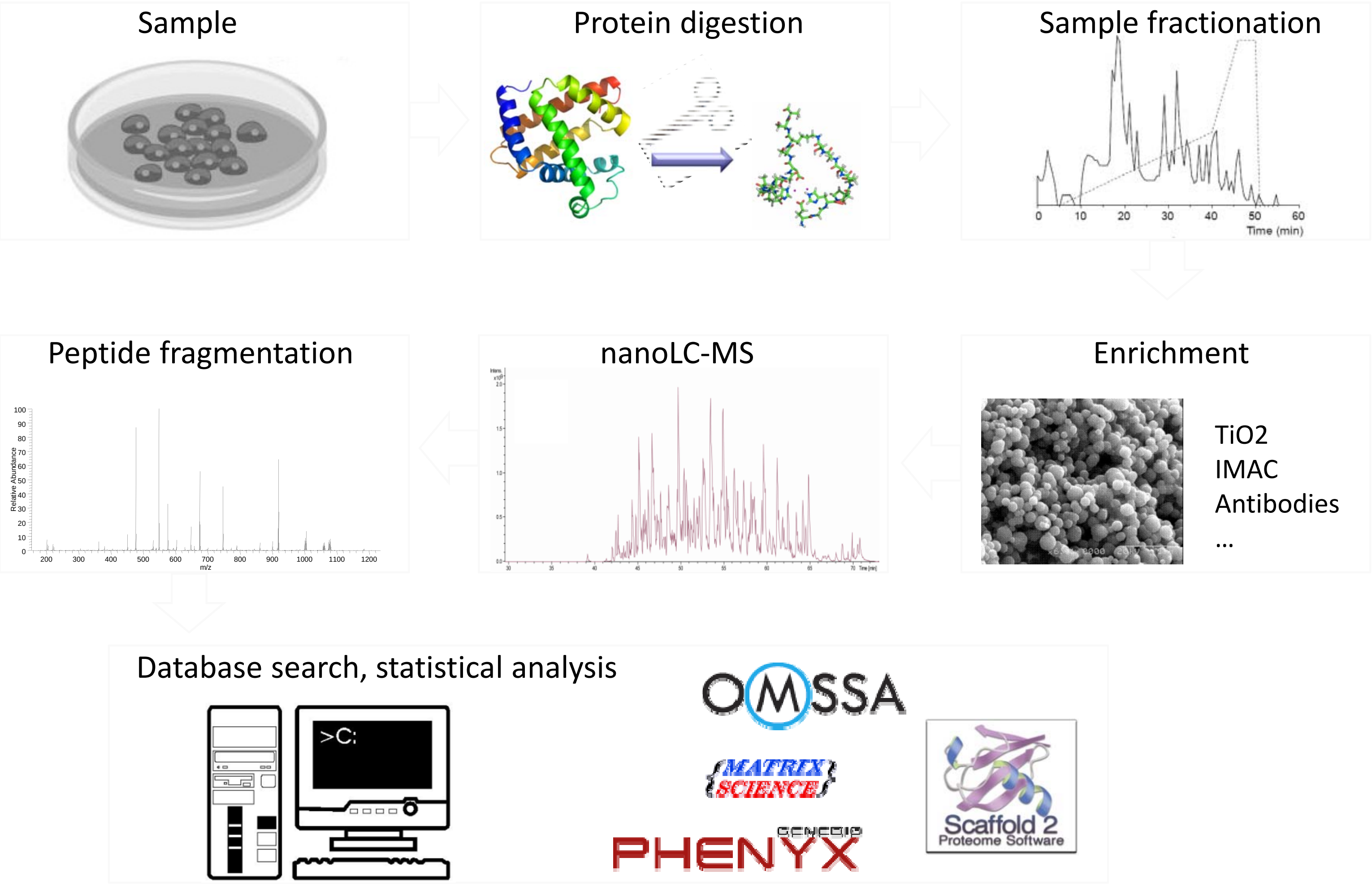
‘I want a camera that captures both in the same picture’

Albumin

Interleukin 6



Conventional proteomics approach: bottom-up



Sample

Protein digestion

Sample fractionation

Enrichment

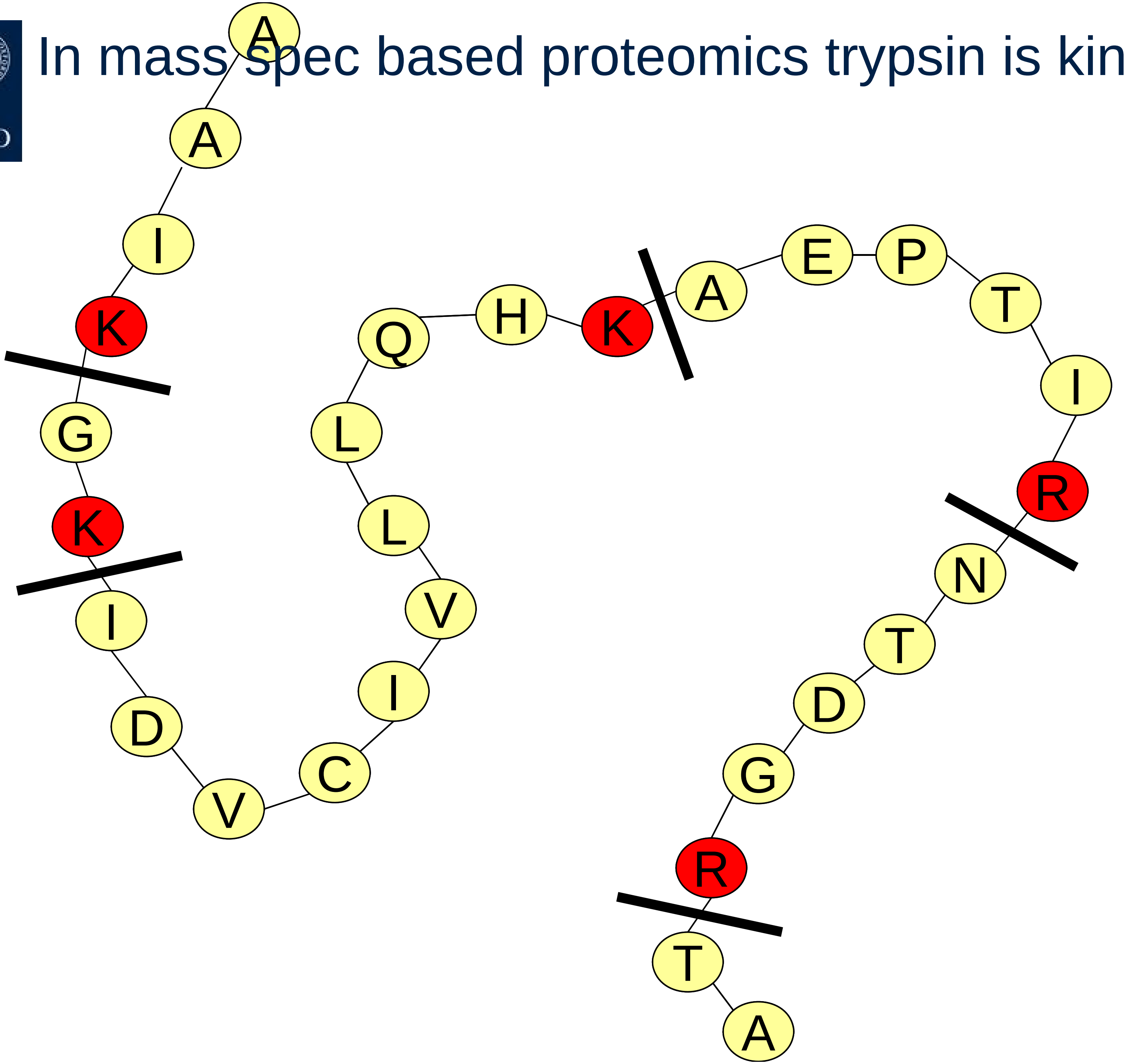
TiO2
IMAC
Antibodies
…

nanoLC-MS

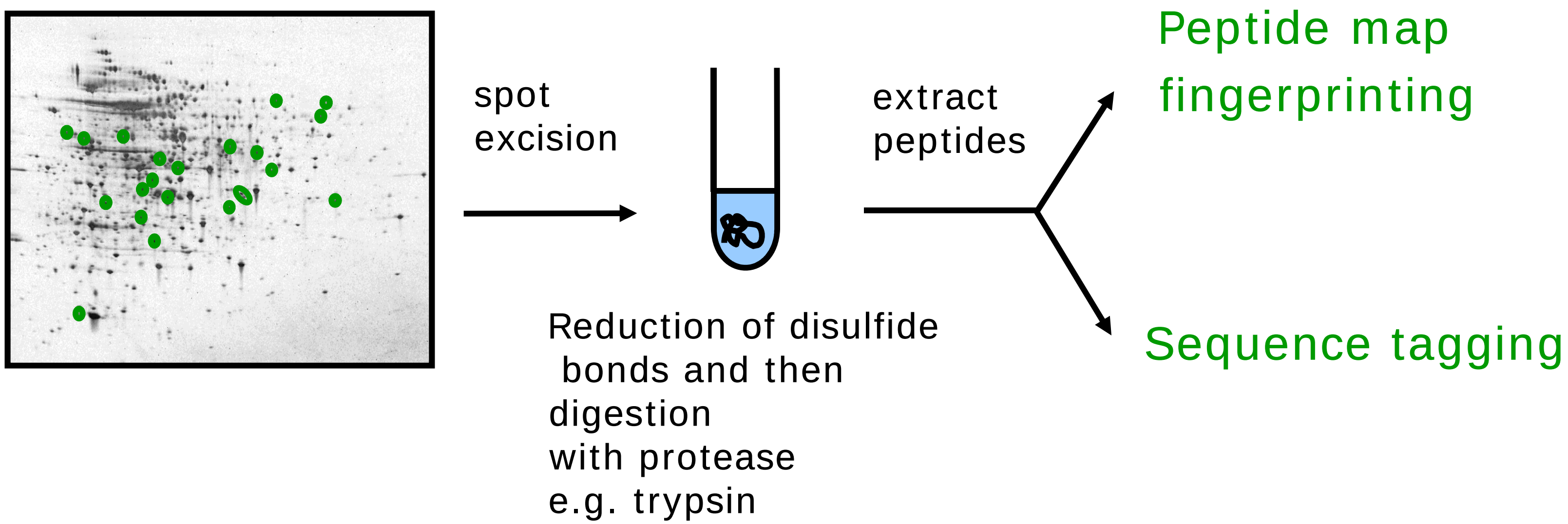
Peptide fragmentation

Database search, statistical analysis

In mass spec based proteomics trypsin is king



Processing of spots & analysis with mass spectrometry



spot
excision

extract
peptides

Peptide map
fingerprinting

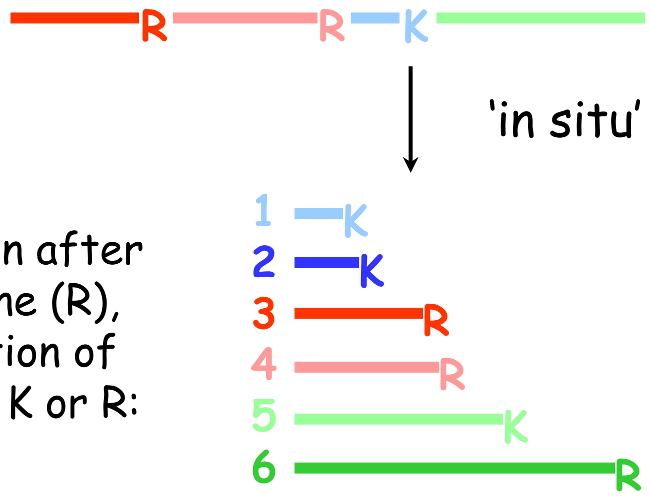
Sequence tagging

Reduction of disulfide
bonds and then
digestion
with protease
e.g. trypsin

mass spectrometry → identification of proteins of interest

Protein identification:
Peptide mass fingerprinting

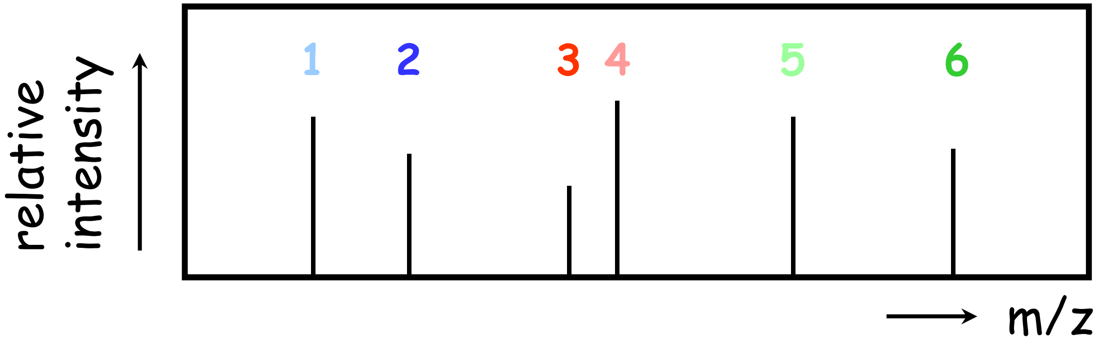
Protein A:



'in situ' digestion with trypsin

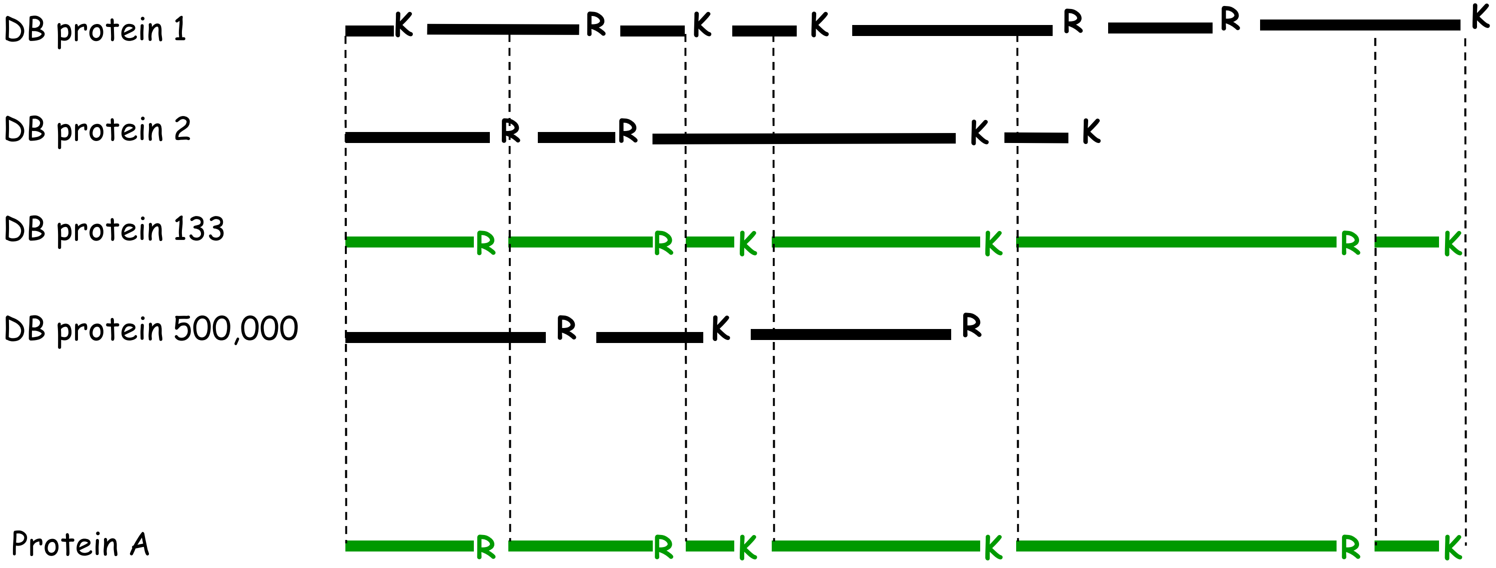
trypsin cuts protein after lysine (K) or arginine (R), resulting in formation of peptides ending at K or R:

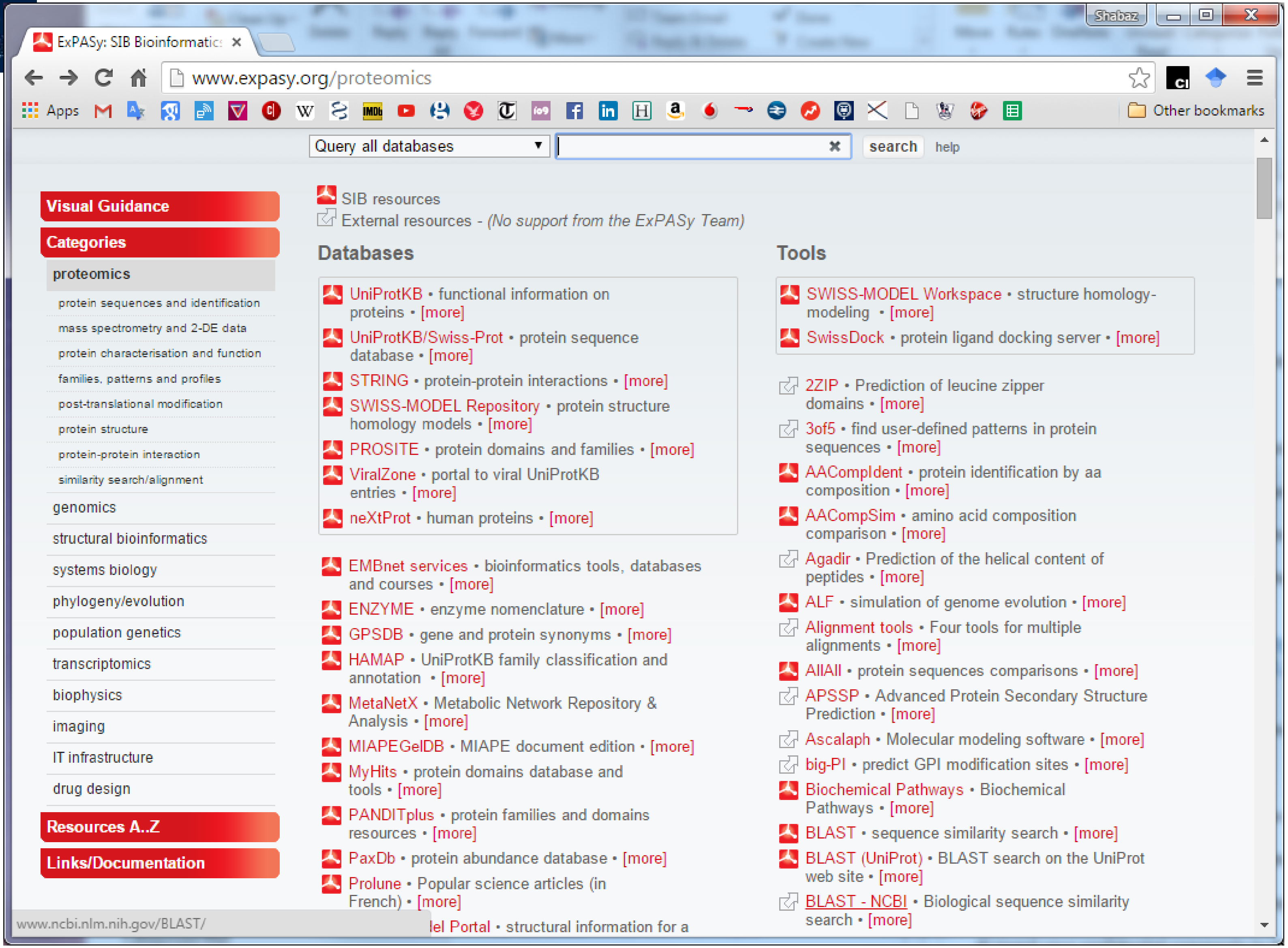
MS spectrum
Shows <u>precise and accurate</u> m/z values
of peptides:



