”MALDI-TOF/TOF Technology - **Excellent Mass Spectrometry** Giving Best Combination of **Resolution**, **Mass Accuracy** and Data Acquisition **Speed for MALDI Imaging**”

Mgr. Michal Boháč, Ph.D., Bruker s.r.o.
presenting work of Bruker Daltonics team

Overview of the talk

- Who is Bruker and who is **Bruker Daltonics?**
- Types of MS detectors for coupling with HPLC, UHPLC and nanoHPLC: **ESI (**and **IonBooster), APCI/APPI, nanoESI or even MALDI?**
- **Applications in metabolomics and food control:**
  - **HPLC-ESI-QTOF (UHR-TOF) (maXis, impact)**
  - ID of unknowns, impurities
  - Multitarget screening and quantitation using hrEIC
- **Applications in proteomics:**
  - **PRIME – combination of nanoLC-ESI (amaZon/impact)** and **nanoLC-MALDI** (ultraflextreme MALDI-TOF/TOF) for Bottom up complex ID of proteins
  - Top Down proteomics
  - MALDI Imaging

Bruker Corporation Overview

	Technology Platforms	Major Applications
Bruker AXS	X-ray Analysis • X-ray Diffraction • X-ray Crystallography • X-ray Fluorescence • EDS Microanalysis • Spark OES	• Materials Identification • Materials Research • Structural Proteomics • Nanotechnology
Bruker Daltonics	Mass Spectrometry • MALDI-TOF(/TOF) • Ion Trap MS^n • ESI-(Qq)-TOF, FTMS • IMS	• Small Molecules Analysis • Proteomics • Clinical Research Tools • Homeland Security/Defense
Bruker Optics	Vibrational Spectroscopy • FT-IR • FT-NIR • Raman	• PAT & Quality Control • Materials Identification • Materials Research • Pharma 'Forensics'
Bruker BioSpin	NMR and EPR spectroscopy • NMR / TD-NMR • EPR • MRI • Analytical Services	• Analytical Chemistry • Pharmaceuticals • Life Science • Food • Metabolomics

Bruker Daltonics

Life Science Mass Spectrometry

- MALDI-TOF and -TOF/TOF Mass Spectrometry
- ESI-(Q)TOF Mass Spectrometry
- ESI- UHR-TOF Mass Spectrometry
- ESI-Ion Trap Mass Spectrometry
- ESI/MALDI-Q-FTMS
- ESI-QqQ (from 2012)

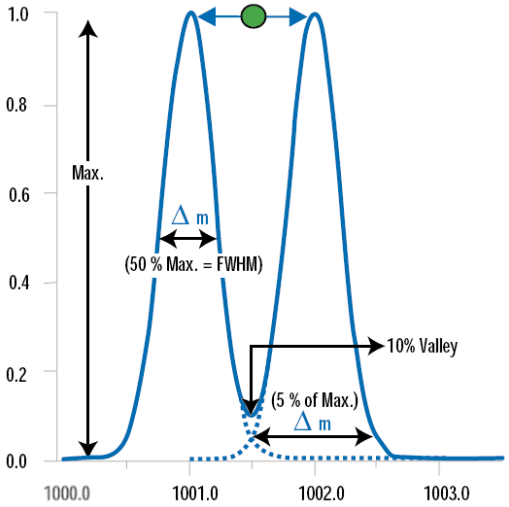
- Unique **Mass Spectrometry Solutions** for
  - **Proteomics** /Biomarker Analysis
  - **MALDI Molecular Imaging**
  - Small Molecules / Metabolite Studies
  - Food, Forensic & Environmental Screening
  - Functional Genomics/SNP Genotyping
  - Microorganism Identification and Classification

Requirements for Modern Mass Spectrometry

- **Performance:**
  - **Mass resolution (separation)**
  - **Mass accuracy (selectivity)**
  - **Sensitivity**
- **Speed:**
  - **Analysis of complex systems (proteome, tissue, screening)**
  - **Compatibility with high speed separation: UHPLC**
- **Robustness & Ease of Use**
- **Complete Solutions**

Basic parameters defining the quality of MS spectra

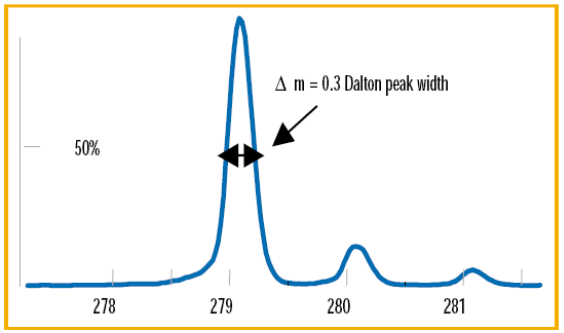
Resolution



Single Ion method

Full Width at Half Maximum (FWHM) or at 5% of the peak height

$$R = \frac{m}{\Delta m}$$



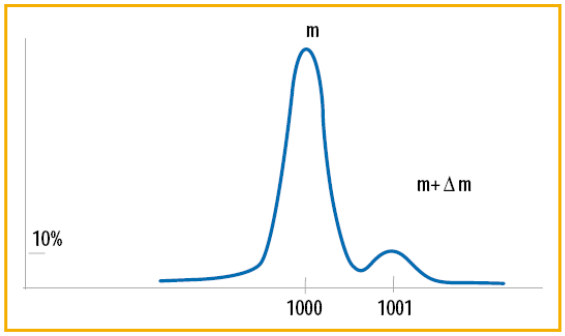
Resolution = m / (FWHM)

In that case R= 279 / 0.3 ~ 1000

Double Ion method

2 adjacent ion peaks with a 10% valley max

$$R = \frac{m}{\Delta mr}$$



In that case R= 1000 / 1 = 1000

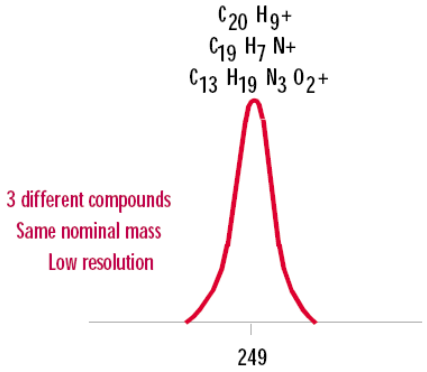
Mass Accuracy

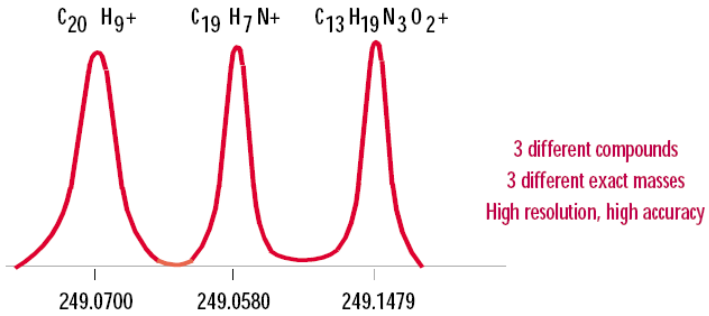
Dm accuracy = $m_{real} - m_{measured}$

It is often expressed in parts per million (ppm)

ppm = 10^6 * Δm accuracy / $m_{measured}$

i.e.: theoretical mass: 1000, measured mass: 999.9 error: 100 ppm





- **S/N Ratio**
- **Dynamic range**
- **Mass Range**
- **Speed**

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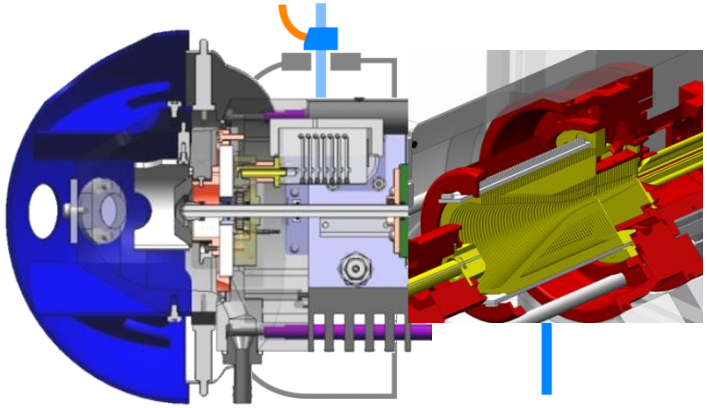
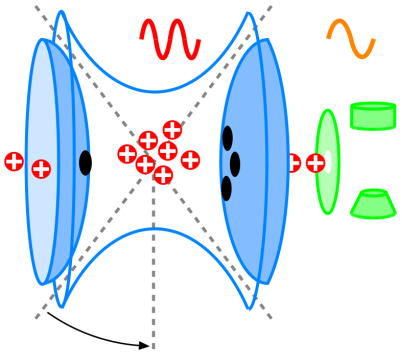
  - Top Down proteomics

ESI–based MS detection

ESI – Electrospray, NanoESI, combination with APCI. APPI

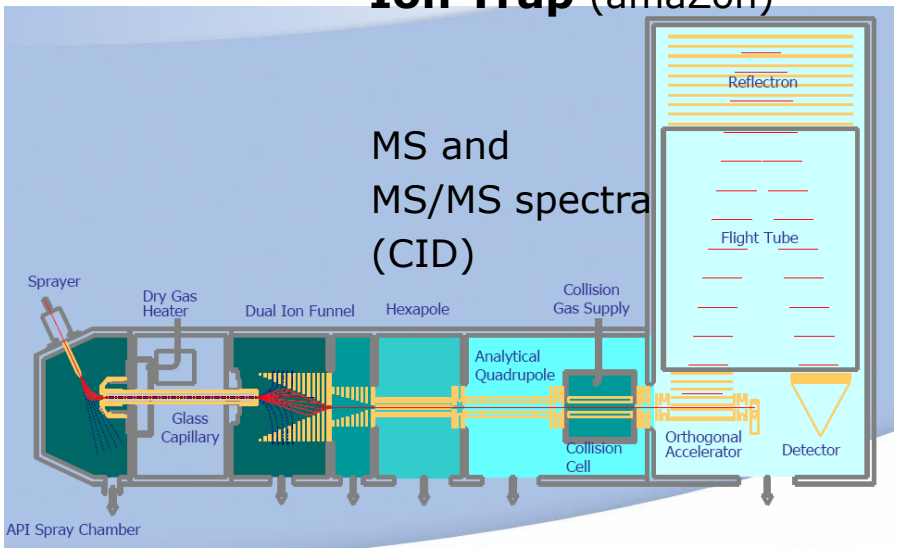




MS and
MS^n spectra
(CID)

Ion Trap (amaZon)

MS and
MS/MS spectra
(CID)



qQ-TOF and UHR-TOF

micrOTOF-Q II

maXis impact

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  - **HPLC-ESI-QTOF and -UHR-TOF (maXis)**
  - ID of unknowns – **SmartFormula 3D, FragmenExplorer, MetFrag**
  - Multitarget screening using **hrEIC**
- **Applications in proteomics:**
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Analytical Platforms LC-MS

Fast chromatography systems e.g. Dionex RSLC coupled to ...

micrOTOF II

micrOTOF-Q II

maXis impact

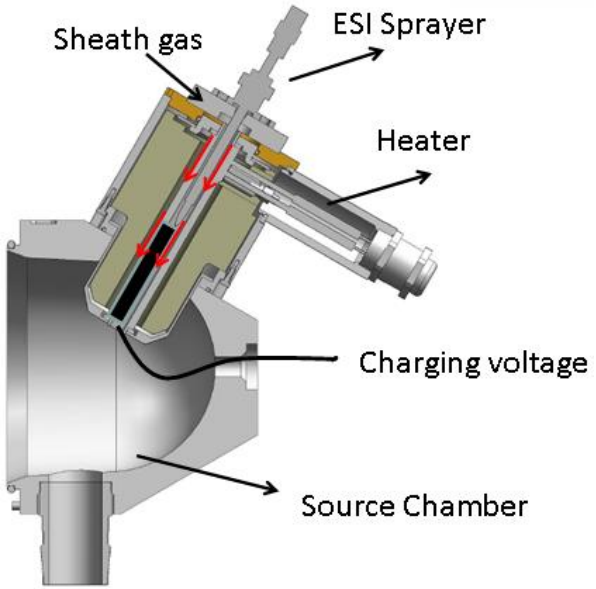
maXis 4G

Analytical Platforms LC-MS

Bruker is unique not only with its Mass Spectrometrs….

Special Ion Sources and Interfaces

Introducing **IonBooster** boost up sensitivity



- Designed to boost electrospray sensitivity
- Compatible with all current Bruker systems (Apollo II source)
- High Flow compatibility: 100 - 1500µl/min
- In general much more sensitive than ESI
- Selective ion source: The „boost" effect reaches a factor of >100 for some compounds!

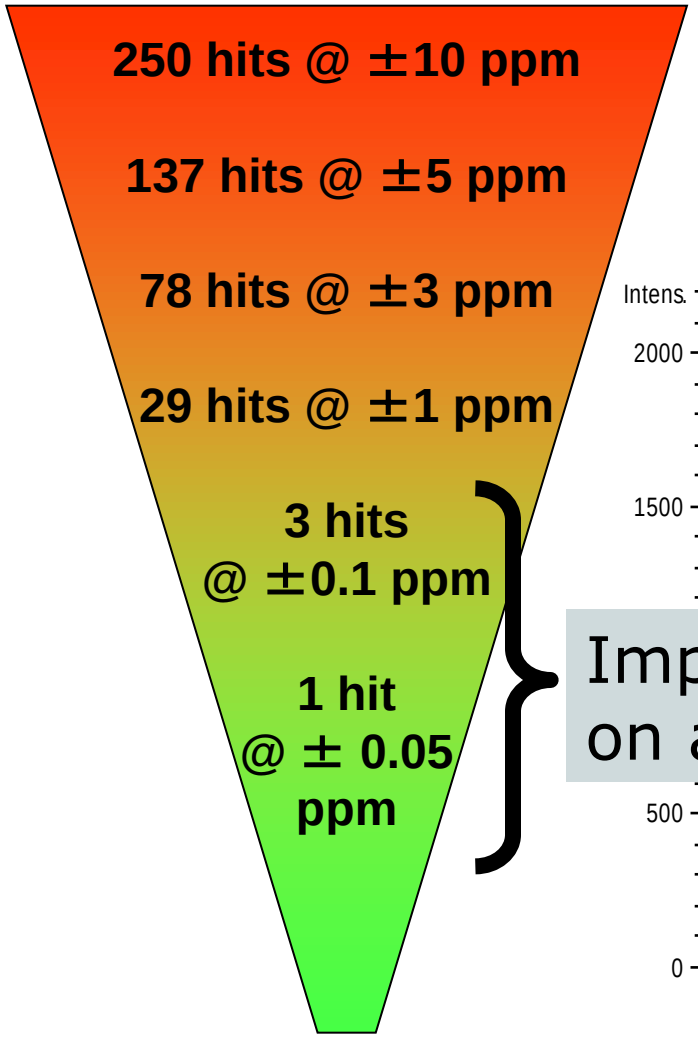
Compound ID: Unambiguous Determination of the Elemental Composition of Unknowns