Database search

- digest all known proteins *in silico* with trypsin
- determine exact theoretical masses
- compare with detected masses of protein A





SIB resources
External resources - *(No support from the ExPASy Team)*

Databases

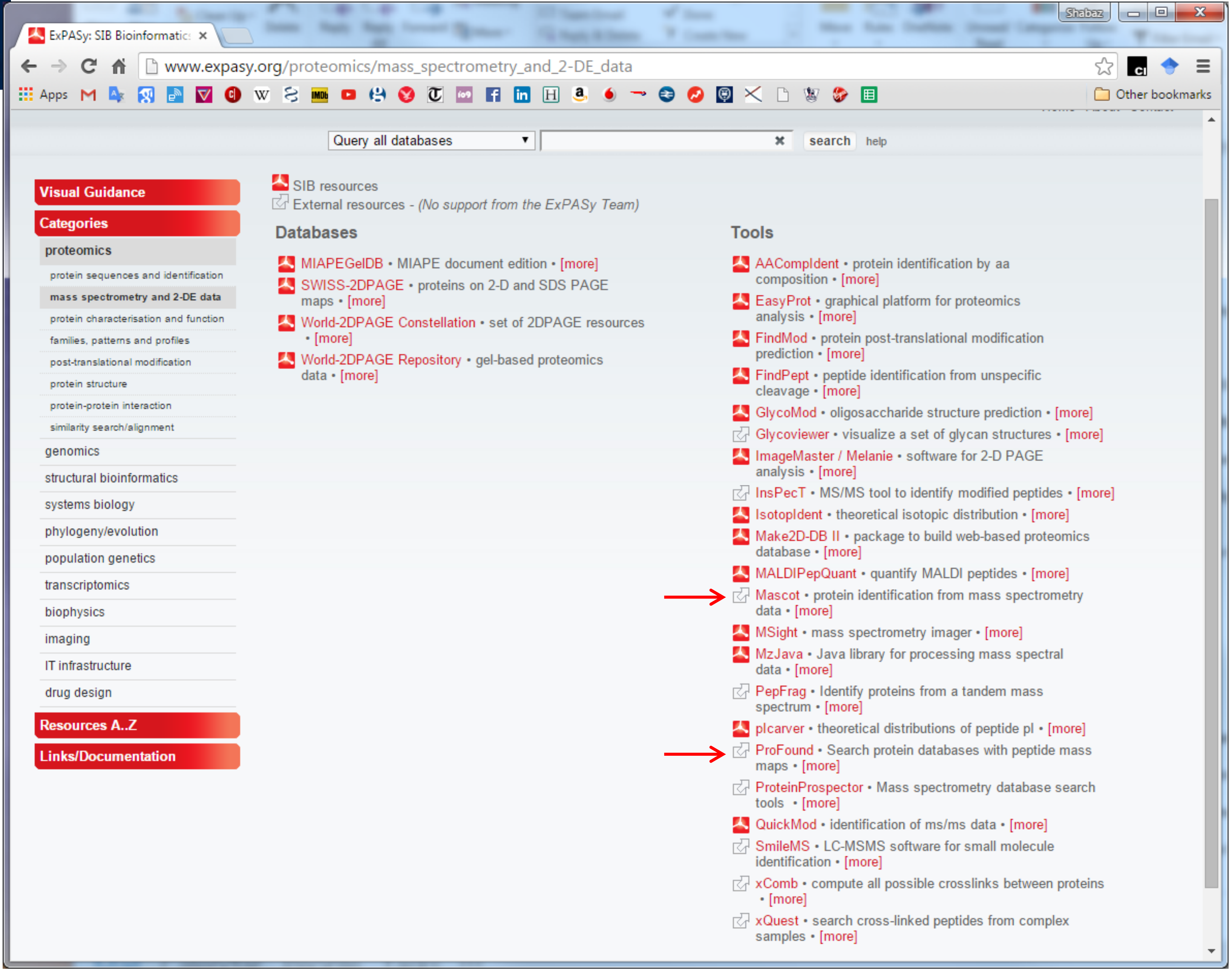
- UniProtKB • functional information on proteins • [more]
- UniProtKB/Swiss-Prot • protein sequence database • [more]
- STRING • protein-protein interactions • [more]
- SWISS-MODEL Repository • protein structure homology models • [more]
- PROSITE • protein domains and families • [more]
- ViralZone • portal to viral UniProtKB entries • [more]
- neXtProt • human proteins • [more]

- EMBnet services • bioinformatics tools, databases and courses • [more]
- ENZYME • enzyme nomenclature • [more]
- GPSDB • gene and protein synonyms • [more]
- HAMAP • UniProtKB family classification and annotation • [more]
- MetaNetX • Metabolic Network Repository & Analysis • [more]
- MIAPEGelDB • MIAPE document edition • [more]
- MyHits • protein domains database and tools • [more]
- PANDITplus • protein families and domains resources • [more]
- PaxDb • protein abundance database • [more]
- Prolune • Popular science articles (in French) • [more]
- lel Portal • structural information for a

Tools

- SWISS-MODEL Workspace • structure homology-modeling • [more]
- SwissDock • protein ligand docking server • [more]

- 2ZIP • Prediction of leucine zipper domains • [more]
- 3of5 • find user-defined patterns in protein sequences • [more]
- AACompIdent • protein identification by aa composition • [more]
- AACompSim • amino acid composition comparison • [more]
- Agadir • Prediction of the helical content of peptides • [more]
- ALF • simulation of genome evolution • [more]
- Alignment tools • Four tools for multiple alignments • [more]
- AllAll • protein sequences comparisons • [more]
- APSSP • Advanced Protein Secondary Structure Prediction • [more]
- Ascalaph • Molecular modeling software • [more]
- big-PI • predict GPI modification sites • [more]
- Biochemical Pathways • Biochemical Pathways • [more]
- BLAST • sequence similarity search • [more]
- BLAST (UniProt) • BLAST search on the UniProt web site • [more]
- BLAST - NCBI • Biological sequence similarity search • [more]



SIB resources
External resources - *(No support from the ExPASy Team)*

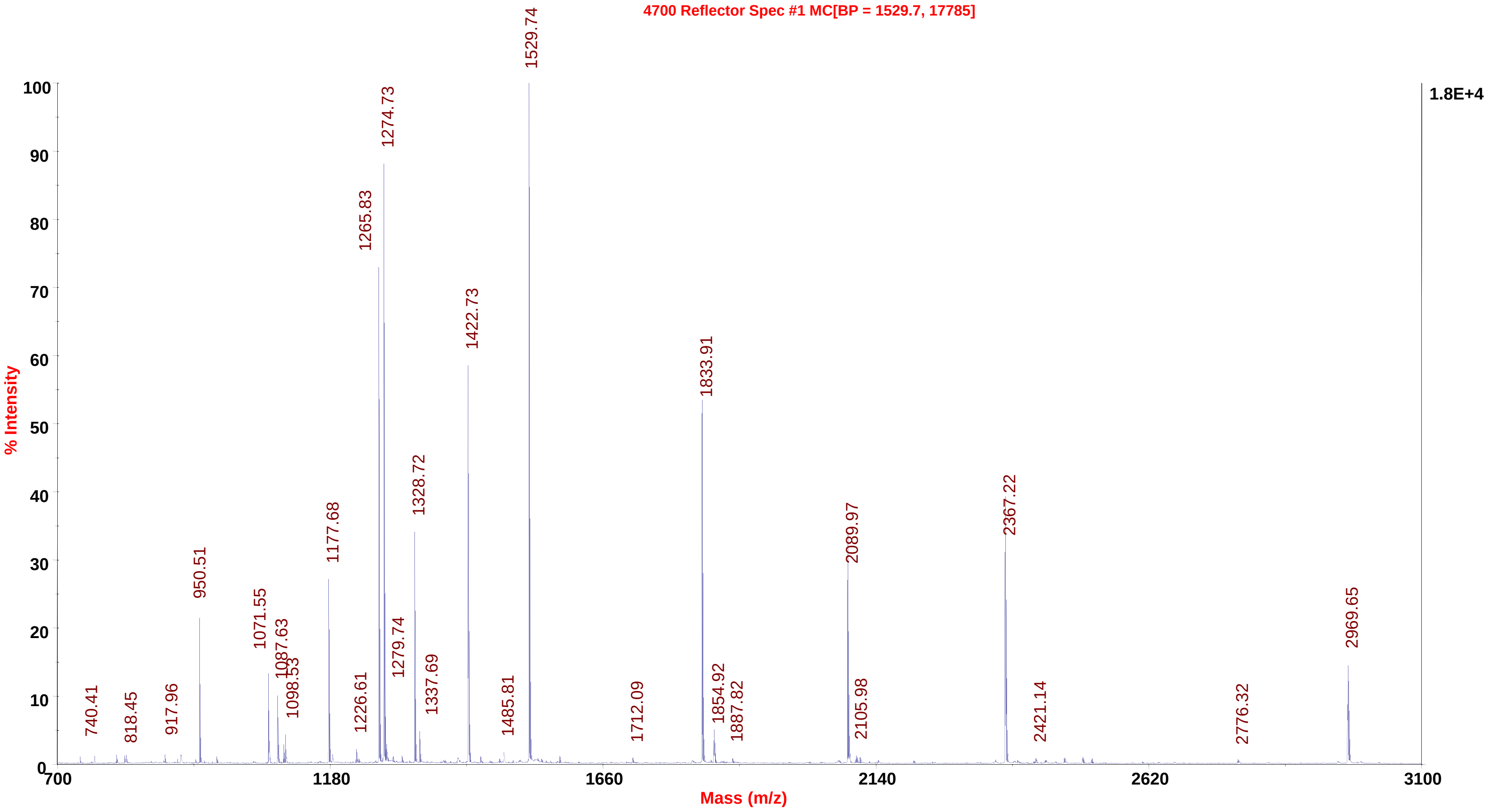
Databases

- MIAPEGelDB • MIAPE document edition • [more]
- SWISS-2DPAGE • proteins on 2-D and SDS PAGE maps • [more]
- World-2DPAGE Constellation • set of 2DPAGE resources • [more]
- World-2DPAGE Repository • gel-based proteomics data • [more]

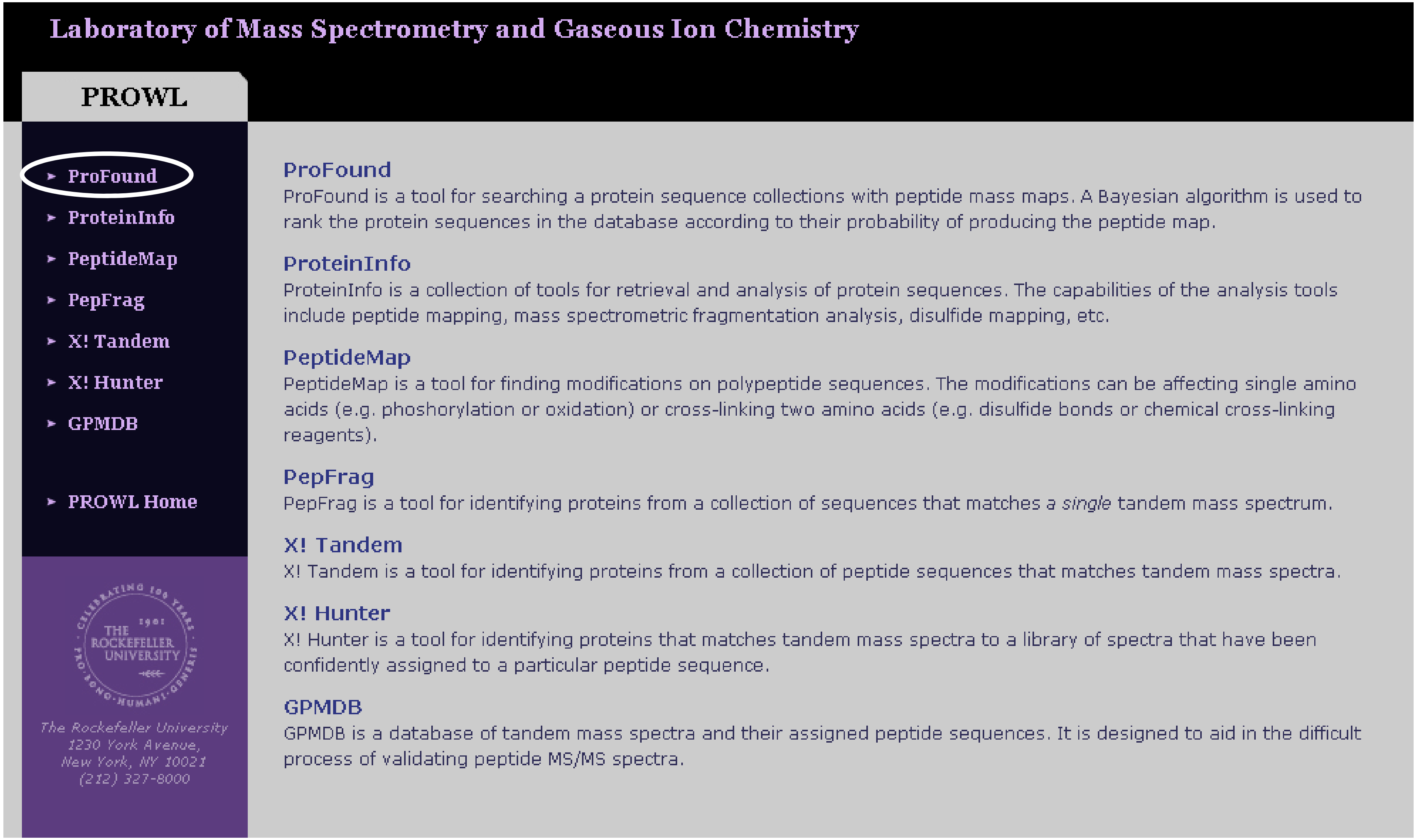
Tools

- AACompIdent • protein identification by aa composition • [more]
- EasyProt • graphical platform for proteomics analysis • [more]
- FindMod • protein post-translational modification prediction • [more]
- FindPept • peptide identification from unspecific cleavage • [more]
- GlycoMod • oligosaccharide structure prediction • [more]
- Glycoviewer • visualize a set of glycan structures • [more]
- ImageMaster / Melanie • software for 2-D PAGE analysis • [more]
- InsPecT • MS/MS tool to identify modified peptides • [more]
- IsotopIdent • theoretical isotopic distribution • [more]
- Make2D-DB II • package to build web-based proteomics database • [more]
- MALDIPepQuant • quantify MALDI peptides • [more]
- Mascot • protein identification from mass spectrometry data • [more]
- MSight • mass spectrometry imager • [more]
- MzJava • Java library for processing mass spectral data • [more]
- PepFrag • Identify proteins from a tandem mass spectrum • [more]
- pIcarver • theoretical distributions of peptide pI • [more]
- ProFound • Search protein databases with peptide mass maps • [more]
- ProteinProspector • Mass spectrometry database search tools • [more]
- QuickMod • identification of ms/ms data • [more]
- SmileMS • LC-MSMS software for small molecule identification • [more]
- xComb • compute all possible crosslinks between proteins • [more]
- xQuest • search cross-linked peptides from complex samples • [more]

Example: MALDI-TOF spectrum of peptides from protein spot



The peptide mass fingerprint program PROWL / PROFOUND



ProFound

ProFound is a tool for searching a protein sequence collections with peptide mass maps. A Bayesian algorithm is used to rank the protein sequences in the database according to their probability of producing the peptide map.

ProteinInfo

ProteinInfo is a collection of tools for retrieval and analysis of protein sequences. The capabilities of the analysis tools include peptide mapping, mass spectrometric fragmentation analysis, disulfide mapping, etc.

PeptideMap

PeptideMap is a tool for finding modifications on polypeptide sequences. The modifications can be affecting single amino acids (e.g. phoshorylation or oxidation) or cross-linking two amino acids (e.g. disulfide bonds or chemical cross-linking reagents).

PepFrag

PepFrag is a tool for identifying proteins from a collection of sequences that matches a *single* tandem mass spectrum.

X! Tandem

X! Tandem is a tool for identifying proteins from a collection of peptide sequences that matches tandem mass spectra.

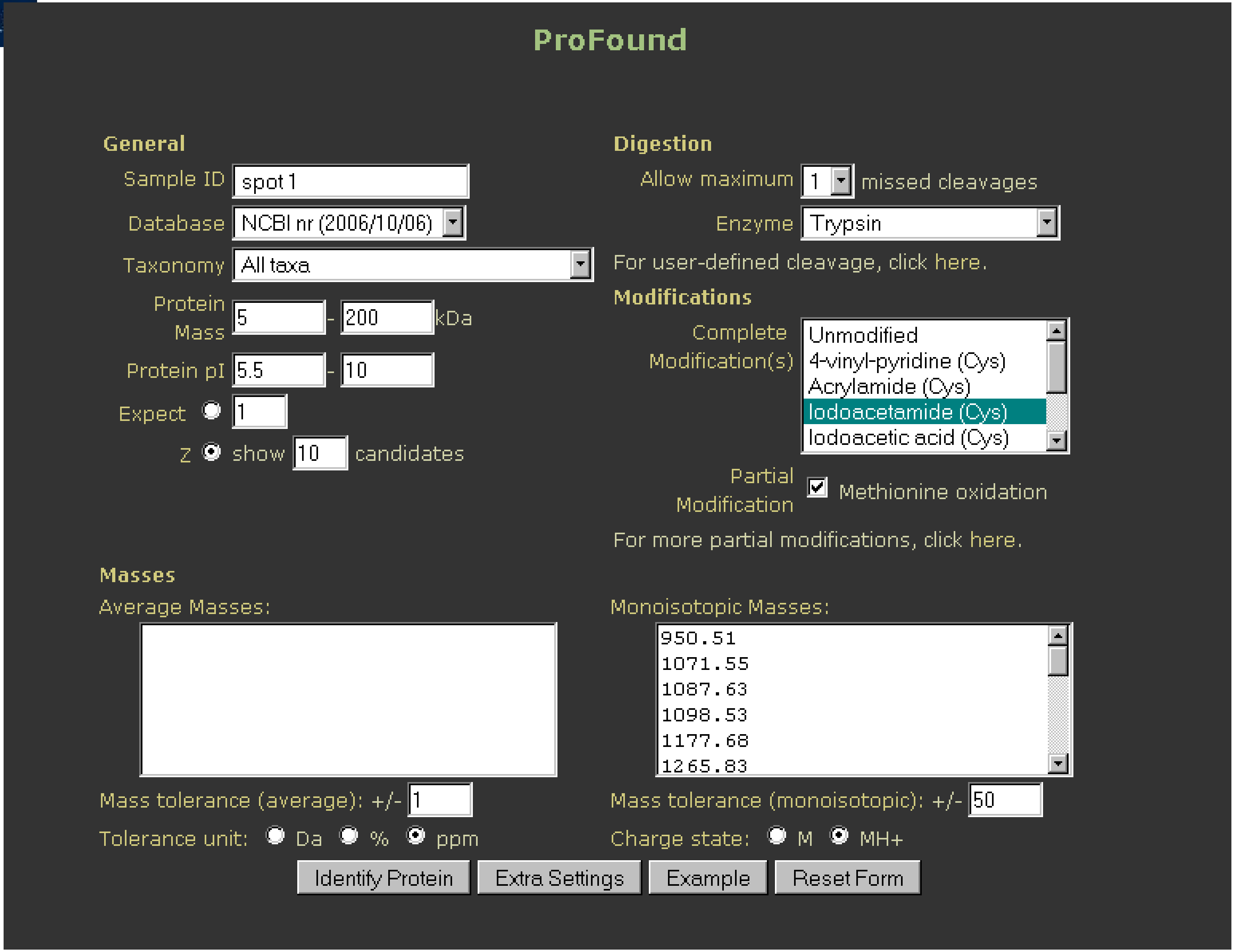
X! Hunter

X! Hunter is a tool for identifying proteins that matches tandem mass spectra to a library of spectra that have been confidently assigned to a particular peptide sequence.

GPMDB

GPMDB is a database of tandem mass spectra and their assigned peptide sequences. It is designed to aid in the difficult process of validating peptide MS/MS spectra.

Input example for protein database search using ProFound



ProFound

General

Sample ID: spot 1

Database: NCBI nr (2006/10/06)

Taxonomy: All taxa

Protein Mass: 5 - 200 kDa

Protein pI: 5.5 - 10

Expect ○ 1

Z ● show 10 candidates

Digestion

Allow maximum 1 missed cleavages

Enzyme: Trypsin

For user-defined cleavage, click here.

Modifications

Complete Modification(s):
- Unmodified
- 4-vinyl-pyridine (Cys)
- Acrylamide (Cys)
- Iodoacetamide (Cys)
- Iodoacetic acid (Cys)

Partial Modification: ☑ Methionine oxidation

For more partial modifications, click here.

Masses

Average Masses:

Monoisotopic Masses:
950.51
1071.55
1087.63
1098.53
1177.68
1265.83

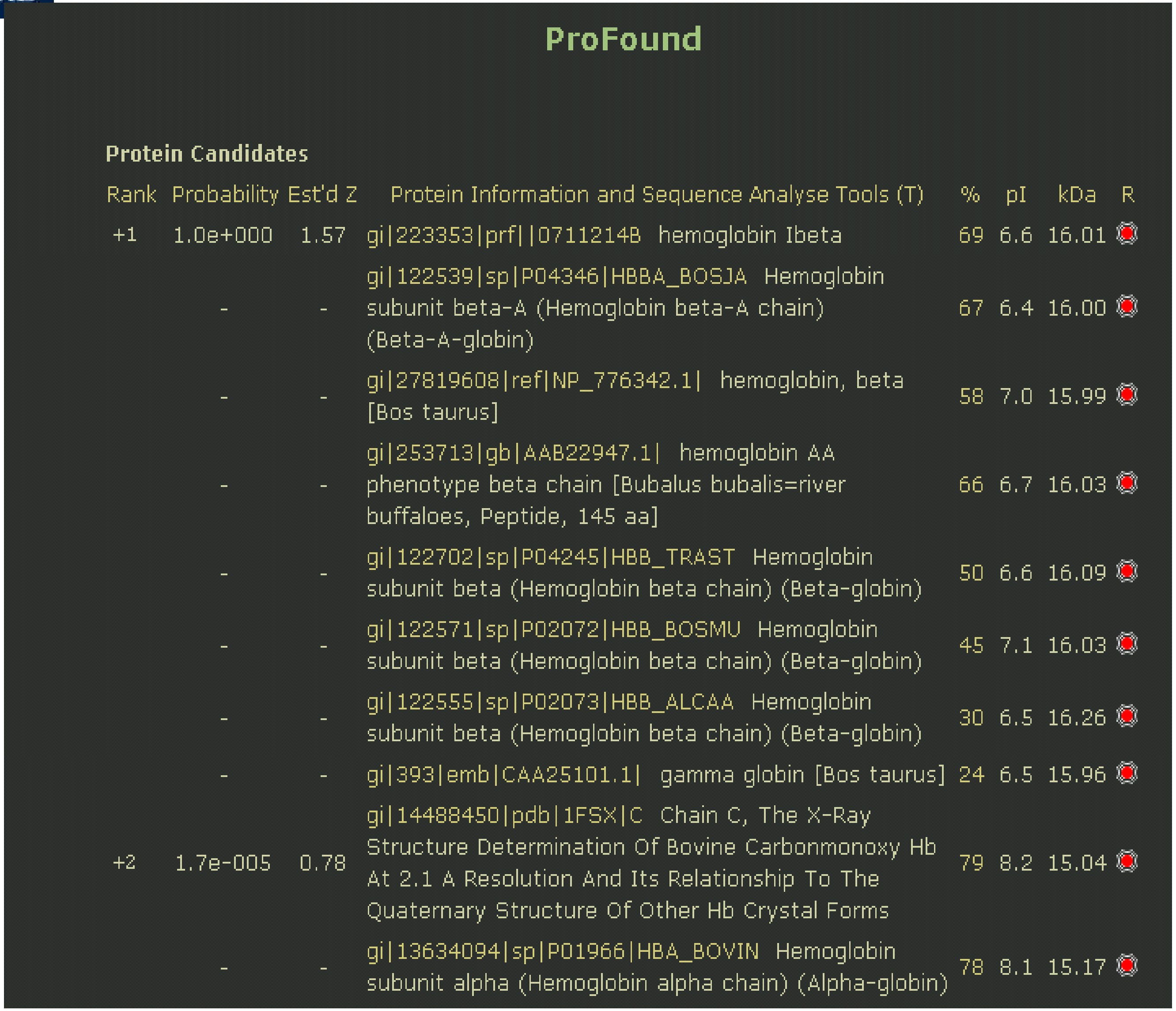
Mass tolerance (average): +/- 1

Tolerance unit: ○ Da ○ % ● ppm

Mass tolerance (monoisotopic): +/- 50

Charge state: ○ M ● MH+

Protein candidates of peptide fingerprint spectrum

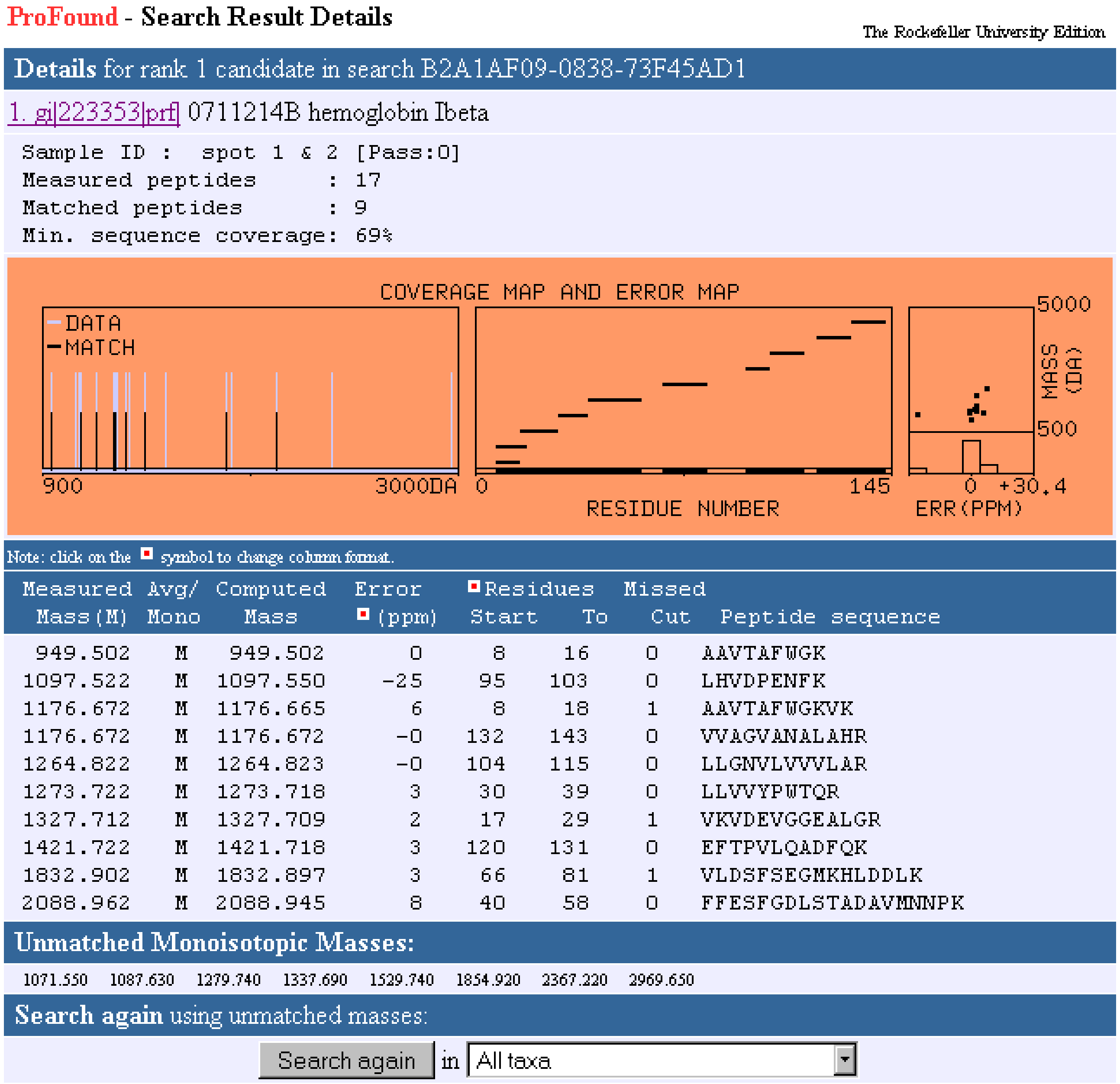


ProFound

Protein Candidates

Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	pI	kDa	R
+1	1.0e+000	1.57	gi|223353|prf||0711214B hemoglobin Ibeta	69	6.6	16.01	●
	-	-	gi|122539|sp|P04346|HBBA_BOSJA Hemoglobin subunit beta-A (Hemoglobin beta-A chain) (Beta-A-globin)	67	6.4	16.00	●
	-	-	gi|27819608|ref|NP_776342.1| hemoglobin, beta [Bos taurus]	58	7.0	15.99	●
	-	-	gi|253713|gb|AAB22947.1| hemoglobin AA phenotype beta chain [Bubalus bubalis=river buffaloes, Peptide, 145 aa]	66	6.7	16.03	●
	-	-	gi|122702|sp|P04245|HBB_TRAST Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)	50	6.6	16.09	●
	-	-	gi|122571|sp|P02072|HBB_BOSMU Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)	45	7.1	16.03	●
	-	-	gi|122555|sp|P02073|HBB_ALCAA Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin)	30	6.5	16.26	●
	-	-	gi|393|emb|CAA25101.1| gamma globin [Bos taurus]	24	6.5	15.96	●
+2	1.7e-005	0.78	gi|14488450|pdb|1FSX|C Chain C, The X-Ray Structure Determination Of Bovine Carbonmonoxy Hb At 2.1 A Resolution And Its Relationship To The Quaternary Structure Of Other Hb Crystal Forms	79	8.2	15.04	●
	-	-	gi|13634094|sp|P01966|HBA_BOVIN Hemoglobin subunit alpha (Hemoglobin alpha chain) (Alpha-globin)	78	8.1	15.17	●

Search result details of hemoglobin beta



ProFound - **Search Result Details**

The Rockefeller University Edition

Details for rank 1 candidate in search B2A1AF09-0838-73F45AD1

1. gi|223353|prf| 0711214B hemoglobin Ibeta

```
Sample ID :  spot 1 & 2 [Pass:0]
Measured peptides      : 17
Matched peptides       : 9
Min. sequence coverage: 69%
```

Note: click on the ▪ symbol to change column format.

Measured Mass (M)	Avg/ Mono	Computed Mass	Error ▪ (ppm)	▪Residues Start	To	Missed Cut	Peptide sequence
949.502	M	949.502	0	8	16	0	AAVTAFWGK
1097.522	M	1097.550	-25	95	103	0	LHVDPENFK
1176.672	M	1176.665	6	8	18	1	AAVTAFWGKVK
1176.672	M	1176.672	-0	132	143	0	VVAGVANALAHR
1264.822	M	1264.823	-0	104	115	0	LLGNVLVVVLAR
1273.722	M	1273.718	3	30	39	0	LLVVYPWTQR
1327.712	M	1327.709	2	17	29	1	VKVDEVGGEALGR
1421.722	M	1421.718	3	120	131	0	EFTPVLQADFQK
1832.902	M	1832.897	3	66	81	1	VLDSFSEGMKHLDDLK
2088.962	M	2088.945	8	40	58	0	FFESFGDLSTADAVMNNPK

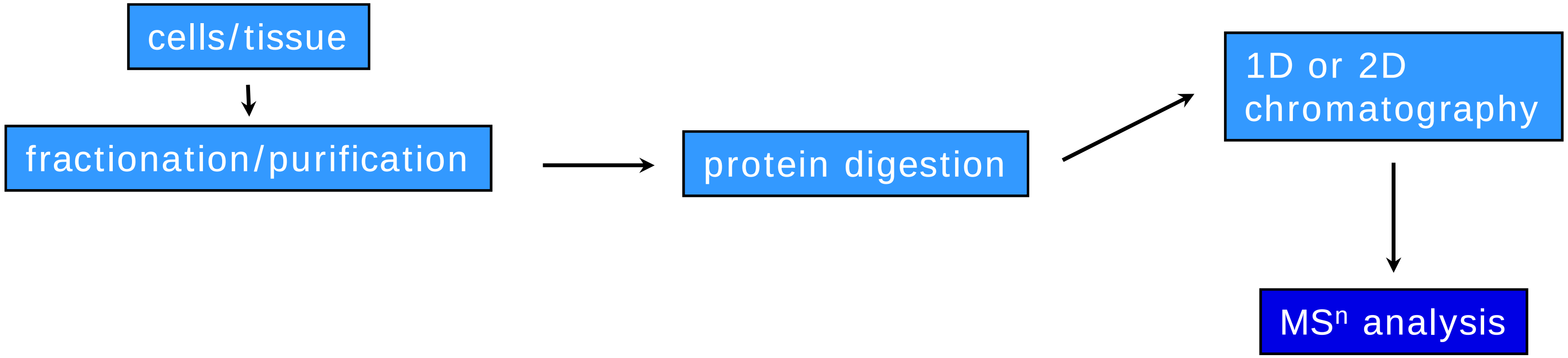
Unmatched Monoisotopic Masses:

1071.550 1087.630 1279.740 1337.690 1529.740 1854.920 2367.220 2969.650

Search again using unmatched masses:

Search again in All taxa

Complexity – samples with more than 1 protein



cells/tissue

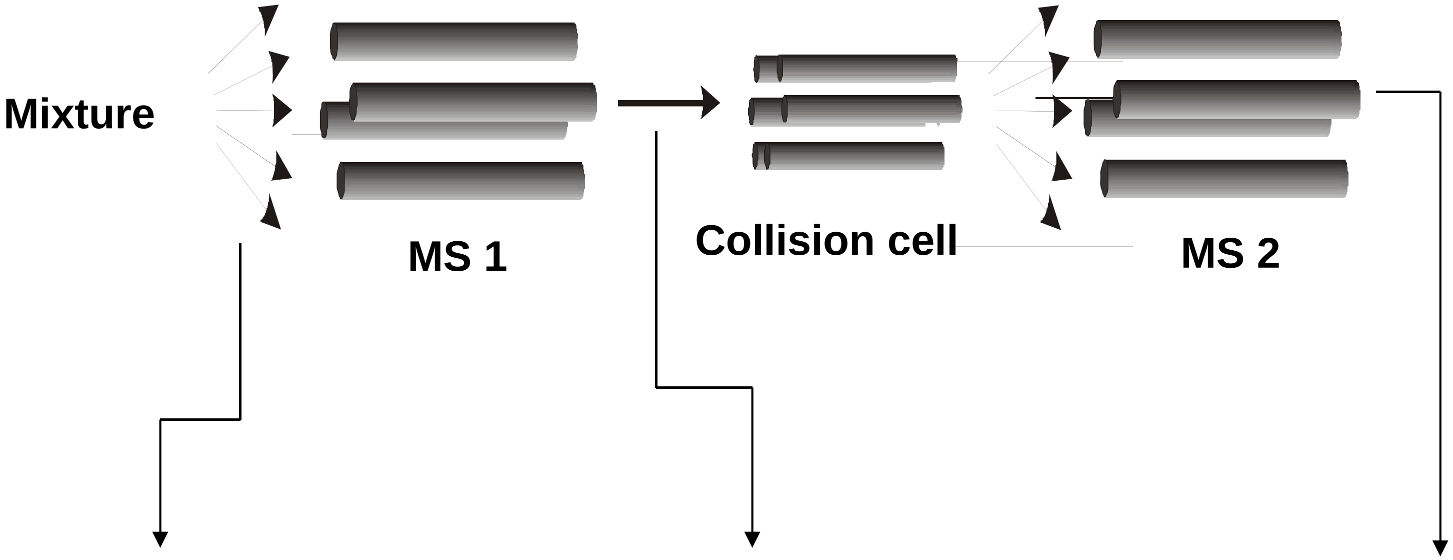
fractionation/purification

protein digestion

1D or 2D chromatography

MS^n analysis

Collision induced dissociation

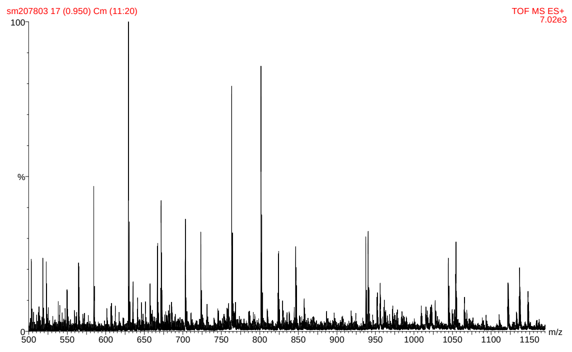


Mixture

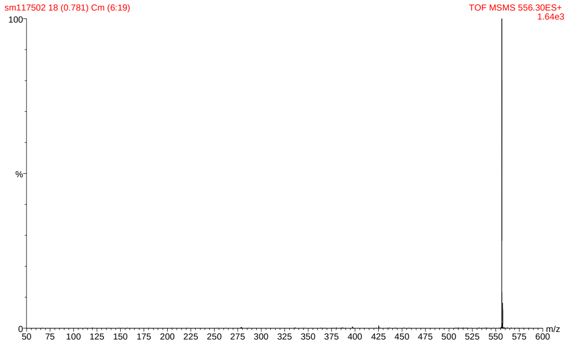
MS 1

Collision cell

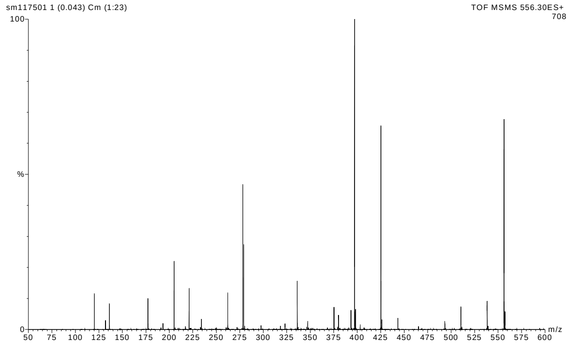
MS 2



A mass spectrum of a mixture

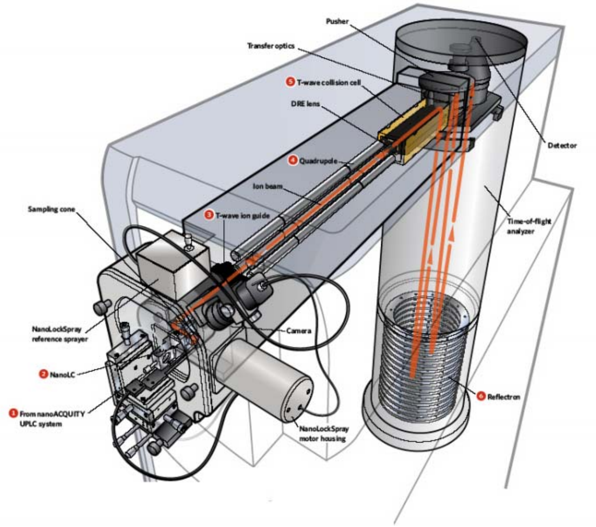


Isolation of an ion
In our case a protonated peptide



A mass spectrum of the fragments
Produced by the ion

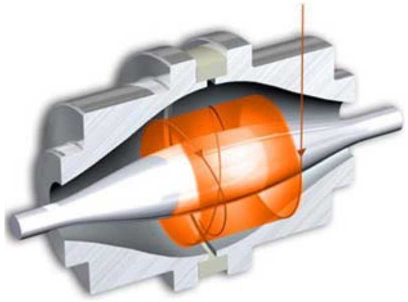
More than one configuration for Collision Induced Dissociation



Trap

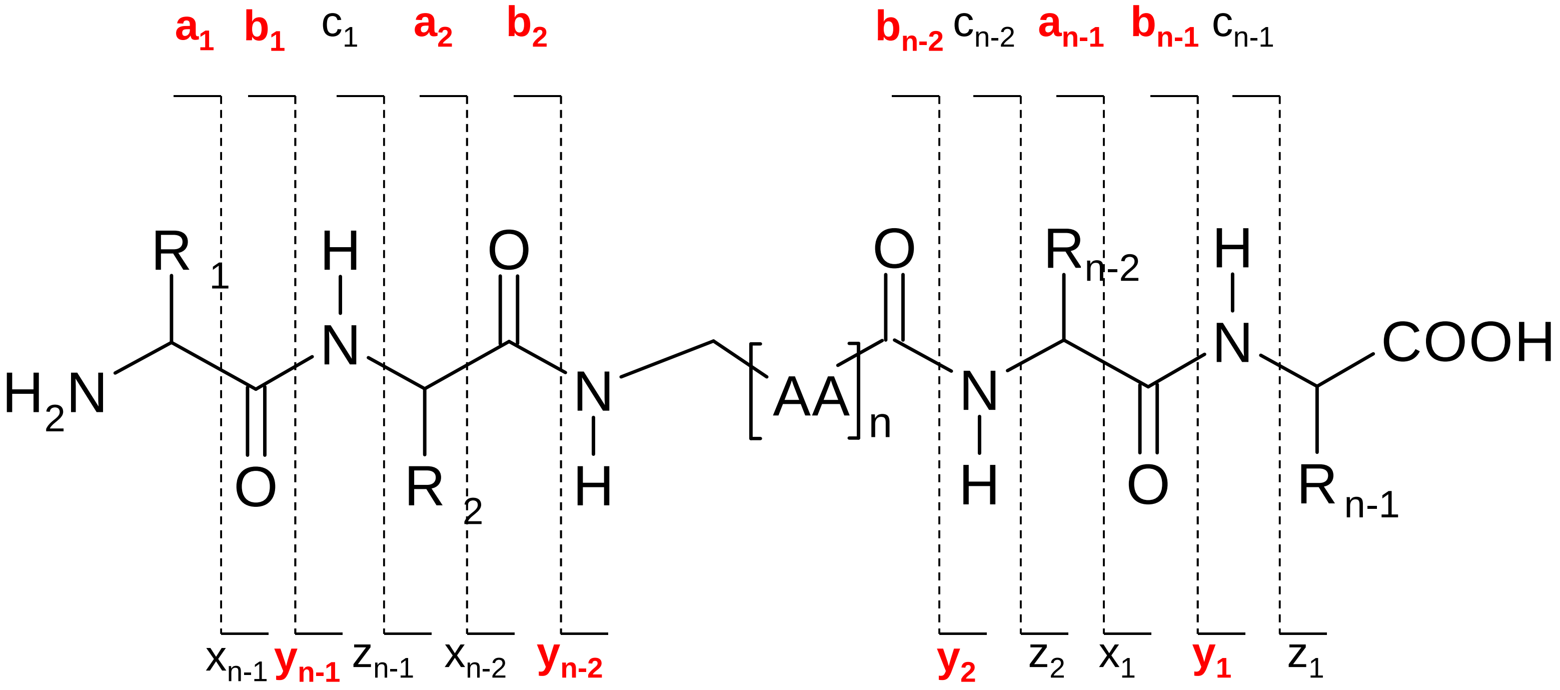
Hybrid

Quad



General Peptide Fragments

Only fragments that are charged are detected!



Fragmentation spectra: complicated and poorly predictable but some things are known

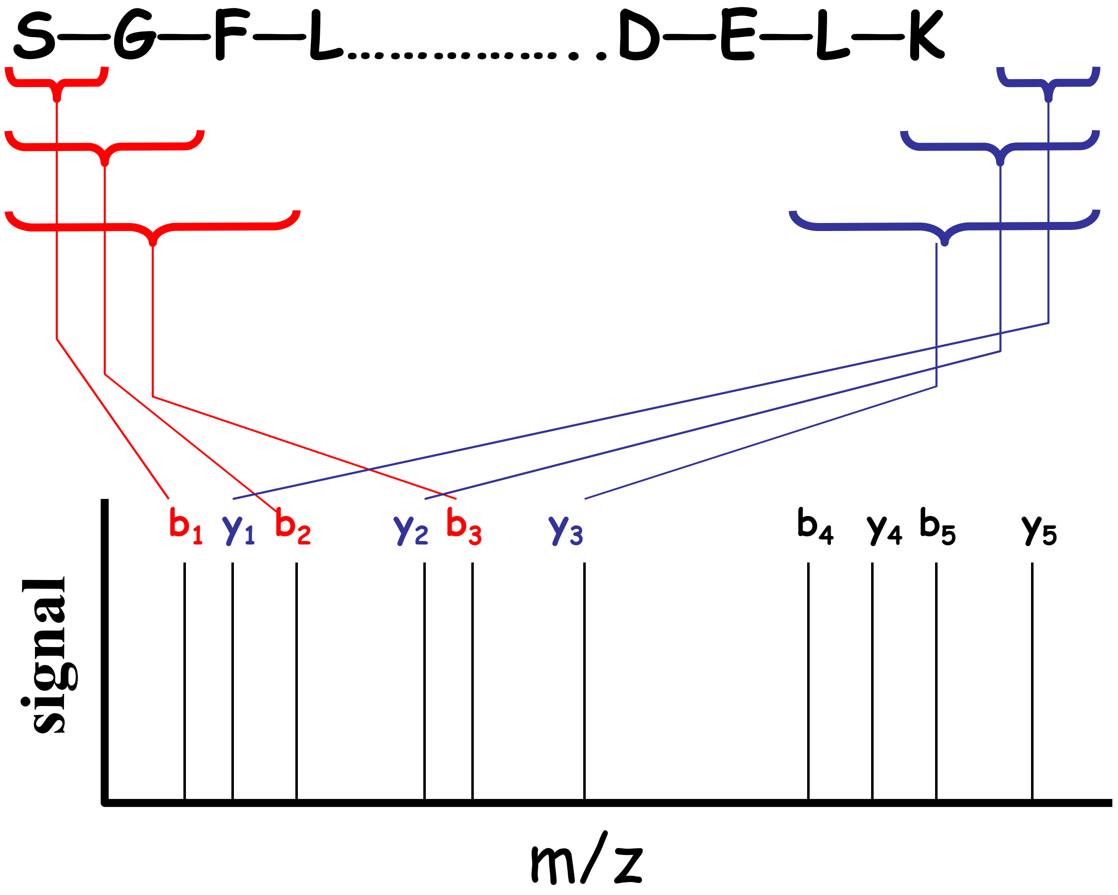
Peptide fragmentation

Any of the peptide bonds *might* break, hard to predict which ones *will* break

Peptide: S-G-F-L-E-E-D-E-L-K

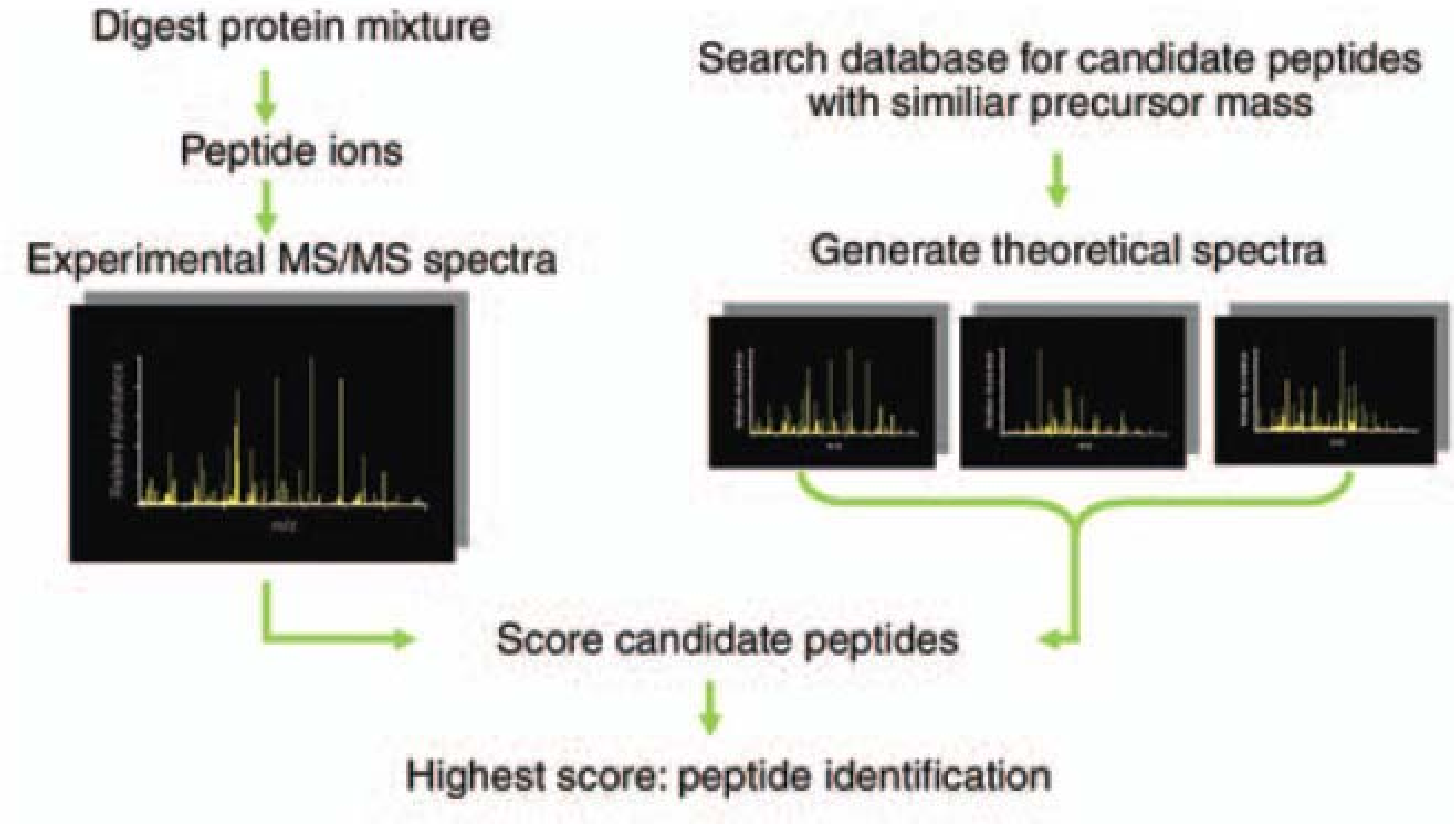
MW	**ion**			**ion**	**MW**
88	b_1	S	GFLEEDELK	y_9	1080
145	b_2	SG	FLEEDELK	y_8	1022
292	b_3	SGF	LEEDELK	y_7	875
405	b_4	SGFL	EEDELK	y_6	762
534	b_5	SGFLE	EDELK	y_5	633
663	b_6	SGFLEE	DELK	y_4	504
778	b_7	SGFLEED	ELK	y_3	389
907	b_8	SGFLEEDE	LK	y_2	260
1020	b_9	SGFLEEDEL	K	y_1	147