Starting point:
each component has its unique mass!!!
e.g. Reserpine, $C_{33}H_{40}N_2O_9$:
609.280657 Da $[M+H]^+$

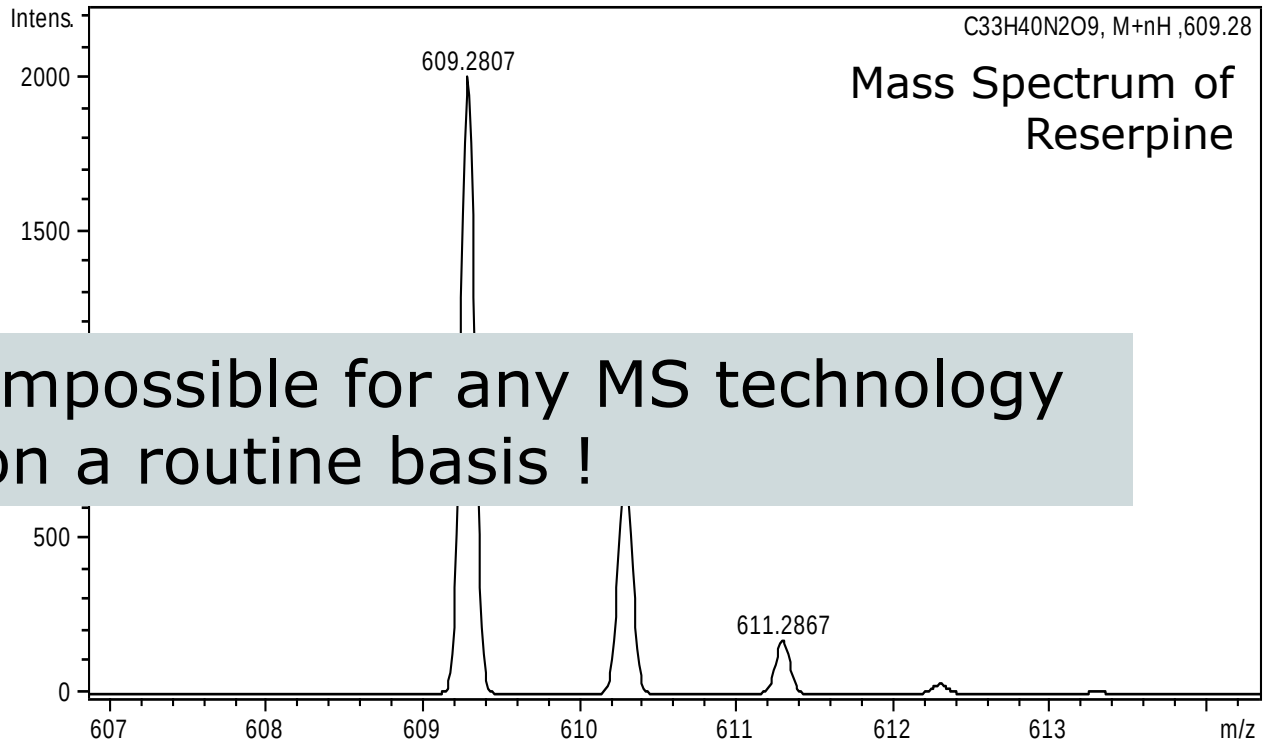
If only it was as easy as it looks on TV…

Theory and Practical Consequences....

Number of hits depending on reachable mass accuracy



Rel. mass accuracy	absolute error
10 ppm	= 0.006 Da
0.05 ppm	= 0.00003 Da (0.03 mDa)



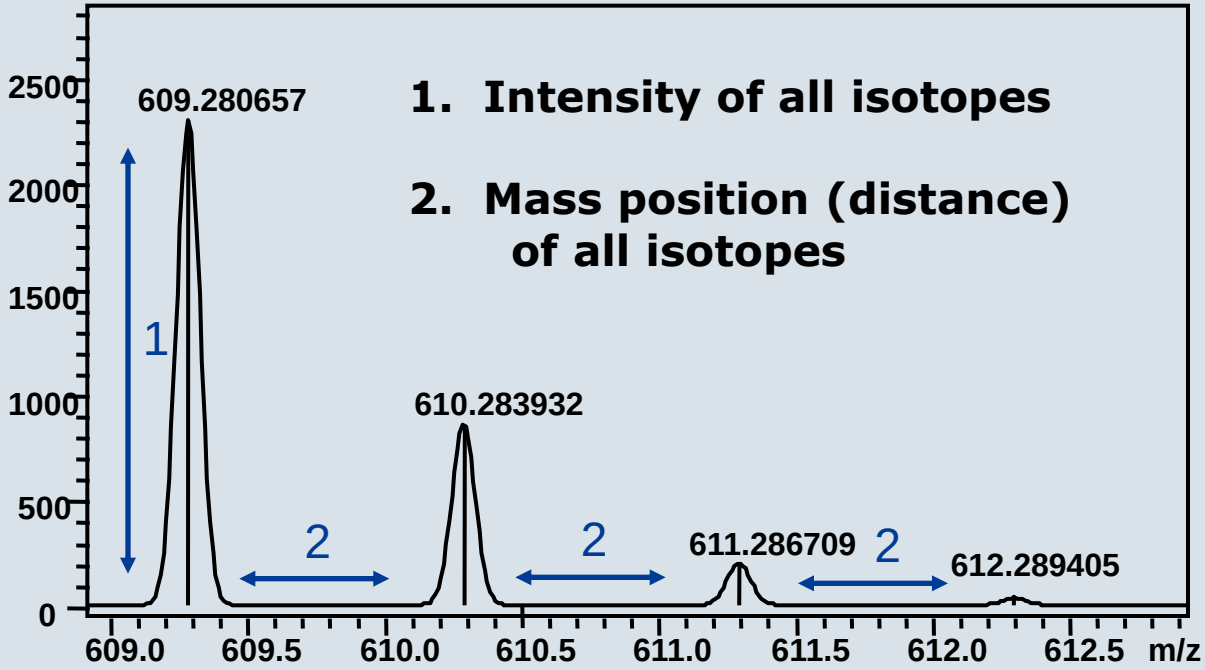
Impossible for any MS technology on a routine basis !

What is Smart Formula?