Peptide fragmentation



Sequence can be read from distance between peaks

Protein identification from complex samples



Digest protein mixture

Peptide ions

Experimental MS/MS spectra

Search database for candidate peptides with similiar precursor mass

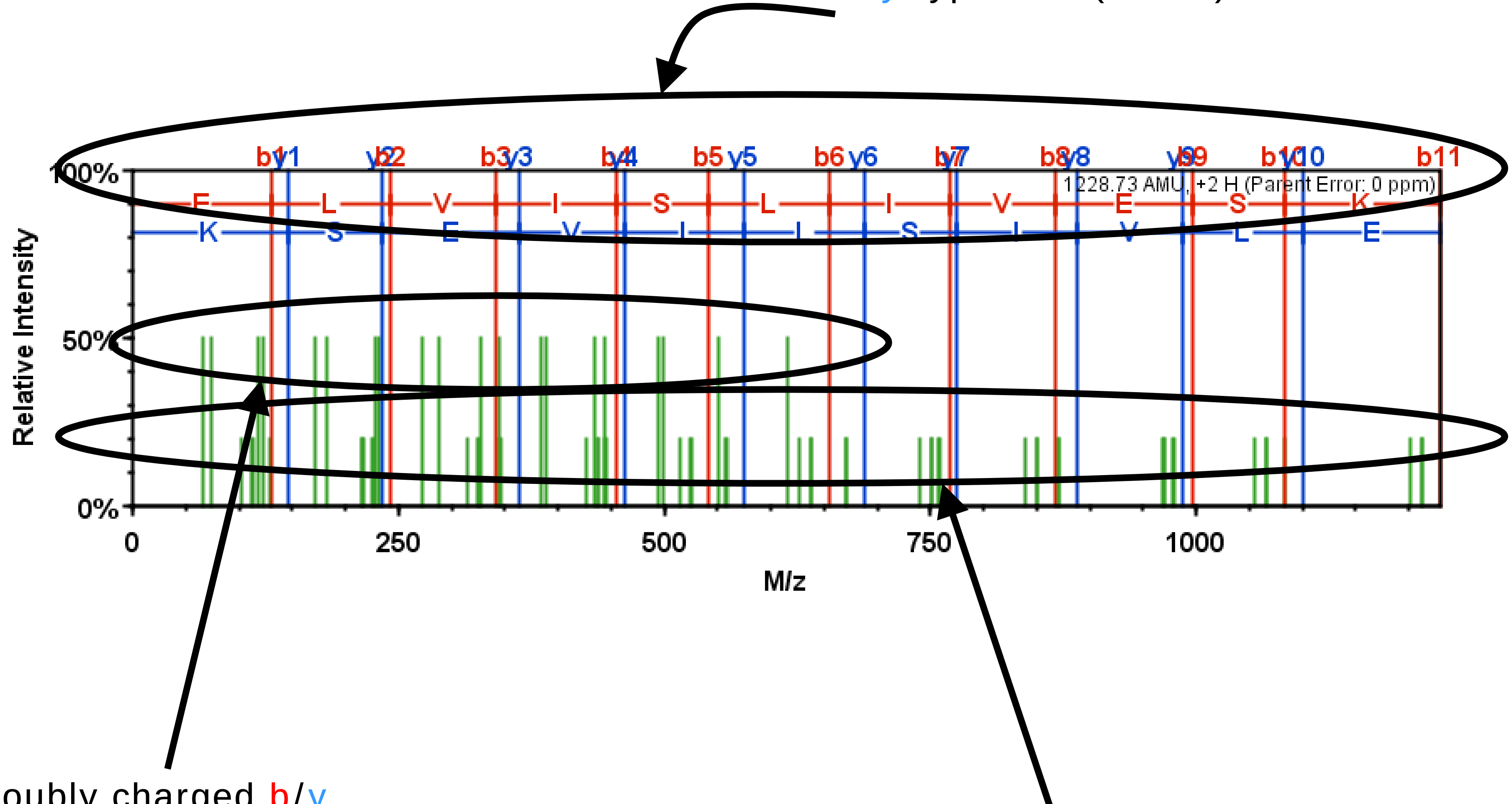
Generate theoretical spectra

Score candidate peptides

Highest score: peptide identification

Model Spectrum

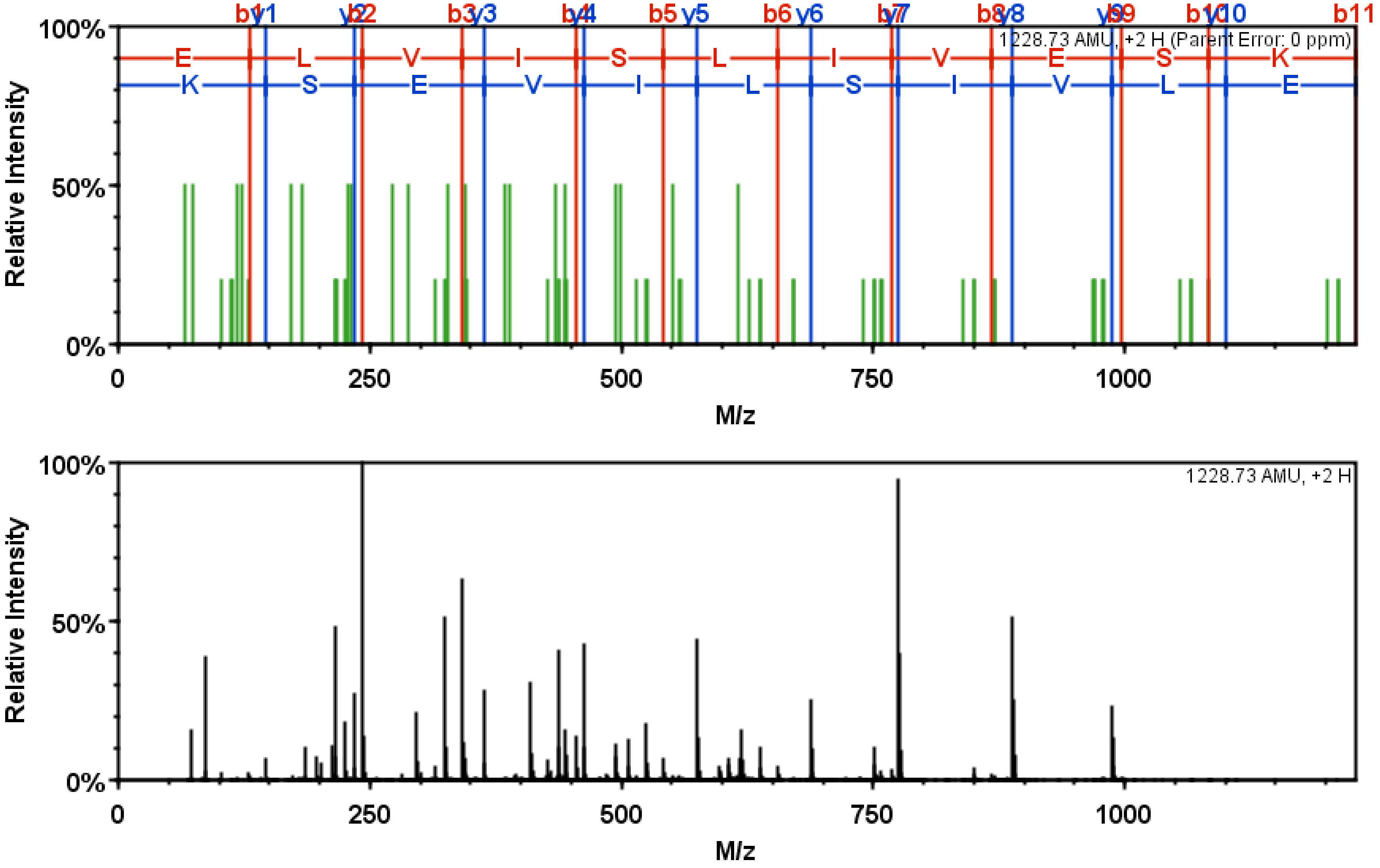
b/y type ions (100%)



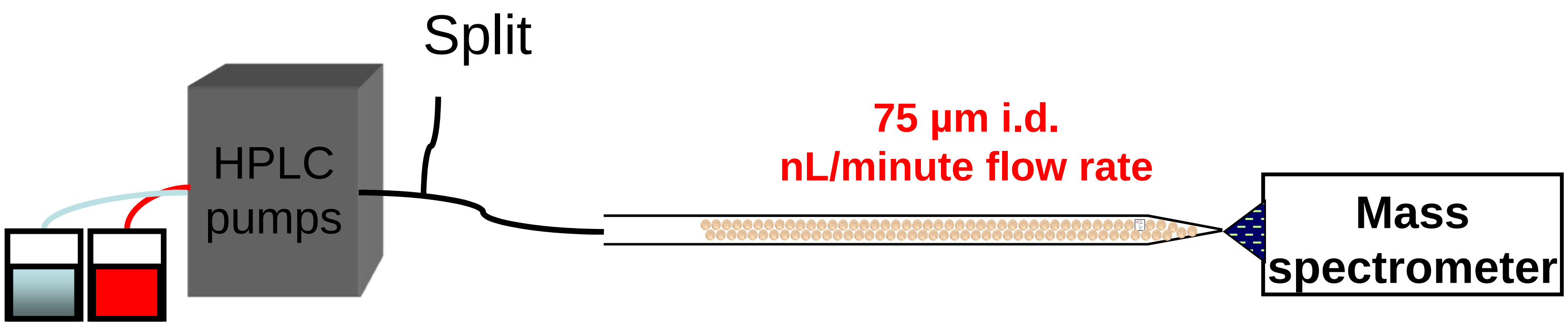
Doubly charged b/y
(50%)

a type ions
b/y $-NH_3/-H_2O$
(20%)

Spectrum Matching



Reverse phase microcapillary LC-MS

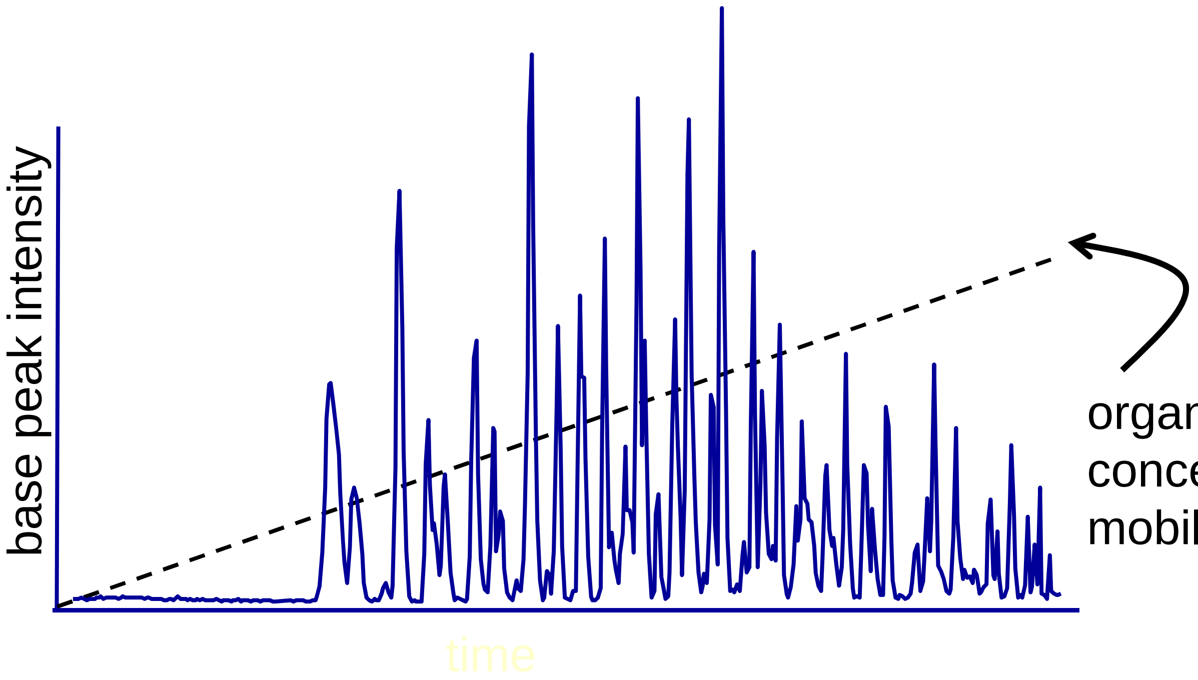


Split

HPLC pumps

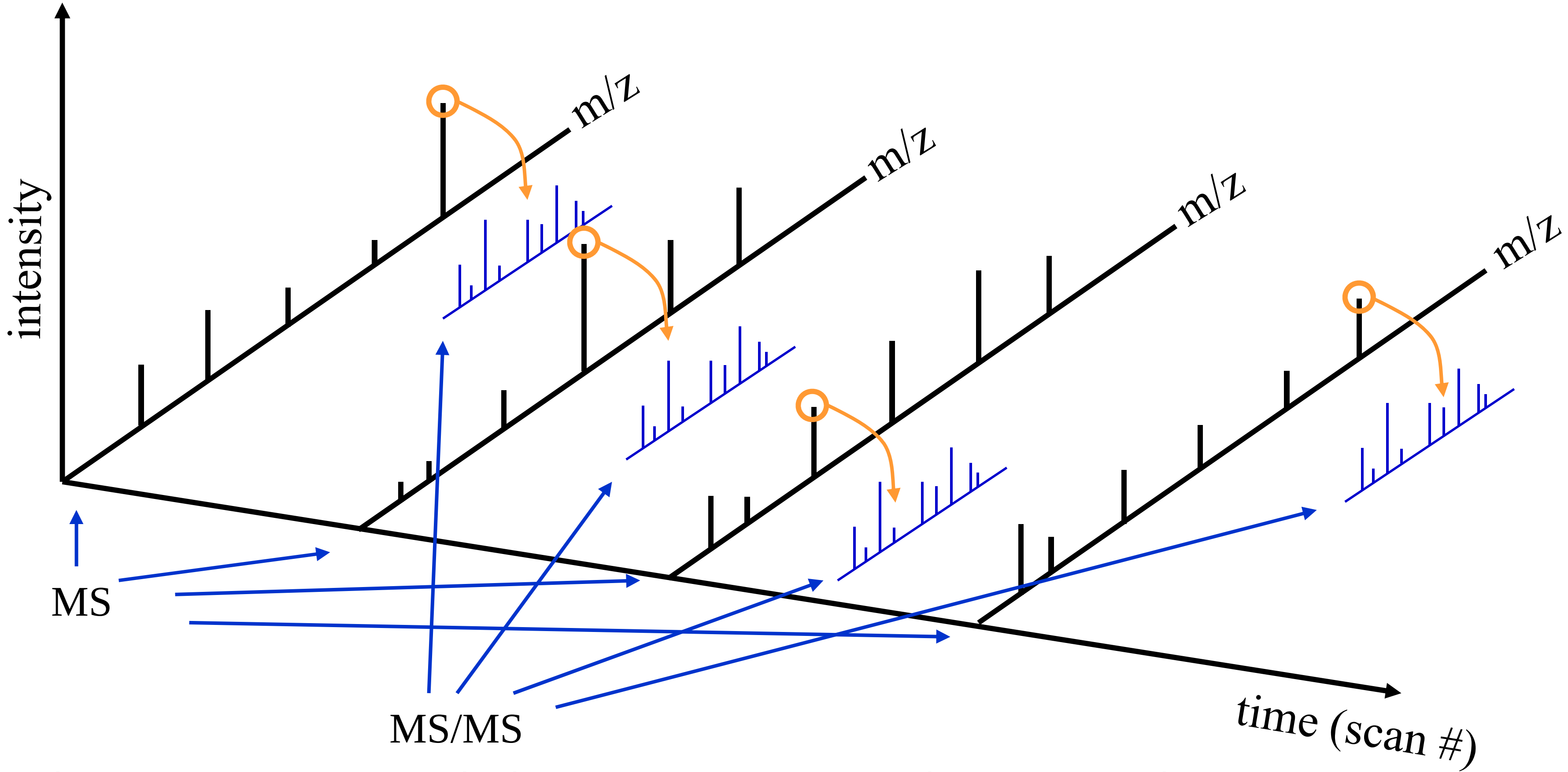
75 µm i.d.
nL/minute flow rate

Mass spectrometer



organic
concentration in
mobile phase

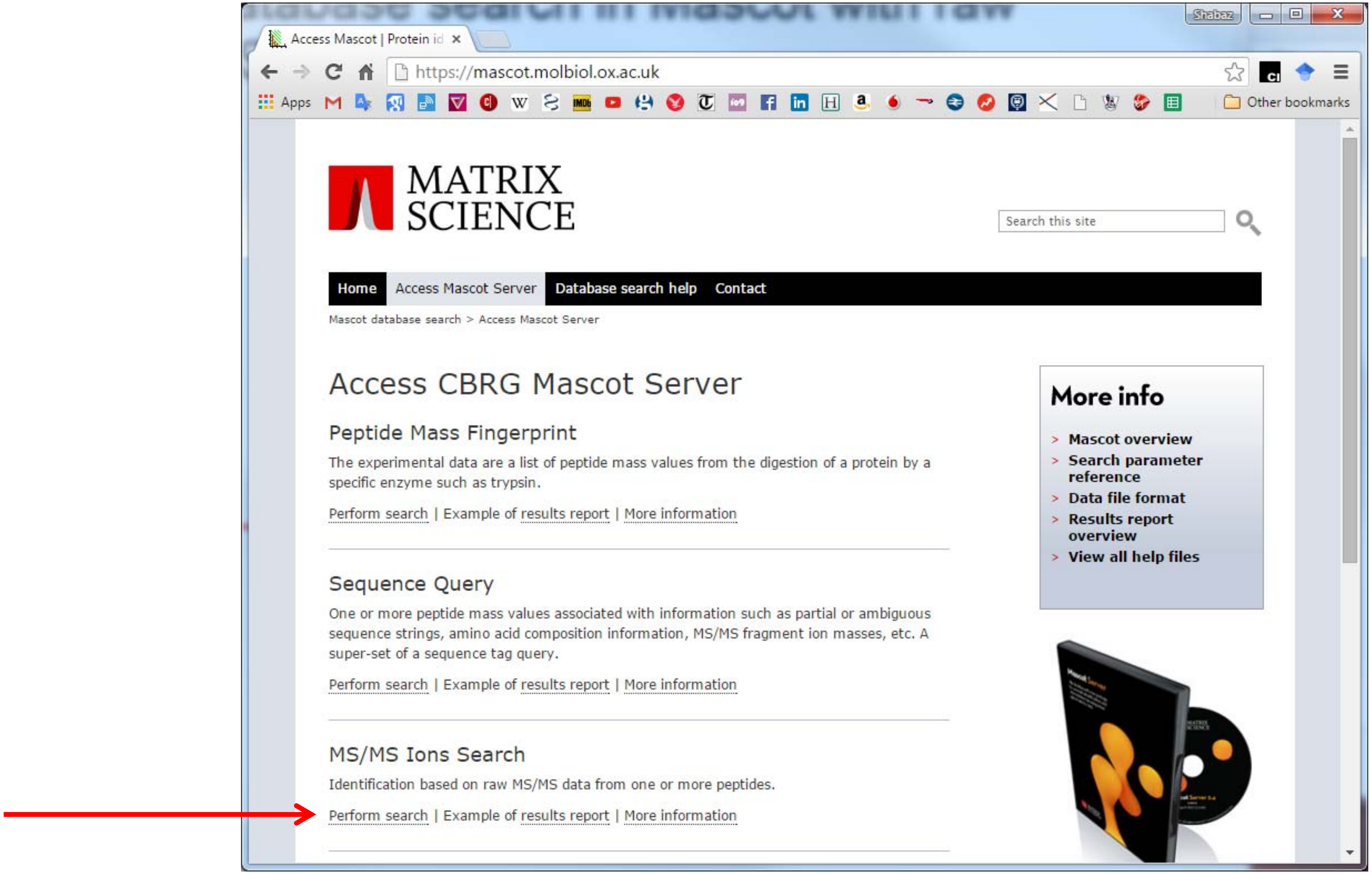
Automated MS/MS



- The mass spectrometer will first perform an MS scan (a survey scan)
- It will then perform MS/MS according to user instructions e.g. 20 most abundant ions
- Once MS/MS is performed it will repeat cycle

- Current technology allows over 100 MS/MS events per minute

Database search in Mascot with raw MS/MS data



Access CBRG Mascot Server

Peptide Mass Fingerprint

The experimental data are a list of peptide mass values from the digestion of a protein by a specific enzyme such as trypsin.

Perform search | Example of results report | More information

Sequence Query

One or more peptide mass values associated with information such as partial or ambiguous sequence strings, amino acid composition information, MS/MS fragment ion masses, etc. A super-set of a sequence tag query.

Perform search | Example of results report | More information

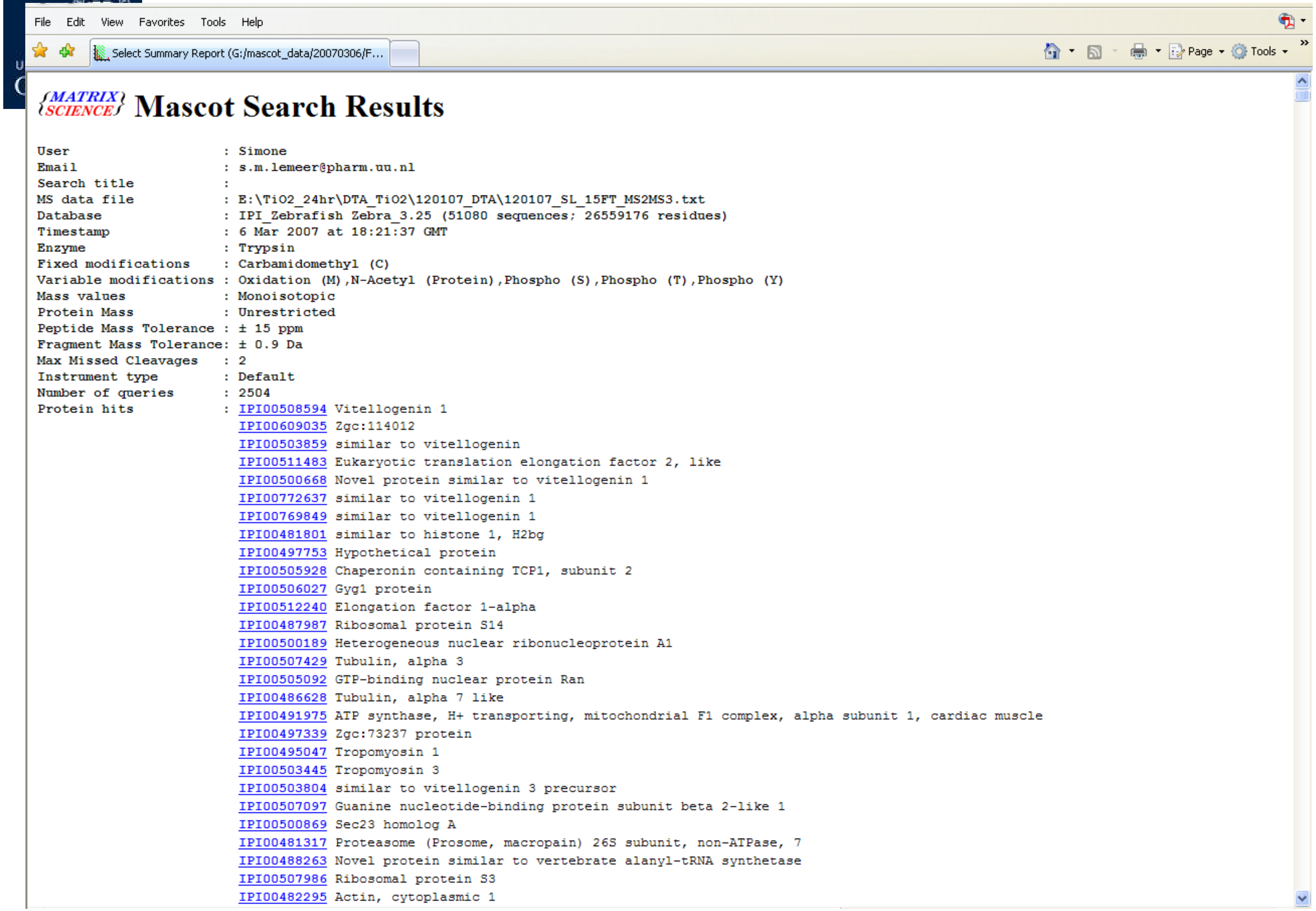
MS/MS Ions Search

Identification based on raw MS/MS data from one or more peptides.

Perform search | Example of results report | More information

More info

- Mascot overview
- Search parameter reference
- Data file format
- Results report overview
- View all help files

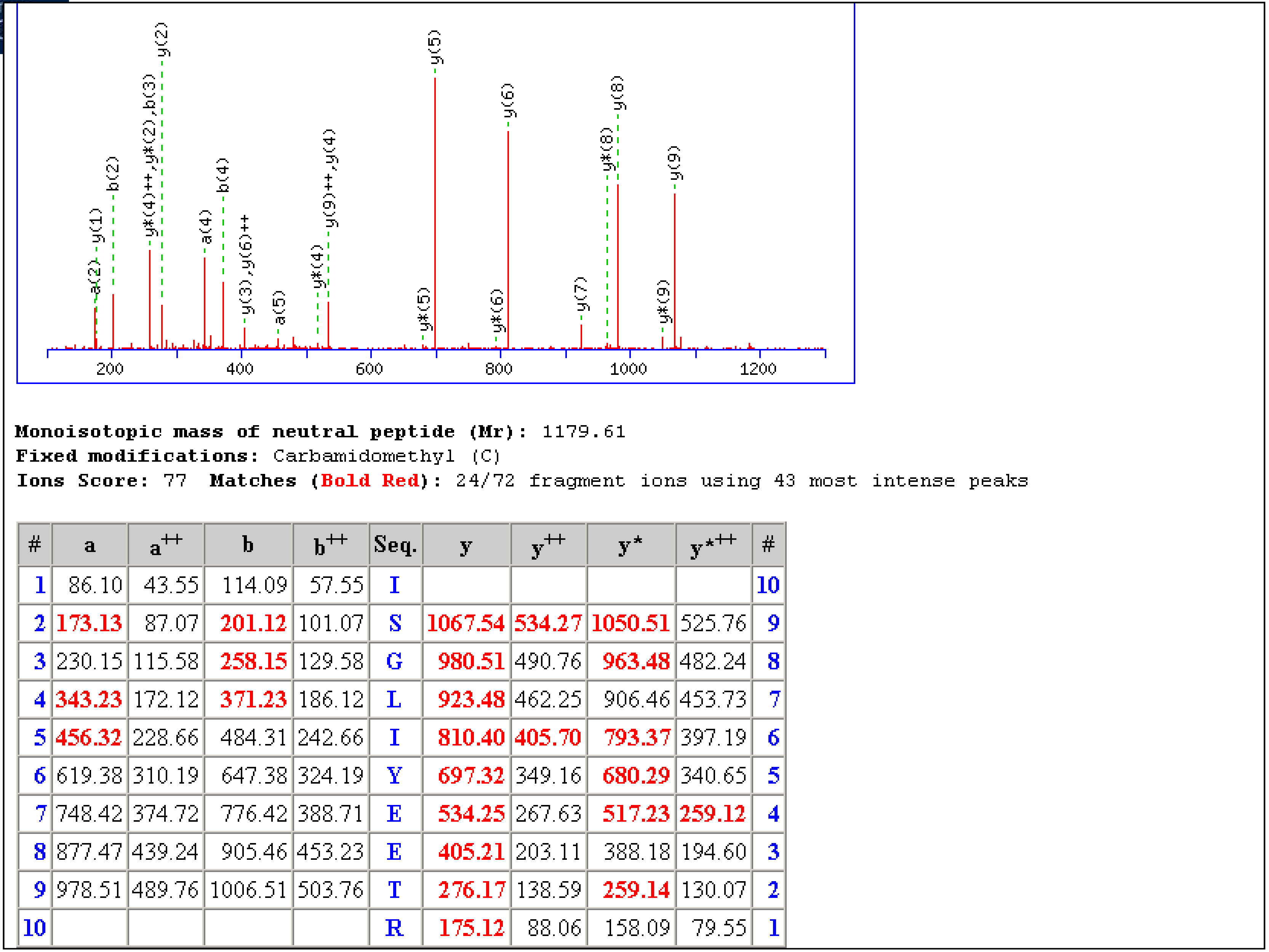


Mascot Search Results

```
User                     : Simone
Email                    : s.m.lemeer@pharm.uu.nl
Search title             :
MS data file             : E:\TiO2_24hr\DTA_TiO2\120107_DTA\120107_SL_15FT_MS2MS3.txt
Database                 : IPI_Zebrafish Zebra_3.25 (51080 sequences; 26559176 residues)
Timestamp                : 6 Mar 2007 at 18:21:37 GMT
Enzyme                   : Trypsin
Fixed modifications      : Carbamidomethyl (C)
Variable modifications   : Oxidation (M),N-Acetyl (Protein),Phospho (S),Phospho (T),Phospho (Y)
Mass values              : Monoisotopic
Protein Mass             : Unrestricted
Peptide Mass Tolerance   : ± 15 ppm
Fragment Mass Tolerance: ± 0.9 Da
Max Missed Cleavages     : 2
Instrument type          : Default
Number of queries        : 2504
Protein hits             : IPI00508594 Vitellogenin 1
                           IPI00609035 Zgc:114012
                           IPI00503859 similar to vitellogenin
                           IPI00511483 Eukaryotic translation elongation factor 2, like
                           IPI00500668 Novel protein similar to vitellogenin 1
                           IPI00772637 similar to vitellogenin 1
                           IPI00769849 similar to vitellogenin 1
                           IPI00481801 similar to histone 1, H2bg
                           IPI00497753 Hypothetical protein
                           IPI00505928 Chaperonin containing TCP1, subunit 2
                           IPI00506027 Gyg1 protein
                           IPI00512240 Elongation factor 1-alpha
                           IPI00487987 Ribosomal protein S14
                           IPI00500189 Heterogeneous nuclear ribonucleoprotein A1
                           IPI00507429 Tubulin, alpha 3
                           IPI00505092 GTP-binding nuclear protein Ran
                           IPI00486628 Tubulin, alpha 7 like
                           IPI00491975 ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
                           IPI00497339 Zgc:73237 protein
                           IPI00495047 Tropomyosin 1
                           IPI00503445 Tropomyosin 3
                           IPI00503804 similar to vitellogenin 3 precursor
                           IPI00507097 Guanine nucleotide-binding protein subunit beta 2-like 1
                           IPI00500869 Sec23 homolog A
                           IPI00481317 Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 7
                           IPI00488263 Novel protein similar to vertebrate alanyl-tRNA synthetase
                           IPI00507986 Ribosomal protein S3
                           IPI00482295 Actin, cytoplasmic 1
```

```
466.  IPI00328343       Mass: 49416    Score: 231    Queries matched: 13
      Spliceosome RNA helicase BAT1
   ☐  Check to include this hit in error tolerant search or archive report

        Query   Observed    Mr(expt)    Mr(calc)      Delta Miss Score    Expect Rank Peptide
        17307  453.73779   905.46103   905.46063    0.00040    0    36    0.0067    1  R.DVQEIFR.M
        46539  552.33282  1102.65109  1102.64981    0.00128    0    31    0.0052    1  R.ILVATNLFGR.G
        79547  653.83569  1305.65683  1305.66113   -0.00430    0    55  7.1e-005    1  K.NCPHIVVGTPGR.I
       105120  756.88940  1511.76425  1511.76193    0.00232    1    48    0.0003    1  R.ELAFQISKEYER.F
       143262  658.30749  1971.90065  1971.89577    0.00488    1    49    0.0004    1  R.KFMQDPMEIFVDDETK.L
       153526  759.75873  2276.25435  2276.24626    0.00809    0    33    0.0031    1  R.CIALAQLLVEQNFPAIAIHR.G
       153990  767.35107  2299.03139  2299.02150    0.00989    0    34     0.015    1  R.VNIAFNYDMPEDSDTYLHR.V

467.  IPI00002255       Mass: 321639   Score: 231    Queries matched: 8
      Lipopolysaccharide-responsive and beige-like anchor protein
   ☐  Check to include this hit in error tolerant search or archive report

        Query   Observed    Mr(expt)    Mr(calc)      Delta Miss Score    Expect Rank Peptide
        49848  561.84997  1121.68539  1121.68079    0.00461    0    58  1.7e-006    1  K.SAAAIALPPIAK.W
        72980  420.21969  1257.63725  1257.64269   -0.00543    0    21      0.23    1  K.ITEMVYAIFR.I
        87415  682.86084  1363.70712  1363.70953   -0.00241    0    51   0.00019    1  R.NPLGSTHPEATLK.T
        89779  462.57345  1384.69853  1384.70200   -0.00347    0    33    0.0088    1  R.TLEGPENCLKPK.L
       136266  620.95001  1859.82820  1859.83189   -0.00369    0    52   0.00021    1  K.LINDCHGSVSEASSEQK.I
       139422  634.64545  1900.91451  1900.91391    0.00060    0    44    0.0014    1  R.QHEQPGQGIAPDAVNGQR.R
       152240  744.99817  2231.97267  2231.97527   -0.00259    0    59  5.5e-005    1  K.DSPVCPHFTTNGNENSSIEK.T


      Proteins matching the same set of peptides:
      IPI00477088       Mass: 321614   Score: 231    Queries matched: 8
      Hypothetical protein DKFZp686K03100

468.  IPI00011857       Mass: 61910    Score: 230    Queries matched: 9
      Chromatin assembly factor 1 subunit B
   ☐  Check to include this hit in error tolerant search or archive report

        Query   Observed    Mr(expt)    Mr(calc)     Delta Miss Score    Expect Rank Peptide
       108526  770.37433  1538.73410  1538.73248  0.00162    0    70  2.7e-006    1  R.TQDPSSPGTTPPQAR.Q
       142561  653.37482  1957.10262  1957.09961  0.00301    1    36    0.0016    1  R.QAPAPTVIRDPPSITPAVK.S
       157885  858.73907  2573.19539  2573.18806  0.00733    1    60  3.9e-005    1  K.VNDNKEPEQIAFQDEDEAQLNK.E
```

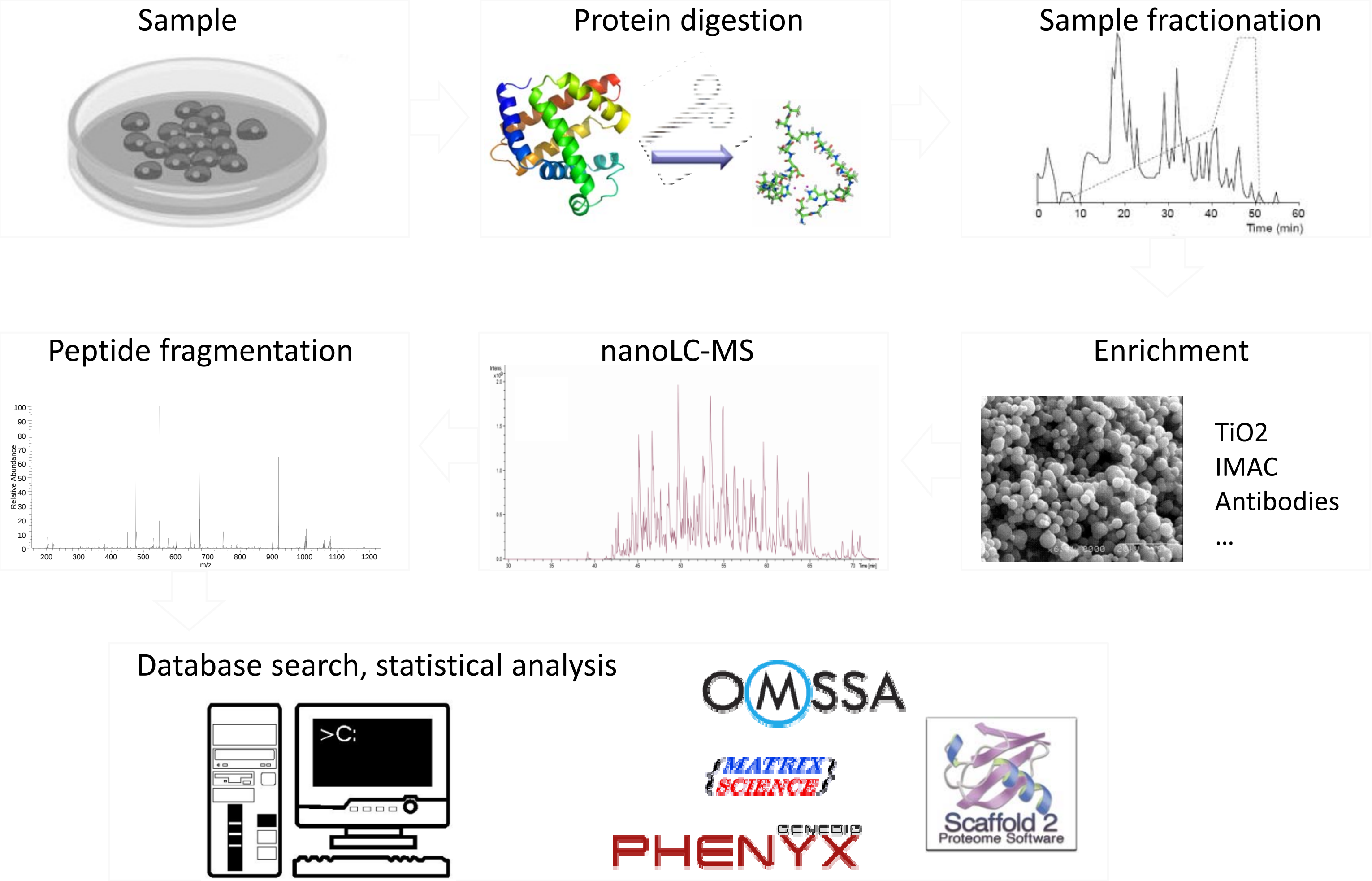
Output details after search in Mascot



Monoisotopic mass of neutral peptide (Mr): 1179.61
Fixed modifications: Carbamidomethyl (C)
Ions Score: 77 **Matches (Bold Red):** 24/72 fragment ions using 43 most intense peaks

#	a	a^{++}	b	b^{++}	Seq.	y	y^{++}	y*	y*$^{++}$	#
1	86.10	43.55	114.09	57.55	I					10
2	**173.13**	87.07	**201.12**	101.07	S	**1067.54**	**534.27**	**1050.51**	525.76	9
3	230.15	115.58	**258.15**	129.58	G	**980.51**	490.76	**963.48**	482.24	8
4	**343.23**	172.12	**371.23**	186.12	L	**923.48**	462.25	906.46	453.73	7
5	**456.32**	228.66	484.31	242.66	I	**810.40**	**405.70**	**793.37**	397.19	6
6	619.38	310.19	647.38	324.19	Y	**697.32**	349.16	**680.29**	340.65	5
7	748.42	374.72	776.42	388.71	E	**534.25**	267.63	**517.23**	**259.12**	4
8	877.47	439.24	905.46	453.23	E	**405.21**	203.11	388.18	194.60	3
9	978.51	489.76	1006.51	503.76	T	**276.17**	138.59	**259.14**	130.07	2
10					R	**175.12**	88.06	158.09	79.55	1