Or known as TIP™, SigmaFit™

with increasing compound mass, a tolerance of 0.001 Da or 5 ppm will not be selective enough to represent just one sum formula. Multiple formula will be possible within this mass window

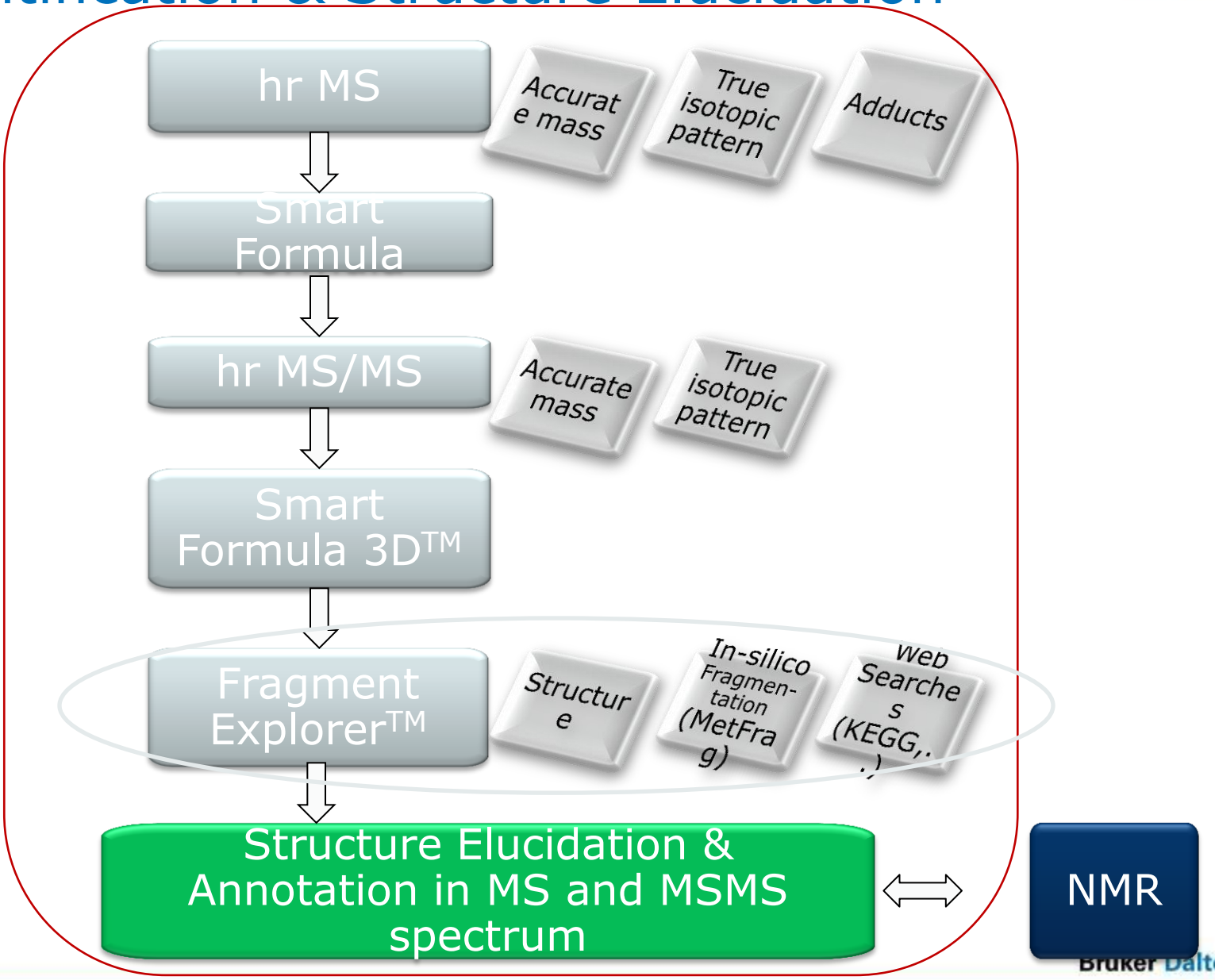
→ **need for another dimension of compound identity confirmation**



simulated spectrum of reserpine $[M+H]^+$, $C_{33}H_{41}N_2O_9$

^{13}C – ^{12}C : 1.0033548 Da
^{2}H – ^{1}H : 1.00627675 Da
^{15}N – ^{14}N : 0.997035 Da
^{34}S – ^{32}S : 1.9957959 Da

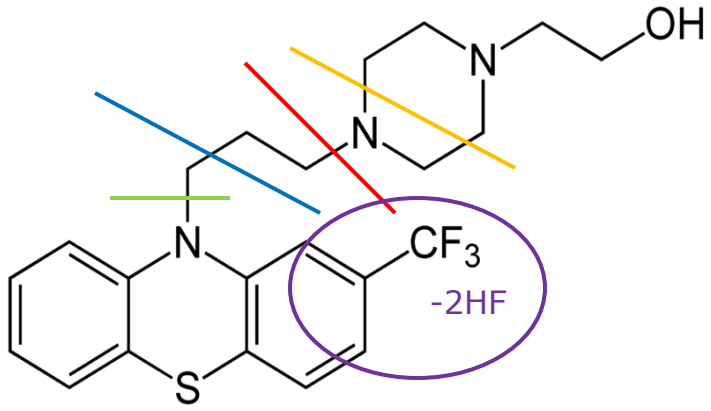
Identification & Structure Elucidation



SmartFormula3D™

Making Sense of the MS/MS Data

- SmartFormula3D result
- 1 ppm window (maXis data)
- Allow unlimited C, H, N, O and up to S_3 and F_3
- SmartFormula3D analyses by mass, isotope pattern, adducts and fragment logic



SumFormula	m/z calc	err [mDa]	err [ppm]	mSigma	eConf	I expl. [%]	Peaks expl.
C 22 H 27 F 3 N 3 O S	438.1821	0.0	0.1	9.8	even	71.6	14

MS Answers

- **One** answer:
- Definitive: it *is* $C_{22}H_{26}F_3N_3OS$

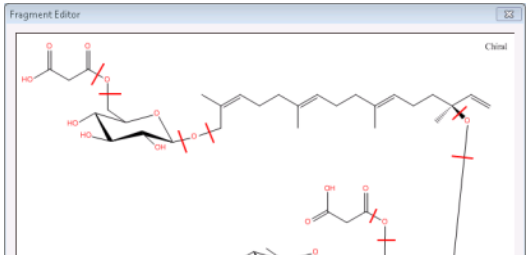
SumFormula	SumFormula Loss	m/z Loss	err [mDa] Loss	m/z calc	err [mDa]	mSigma	eConf
C 22 H 25 F N 3 O S	H 2 F 2	40.0123	0.1	398.1697	-0.1	47.1	even
C 16 H 13 F 3 N S	C 6 H 14 N 2 O	130.1109	-0.3	308.0715	0.3	22.4	even
C 14 H 9 F 3 N S	C 8 H 18 N 2 O	158.1421	-0.2	280.0402	0.2	13.6	even
C 14 H 8 F 2 N S	C 8 H 19 F N 2 O	178.1479	0.2	260.0340	-0.2	37.1	even
C 14 H 9 F 3 N	C 8 H 18 N 2 O S	190.1140	-0.0	248.0682	0.0	6.6	even
C 15 H 13 N S	C 7 H 14 F 3 N 2 O	199.1060	-0.2	239.0763	0.2	21.5	odd
C 14 H 9 F N O	C 8 H 18 F 2 N 2 S	212.1161	-0.2	226.0663	0.2	9.1	even
C 9 H 19 N 2 O	C 13 H 8 F 3 N S	267.0330	-0.1	171.1492	0.1	12.6	even
C 7 H 15 N 2 O	C 15 H 12 F 3 N S	295.0643	-0.1	143.1179	0.1	1.4	even
C 8 H 17 N 2	C 14 H 10 F 3 N O S	297.0434	0.2	141.1386	-0.1	5.9	even
C 7 H 13 N 2 O	C 15 H 14 F 3 N S	297.0798	0.1	141.1022	-0.1	19.3	even
C 5 H 10 N O	C 17 H 17 F 3 N 2 S	338.1064	0.0	100.0757	0.0	9.9	even
C 5 H 10 N	C 17 H 17 F 3 N 2 O S	354.1012	0.2	84.0808	-0.1	33.4	even
C 4 H 8 N	C 18 H 19 F 3 N 2 O S	368.1170	0.0	70.0651	0.0	48.1	even

MS/MS Interpretation

- Fragments and neutral losses can also be checked – relate to a structure

Identification & Structure Elucidation

SmartFormula(3D) & Fragment Explorer

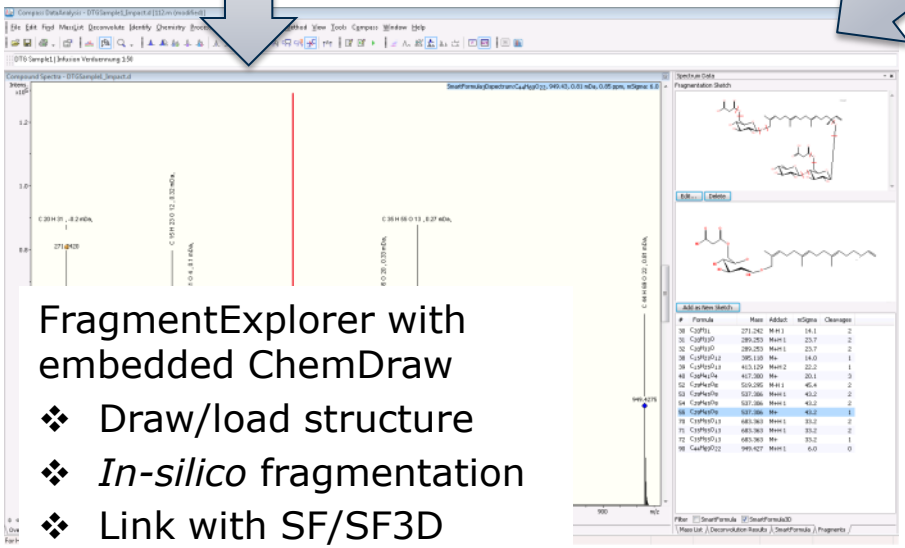


Possible structure from database query – displayed in FragmentExplorer

SumFormula	m/z calc	Score	err[ppm]	mSigma
C44H69O22	949.4275	100	0.9	6.0

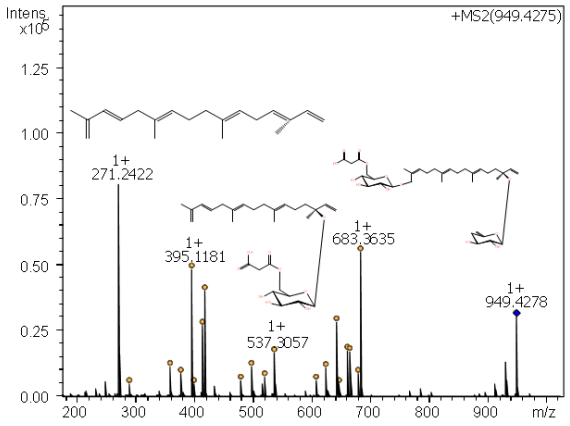
SumFormula	m/z Calc.	SumFormula Loss	err[mDa] Loss
C35H55O13	683.3637	C9H14O9	0.5
C24H39O22	679.1927	C20H32O	0.4
C24H37O21	661.1822	C20H32O	0.6
C24H35O20	643.1716	C20H34O2	0.5
C24H33O19	625.1611	C20H36O3	0.4
C24H31O18	607.1505	C20H38O4	0.3
C29H45O9	537.3058	C15H24O13	0.7
C29H43O8	519.2952	C15H26O14	0.5
C18H25O16	497.1137	C26H44O6	0.4
C18H23O15	479.1031	C26H46O7	0.4
C26H41O4	417.2999	C18H28O18	0.7
C15H25O13	413.1290	C29H44O9	0.5
C26H39O3	399.2894	C18H30O19	0.7
C15H23O12	395.1184	C29H46O10	0.5
C15H21O11	377.1078	C29H48O11	0.7
C15H19O10	359.0973	C29H50O12	0.6
C29H33O	289.2526	C24H36O21	1.0
C29H31	271.2420	C24H38O22	1.0