Conventional proteomics approach: bottom-up



Sample

Protein digestion

Sample fractionation

Enrichment

TiO2
IMAC
Antibodies
…

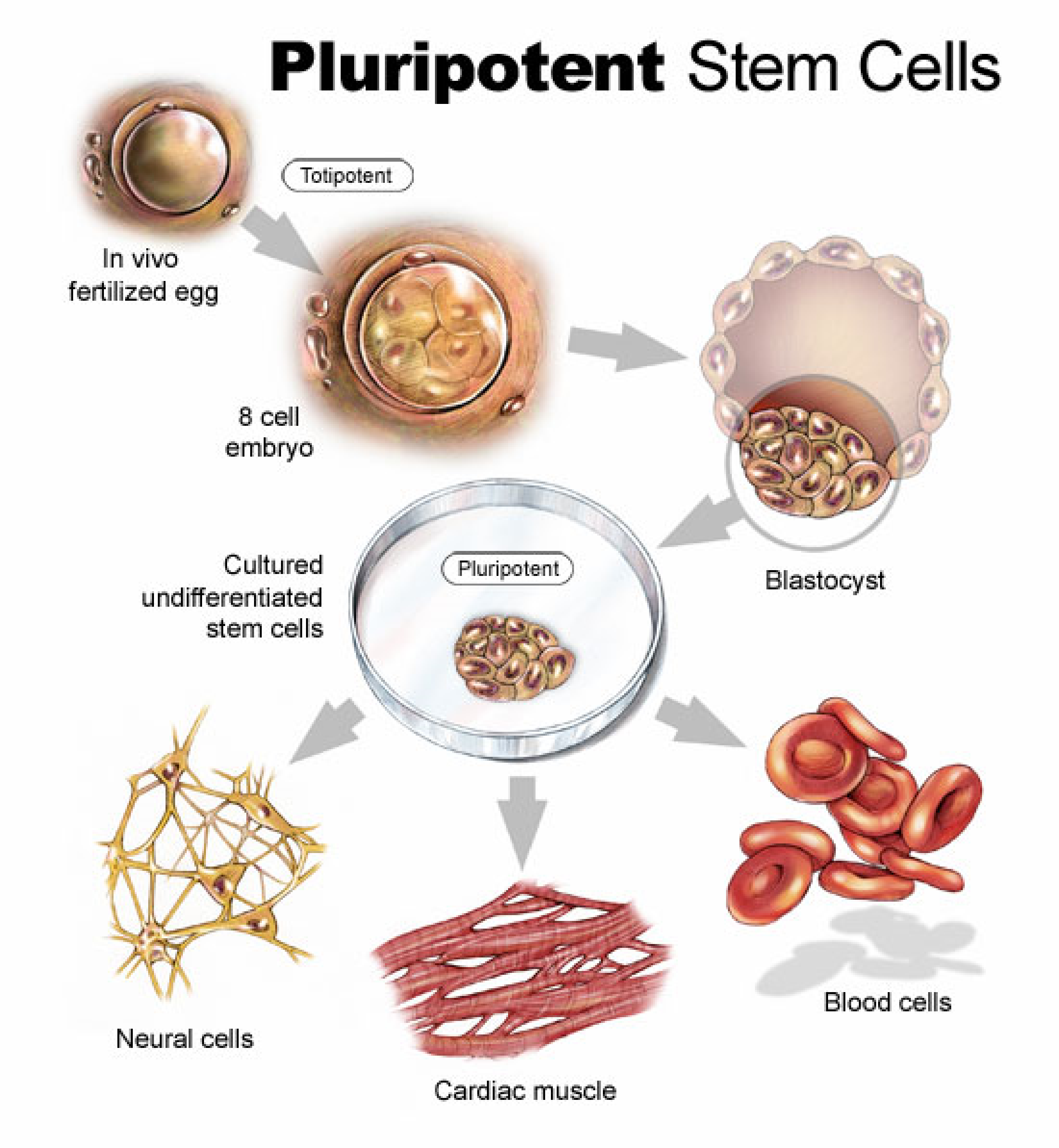
nanoLC-MS

Peptide fragmentation

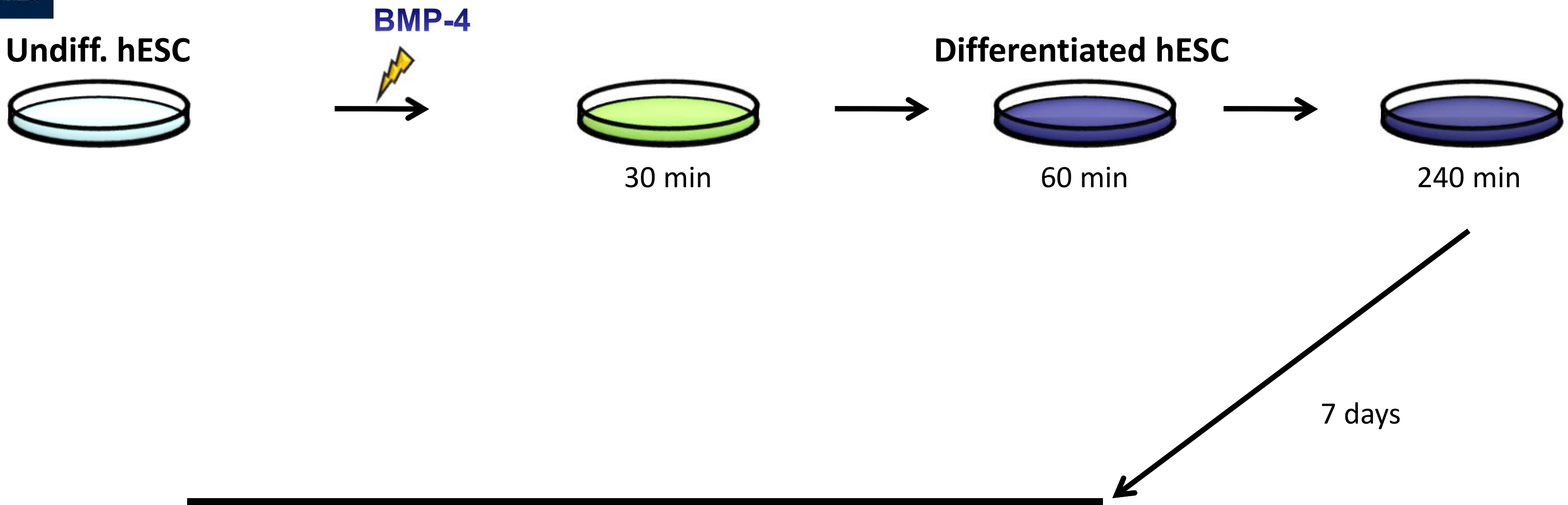
Database search, statistical analysis

Embryonic Stem Cells

- Embryonic Stem Cells (ESCs) can give rise to more than 200 cell types
- Theoretically potential source for regenerative medicine and tissue replacement after injury or disease
- After nearly ten years of research, there are no approved treatments or human trials using ESCs
- Research in ESC will enable us to optimize cell cultures to specific fates



Experimental Workflow



Undiff. hESC

BMP-4

30 min

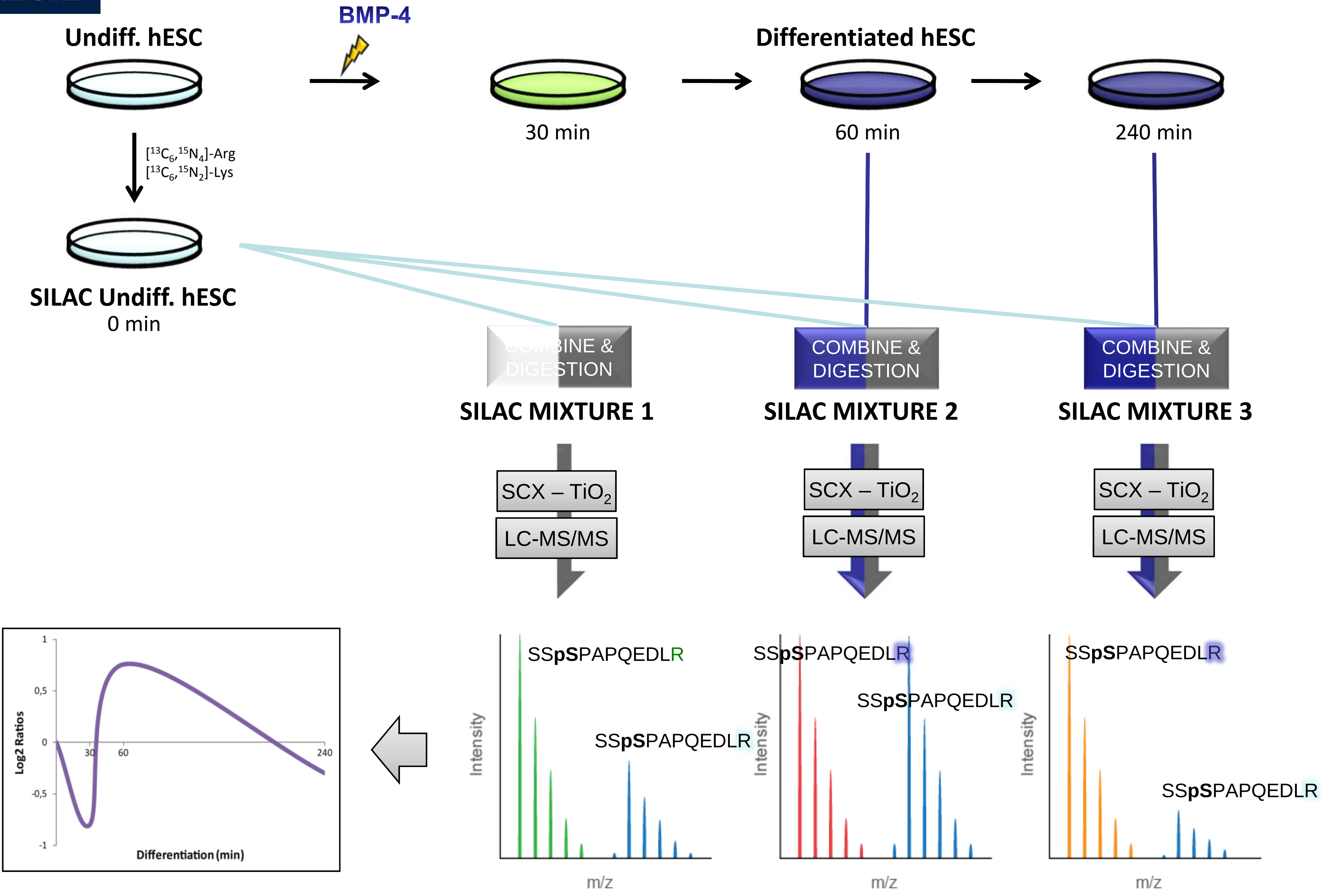
Differentiated hESC

60 min

240 min

7 days

Experimental Workflow



Undiff. hESC

BMP-4

Differentiated hESC

30 min

60 min

240 min

[$^{13}C_6$,$^{15}N_4$]-Arg
[$^{13}C_6$,$^{15}N_2$]-Lys

SILAC Undiff. hESC
0 min

COMBINE & DIGESTION

SILAC MIXTURE 1

COMBINE & DIGESTION

SILAC MIXTURE 2

COMBINE & DIGESTION

SILAC MIXTURE 3

SCX – TiO_2

LC-MS/MS

SS**pS**PAPQEDLR

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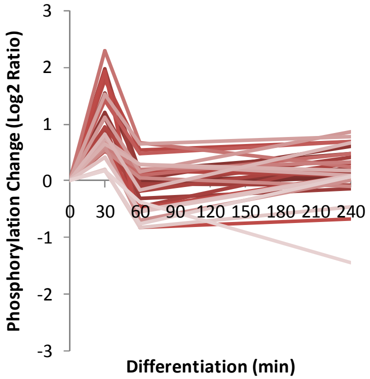
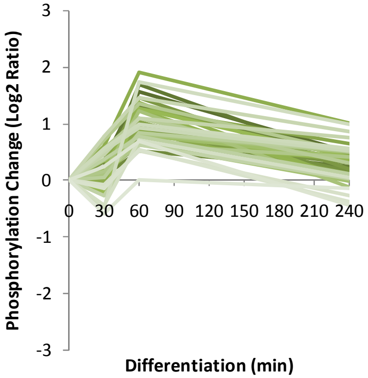
OXFORD

Phosphorylation Dynamics

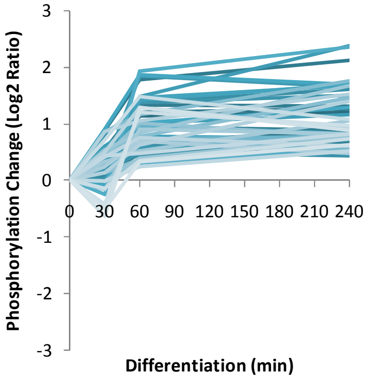
Immediate Activation



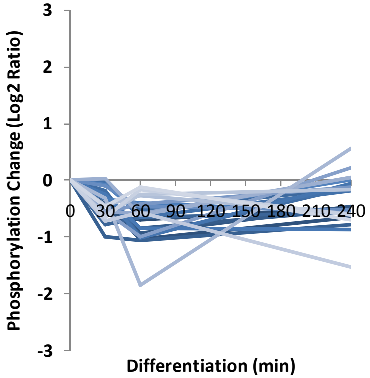
Delayed Activation



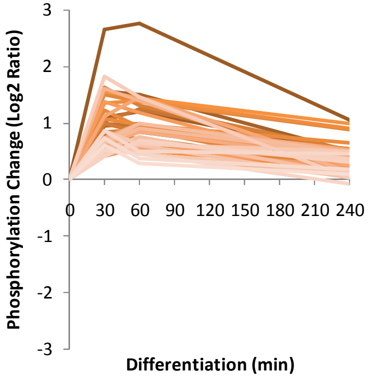
Progressive Activation



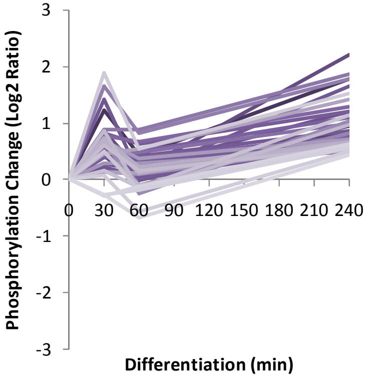
Down-Regulated



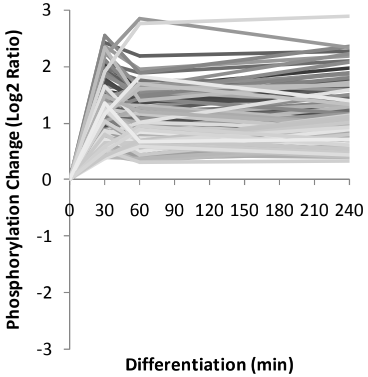
Temporal Activation



Intermitted Activation



Sustained Activation

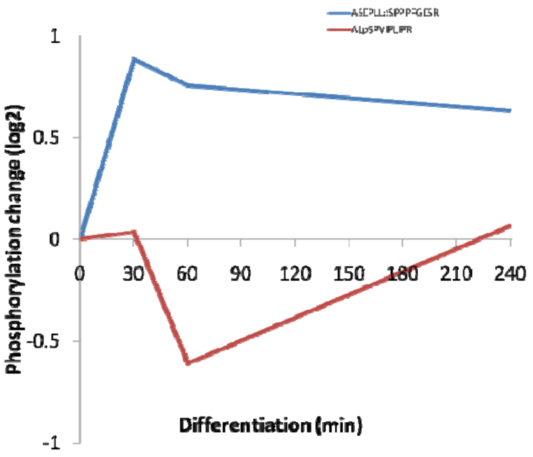


Van Hoof D, Muñoz J, Braam SR, Pinkse MW,
Linding R, Heck AJ, Mummery CL, Krijgsveld J.
Cell Stem Cell. 2009 Aug 7;5(2):214-26.

Site-Specific Regulation

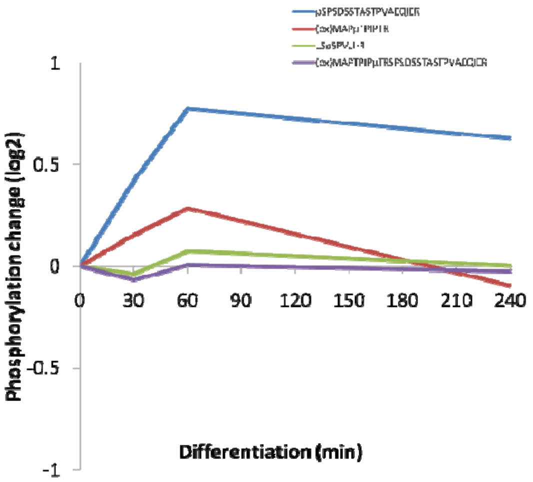
Histone-lysine N-methyltransferase (MLL2)

Histone methyltransferase. Methylates H3K4. Epigenetic control



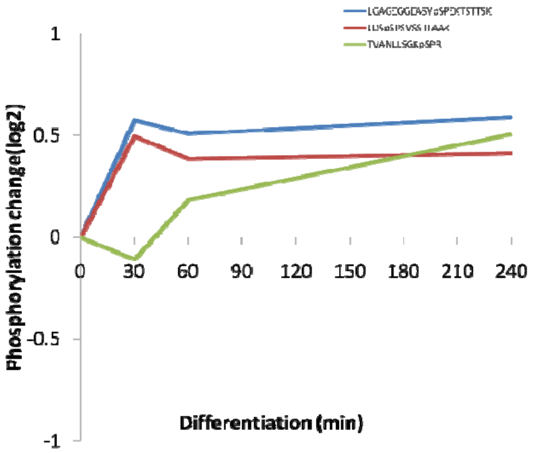
Treacle protein (TCOF1)

Nucleolar-cytoplasmic transport. May play a fundamental role in early embryonic development



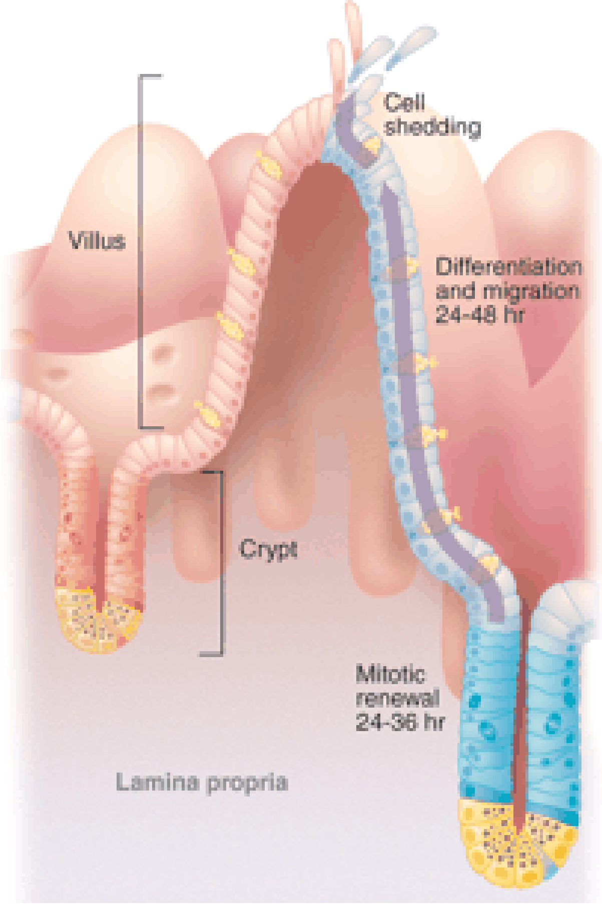
Developmentally-regulated brain protein (DBN1)

Differentiation and neurogenesis



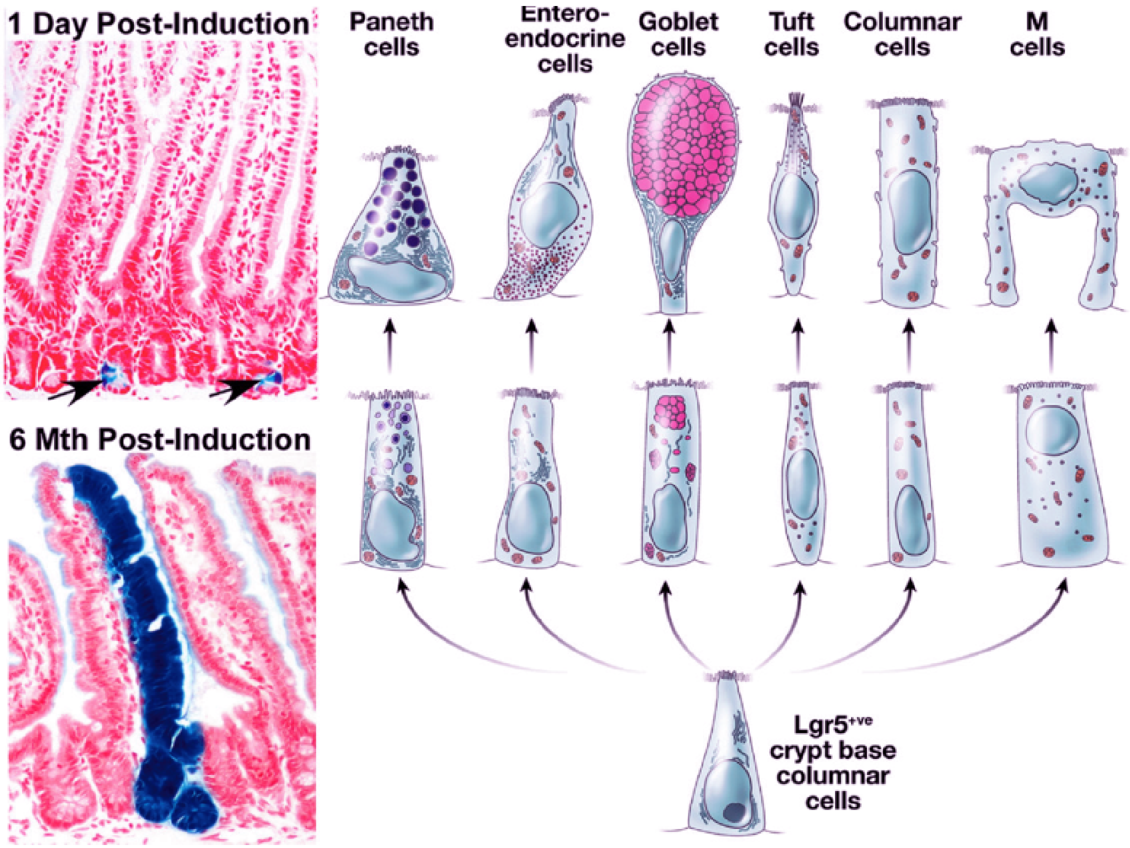
Van Hoof D, Muñoz J, Braam SR, Pinkse MW, Linding R, Heck AJ, Mummery CL, Krijgsveld J. Cell Stem Cell. 2009 Aug 7;5(2):214-26.