Correct sum formula for precursor and fragment ions – SmartFormula3D



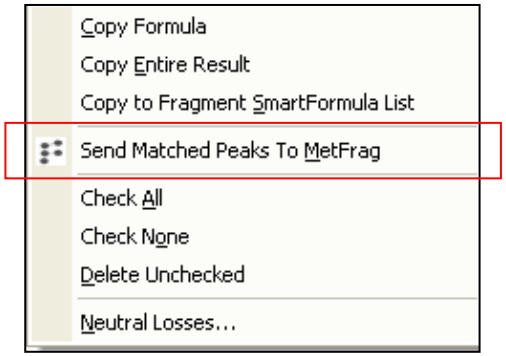
FragmentExplorer with embedded ChemDraw

- Draw/load structure
- *In-silico* fragmentation
- Link with SF/SF3D results

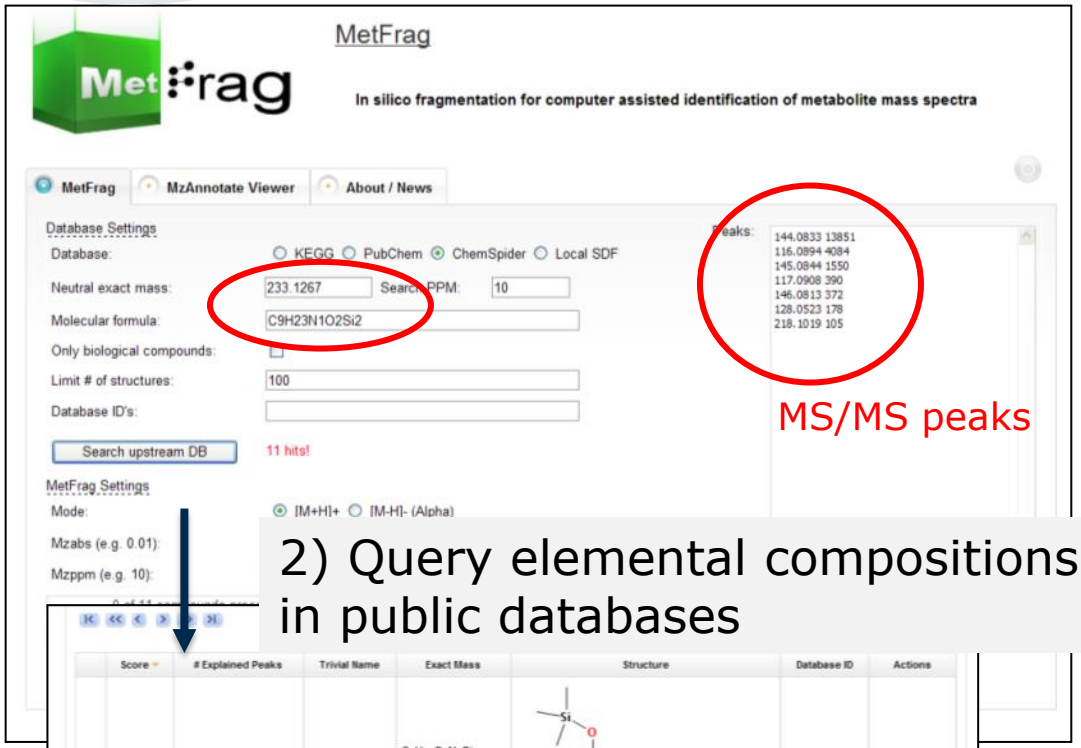


Structure Annotation in MS and MSMS data for your publication

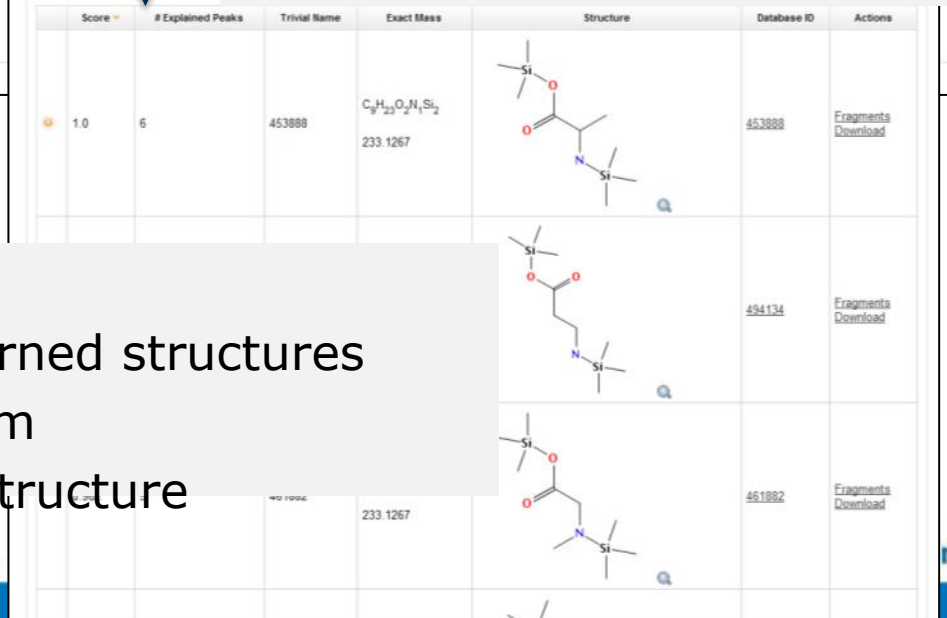
"De-novo" structure elucidation in-silico fragmentation with MetFrag



1) Send
- elemental formula
- exact mass
- MS/MS info



2) Query elemental compositions in public databases

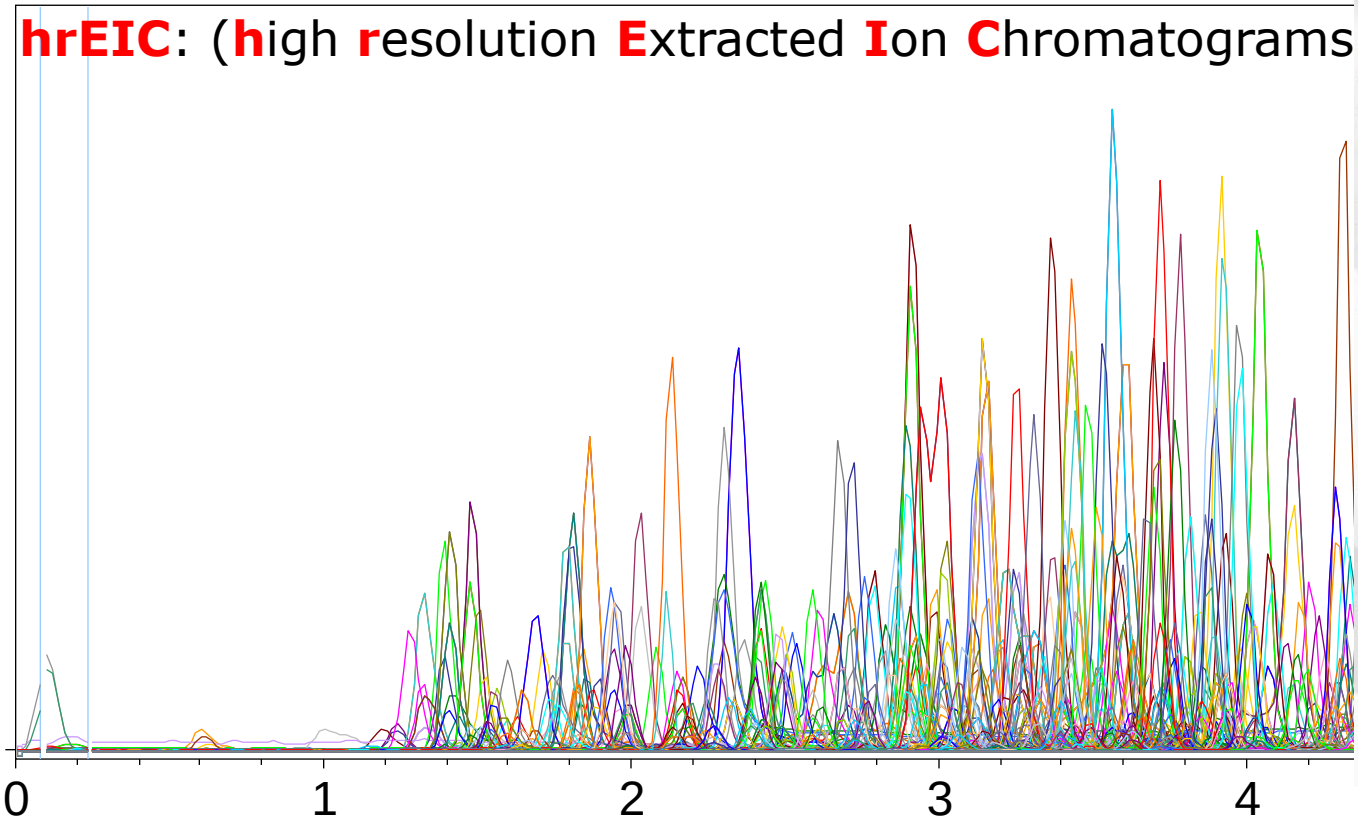


3)
- ✓ In-silico fragmentation of returned structures
- ✓ match against MS/MS spectrum
- ✓ sort according to most likely structure

http://msbi.ipb-halle.de/MetFrag/

PesticideScreener: Multi-Target Screening of 750 Pesticides in a Single LC-MS Run

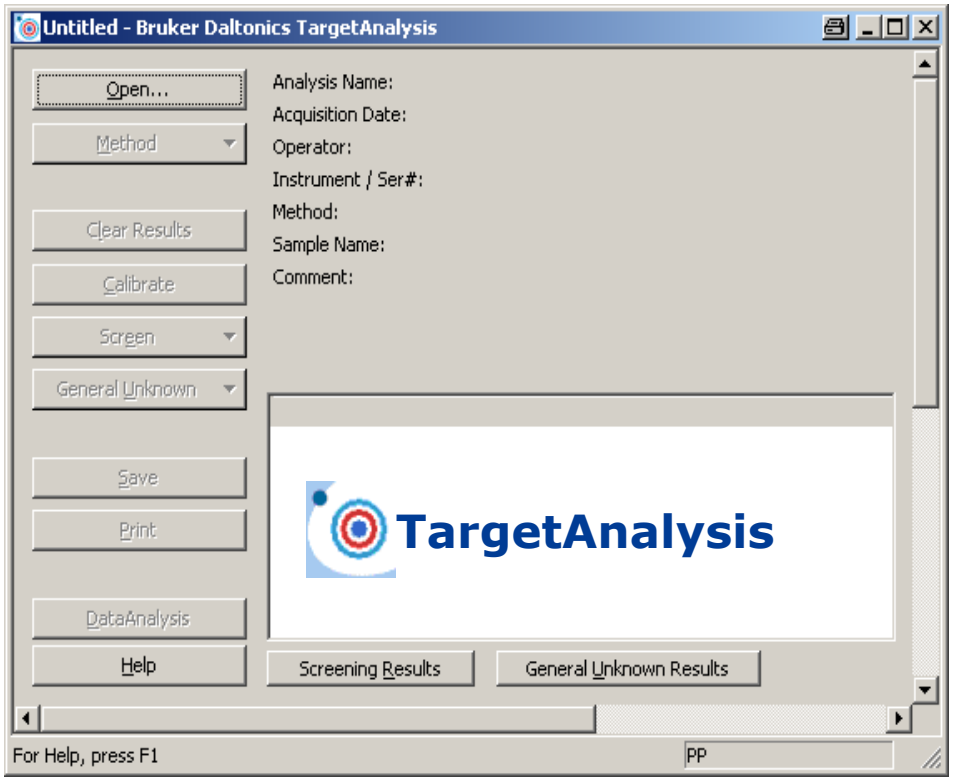
Database setup with standards:

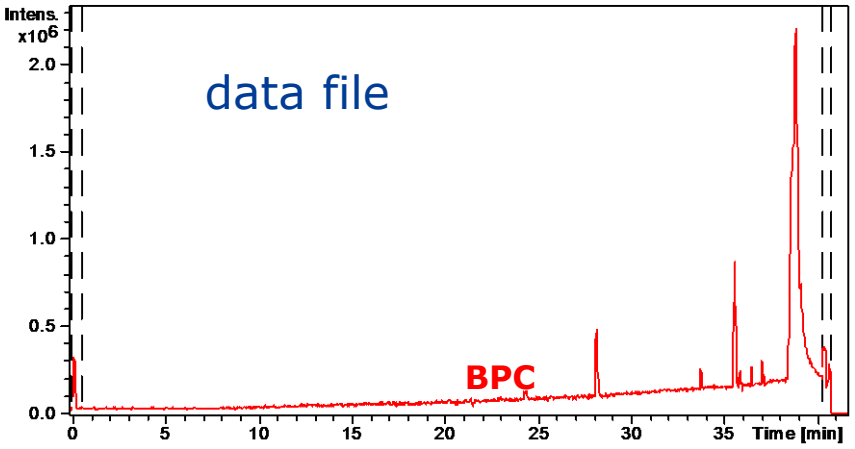


Multi compound standard of 750 pesticides, 7 min gradient. Overlayed compound EICs, complete pesticide elution in about 5 min.

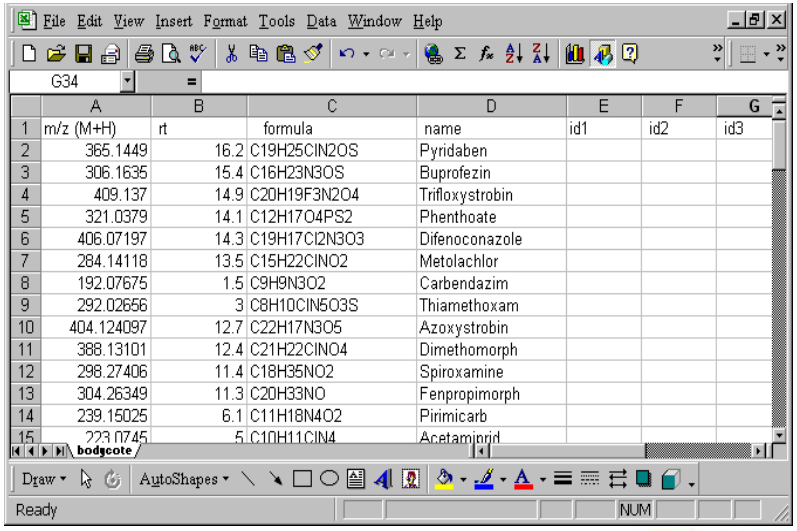
TargetAnalysis Workflow

What do we need?





database



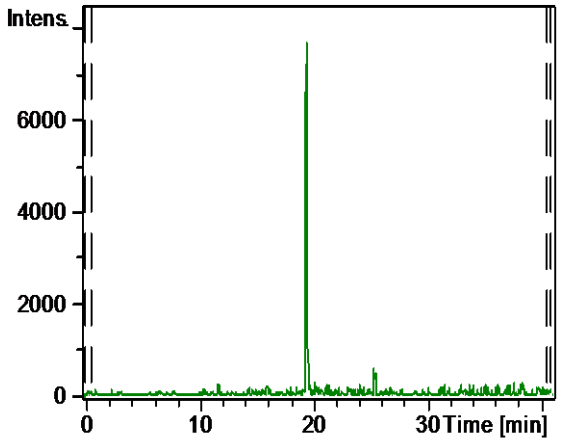
	A	B	C	D	E	F	G
1	m/z (M+H)	rt	formula	name	id1	id2	id3
2	365.1449	16.2	C19H25ClN2OS	Pyridaben			
3	306.1635	15.4	C16H23N3OS	Buprofezin			
4	409.137	14.9	C20H19F3N2O4	Trifloxystrobin			
5	321.0379	14.1	C12H17O4PS2	Phenthoate			
6	406.07197	14.3	C19H17Cl2N3O3	Difenoconazole			
7	284.14118	13.5	C15H22ClNO2	Metolachlor			
8	192.07675	1.5	C9H9N3O2	Carbendazim			
9	292.02656	3	C8H10ClN5O3S	Thiamethoxam			
10	404.124097	12.7	C22H17N3O5	Azoxystrobin			
11	388.13101	12.4	C21H22ClNO4	Dimethomorph			
12	298.27406	11.4	C18H35NO2	Spiroxamine			
13	304.26349	11.3	C20H33NO	Fenpropimorph			
14	239.15025	6.1	C11H18N4O2	Pirimicarb			
15	223.0745	5	C10H11ClN4	Acetamiprid			

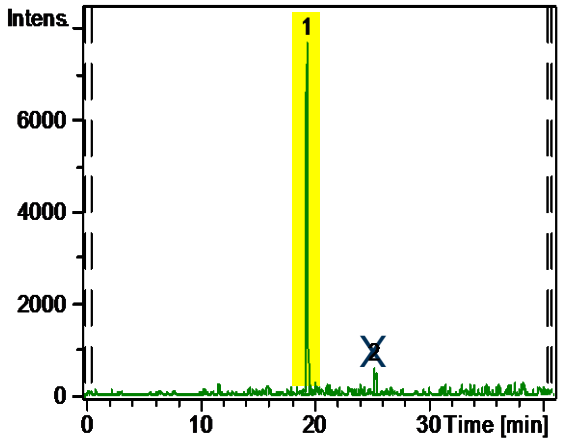
TargetAnalysis Workflow

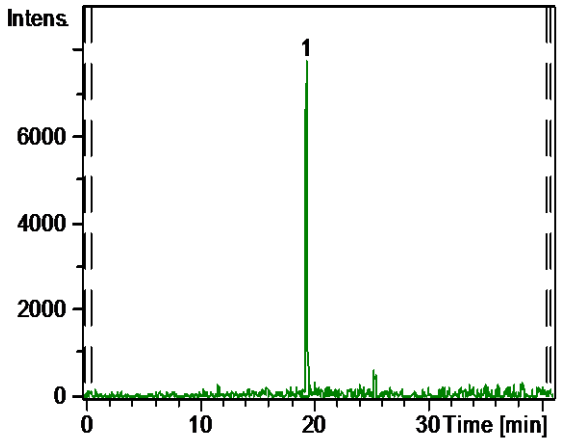
Compound Seeking

	A	B	C	D	E	F	G	H	I	J	K
14	203.0927	20.4	C10H10N4O	METAMITRON	41394-05-2	x	Ehrenstorfer 47			0.03	3