Lgr5 and the intestine

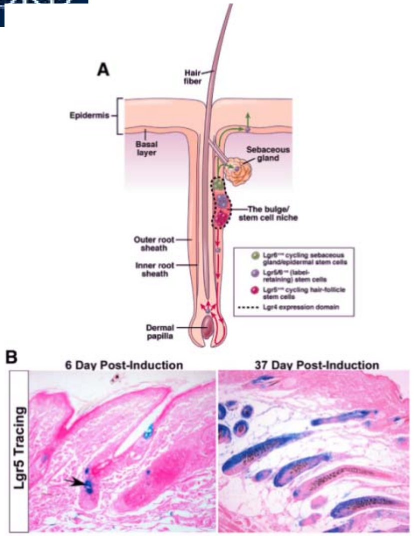


- The **epithelium** of small intestine is **renewed** every **5 days**
- **Lgr5** marker is expressed at the crypt base in **CBC cells**

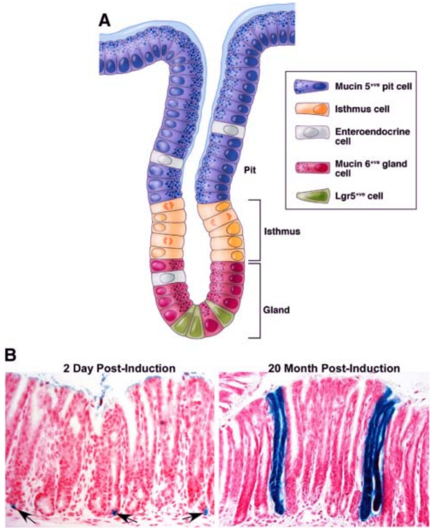
Lineage tracing experiments: cells expressing Lgr5 and its progeny were irreversibly labeled with LacZ reporter gene in small intestine (Barker *et al.* Nature 2007).



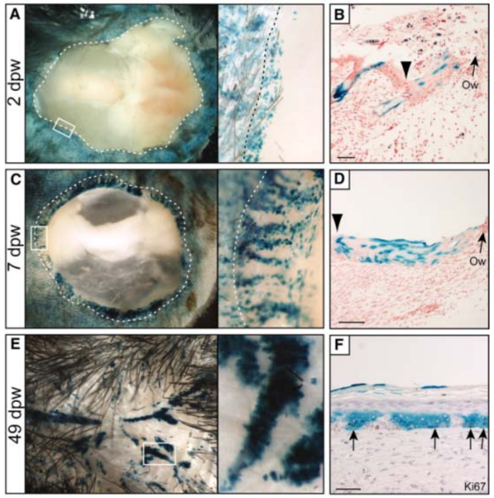
Other Tissues, Cancer And Multipotency



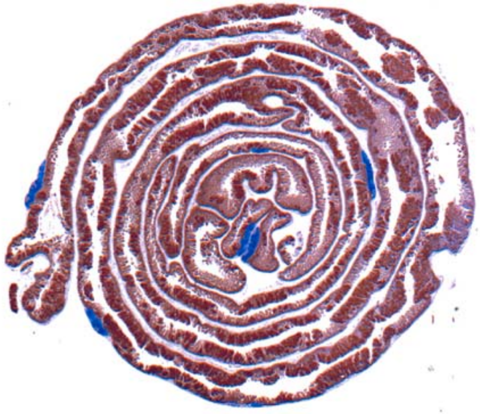
Hair follicles
(Jacks *et al.* Nat. Genetics 2008)



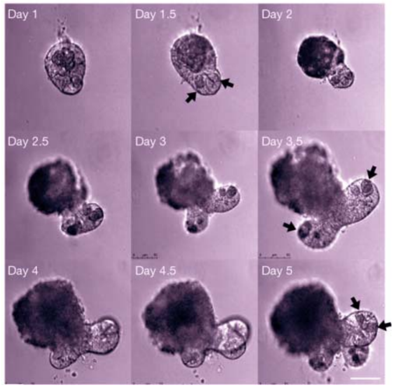
Stomach
(Jacks *et al.* Cell Stem Cell 2010)



Skin-Lgr6*
(Snippert *et al.* Science 2010)

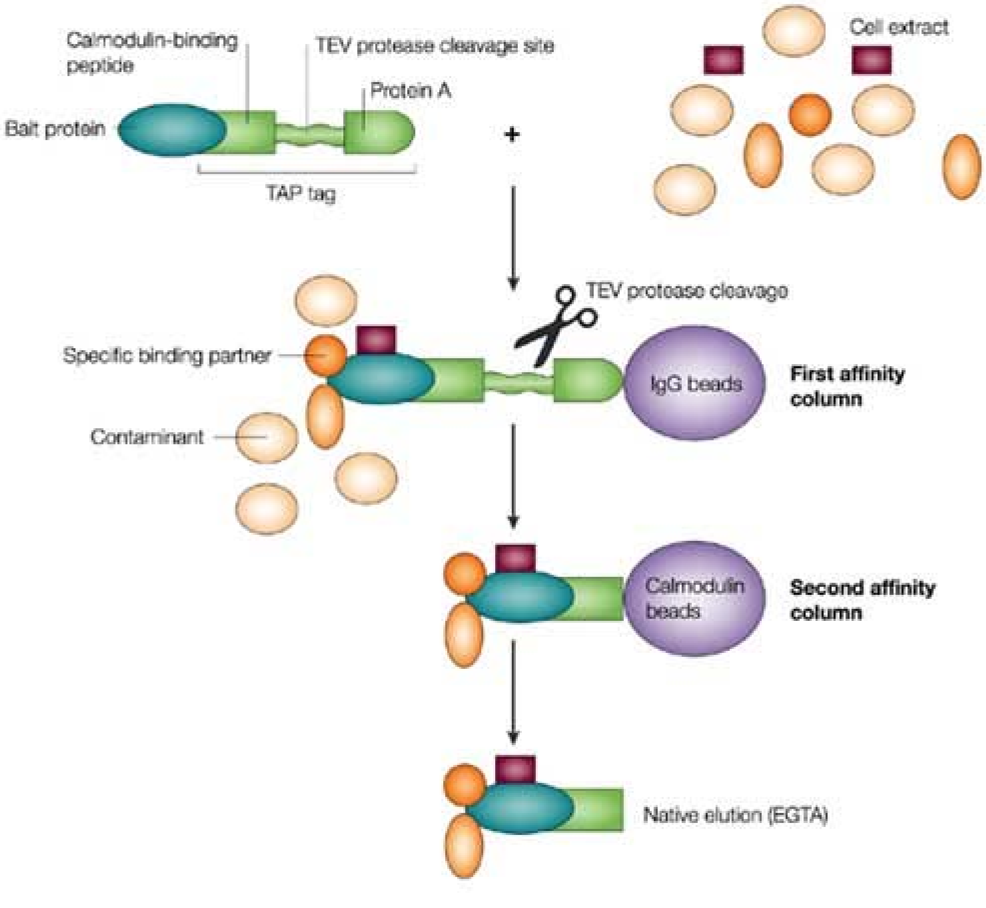


Crypt stem cells as the cells-of-**origin** of **intestinal cancer** (Barker *et al.* Nature 2009)



Single Lgr5 stem cells build crypt–villus structures without a mesenchymal niche (Sato *et al.* Nature 2009)

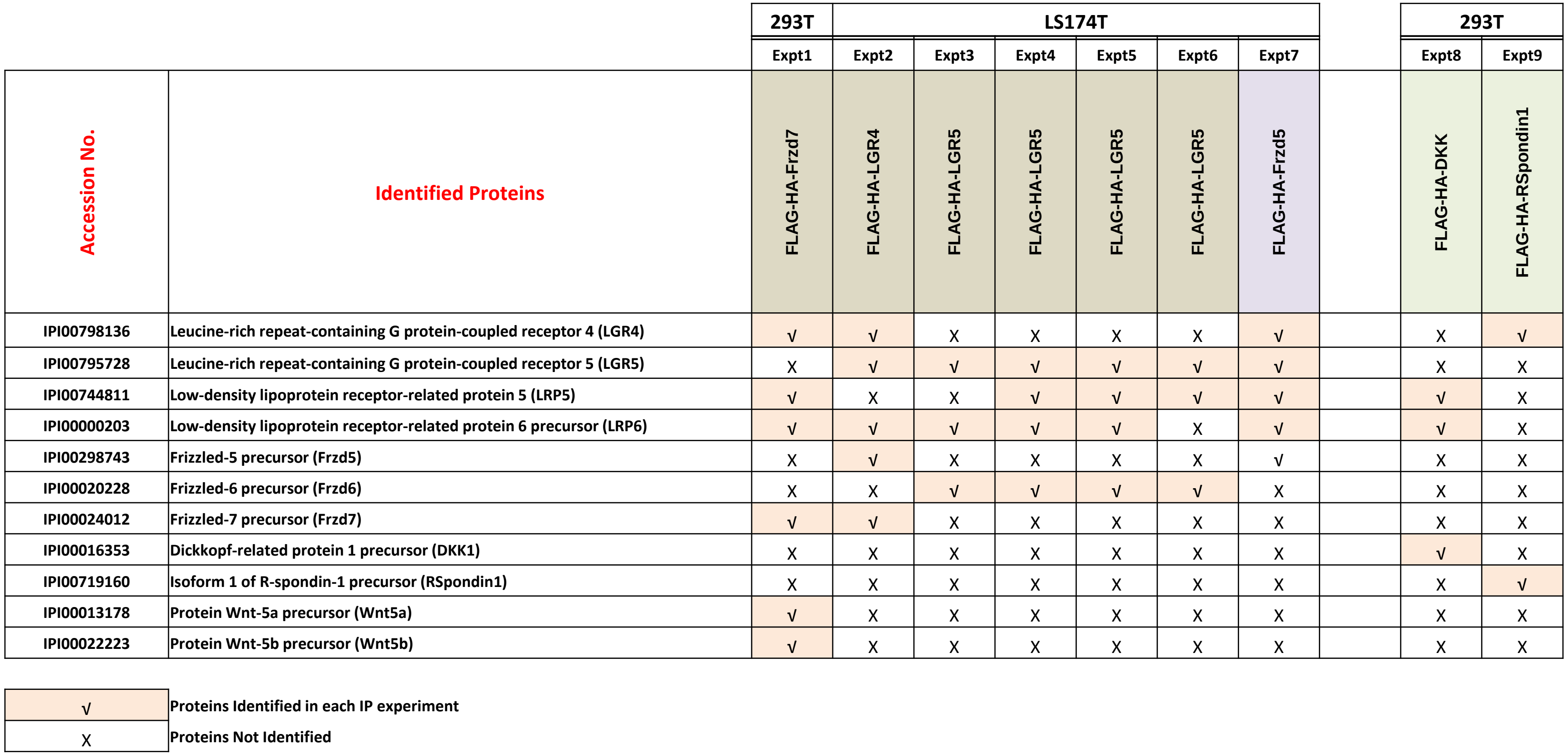
Hunt for Lgr5 and its ligand



Nature Reviews | Molecular Cell Biology

Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling

Lots of IPs with label free LCMS

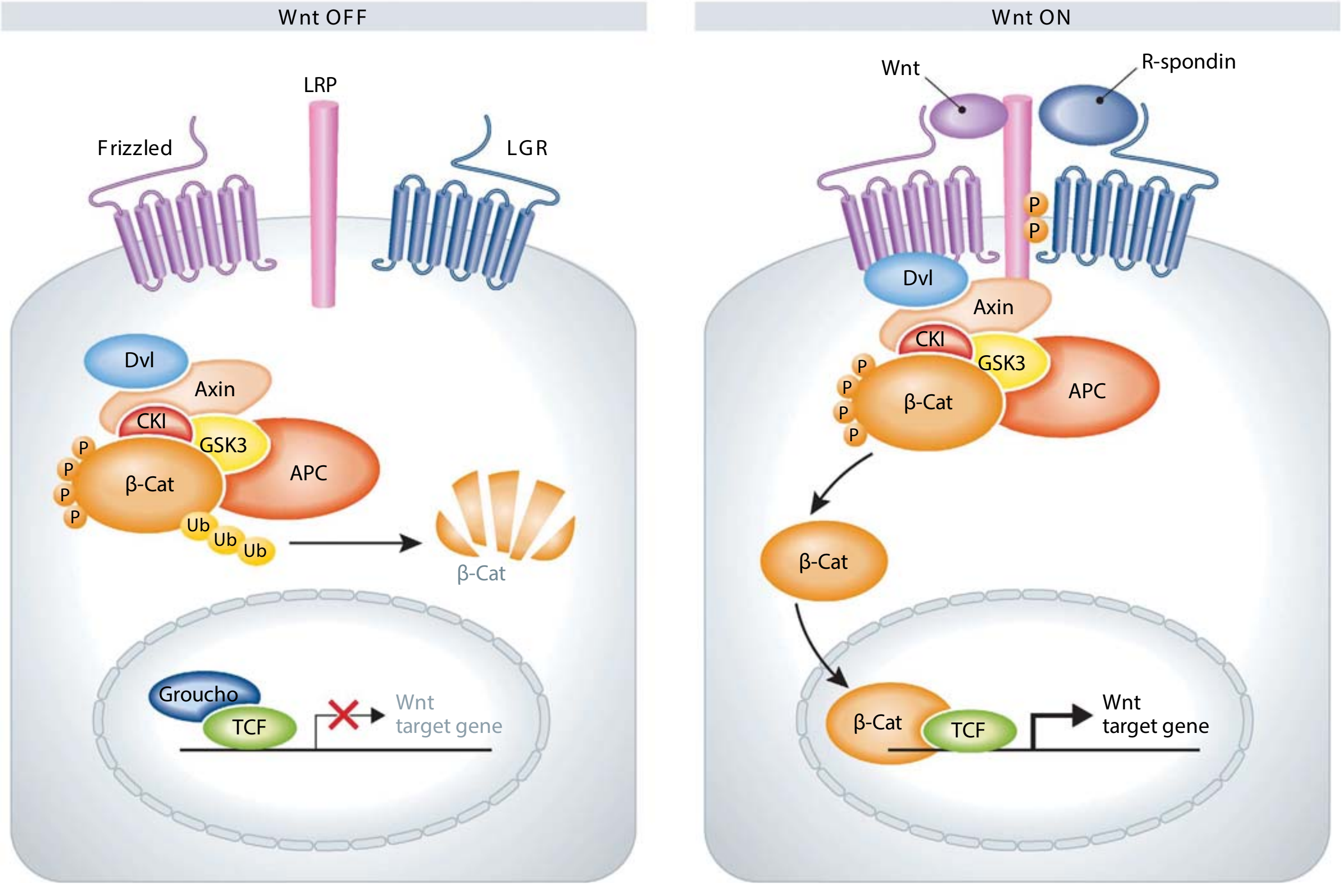


		293T	LS174T							293T	
		Expt1	Expt2	Expt3	Expt4	Expt5	Expt6	Expt7		Expt8	Expt9
Accession No.	**Identified Proteins**	FLAG-HA-Frzd7	FLAG-HA-LGR4	FLAG-HA-LGR5	FLAG-HA-LGR5	FLAG-HA-LGR5	FLAG-HA-LGR5	FLAG-HA-Frzd5		FLAG-HA-DKK	FLAG-HA-RSpondin1
IPI00798136	Leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4)	√	√	X	X	X	X	√		X	√
IPI00795728	Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)	X	√	√	√	√	√	√		X	X
IPI00744811	Low-density lipoprotein receptor-related protein 5 (LRP5)	√	X	X	√	√	√	√		√	X
IPI00000203	Low-density lipoprotein receptor-related protein 6 precursor (LRP6)	√	√	√	√	√	X	√		√	X
IPI00298743	Frizzled-5 precursor (Frzd5)	X	√	X	X	X	X	√		X	X
IPI00020228	Frizzled-6 precursor (Frzd6)	X	X	√	√	√	√	X		X	X
IPI00024012	Frizzled-7 precursor (Frzd7)	√	√	X	X	X	X	X		X	X
IPI00016353	Dickkopf-related protein 1 precursor (DKK1)	X	X	X	X	X	X	X		√	X
IPI00719160	Isoform 1 of R-spondin-1 precursor (RSpondin1)	X	X	X	X	X	X	X		X	√
IPI00013178	Protein Wnt-5a precursor (Wnt5a)	√	X	X	X	X	X	X		X	X
IPI00022223	Protein Wnt-5b precursor (Wnt5b)	√	X	X	X	X	X	X		X	X

√	Proteins Identified in each IP experiment
X	Proteins Not Identified

De Lau *et al*. Nature. 2011 Jul 4;476(7360):293-7.

Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling



Schuijers *et al*. EMBO J (2013)