$[M+H]^+$: 203.0927



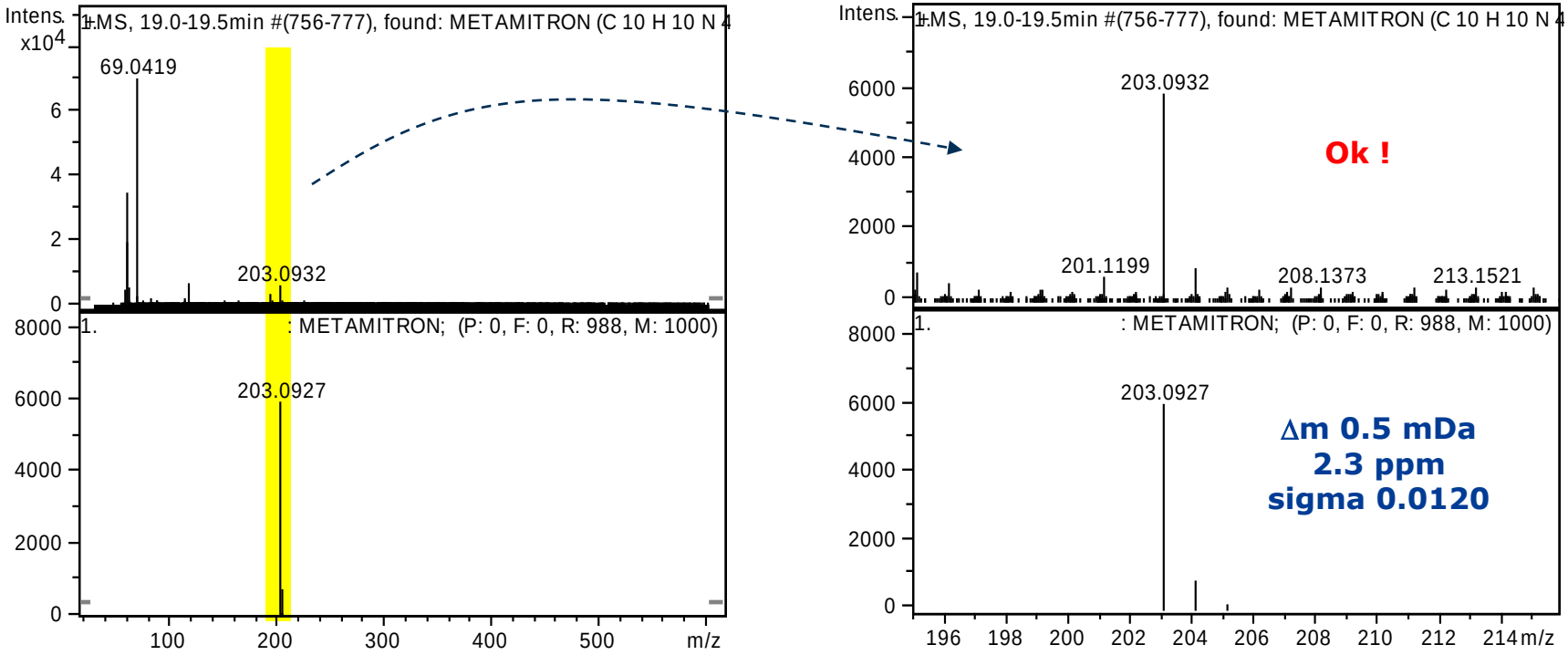




- Calculation of the theoretical mass of $[M+H]^+$ from the sum formula
- Calculation of the corresponding EIC (smoothed, mass window specified in TA)
- "Find compounds"
- Reject peaks which are not within the allowed retention time window

TargetAnalysis Workflow

Compound identification



- Calculate mass spectrum, check mass accuracy and SigmaFit™
- Reject/accept compounds using criteria for mass accuracy and sigma value
- Rating and display of results

Multi-Target Screening of 750 Pesticides in a Single LC/MS Run

Application on real samples:

Sweet pepper :

Found	Compound Name	Reg.No.	Mol.Formula	PMI	d RT [min]	Err [ppm]	Err [mDa]	mSigma	Area	Intens.	RT,exp.[min]	RT,meas.[min]	m/z,calc.	m/z,meas.	Algorithm
+++	Azoxystrobin	13186008	C 22 H 18 N 3 O 5	[M+H]+	-0.03	2.2	0.9	7.5	43393	8738	8.94	8.97	404.1241	404.1232	Chromatogram
+++	Chlorpyriphos	292102	C 9 H 12 Cl 3 N 1 O 3 P 1 S 1	[M+H]+	-0.02	1.2	0.4	19.6	54738	12476	12.66	12.68	349.9336	349.9332	Chromatogram
+++	Imidacloprid	13826103	C 9 H 11 Cl 1 N 5 O 2	[M+H]+	0.00	2.1	-0.5	44.0	5810	1259	4.71	4.71	256.0596	256.0601	Chromatogram
+++	Iprodione	3673407	C 13 H 14 Cl 2 N 3 O 3	[M+H]+	-0.00	0.7	0.2	39.6	1276	357	10.44	10.44	330.0407	330.0405	Chromatogram
+++	Iprodione (Na)	3673407	C 13 H 13 Cl 2 N 3 Na 1 O 3	[I]1+	-0.00	1.0	-0.4	43.5	1005	270	10.44	10.44	352.0226	352.0230	Chromatogram
+++	Kresoxim-methyl	14339000	C 18 H 20 N 1 O 4	[M+H]+	0.00	1.6	0.5	2.0	94128	19979	10.73	10.73	314.1387	314.1382	Chromatogram
+++	Kresoxim-methyl (NH4)	14339000	C 18 H 23 N 2 O 4	[M+H]+	0.01	2.3	0.8	16.0	52705	11214	10.73	10.72	331.1652	331.1645	Chromatogram
---	Metalaxyl	5783701	C 15 H 22 N 1 O 4	[M+H]+	-0.02	5.2	-1.5	296.6	3103	678	8.18	8.20	280.1543	280.1558	Chromatogram
---	Methomyl	1675205	C 5 H 11 N 2 O 2 S 1	[M+H]+	0.04	4.4	-0.7	199.5	3217	386	4.13	4.09	163.0536	163.0543	Chromatogram
++	Methomyl Fragm 88	1675205	C 3 H 6 N 1 S 1	[M+H]+	0.04	4.3	0.4	36.6	542	138	4.13	4.09	88.0215	88.0212	Chromatogram
++	Oxamyl	2313500	C 7 H 14 N 3 O 3 S 1	[M+H]+	0.00	4.4	1.0	82.7	2849	503	3.73	3.73	220.0750	220.0741	Chromatogram
+++	Oxamyl (NH4)	2313500	C 7 H 17 N 4 O 3 S 1	[M+H]+	-0.00	0.2	-0.0	32.1	60032	10346	3.73	3.73	237.1016	237.1016	Chromatogram
---	Oxamyl Fragm 72	2313500	C 3 H 6 N 1 O 1	[M+H]+	-0.00	10.3	0.7	9.6	3743	639	3.73	3.73	72.0444	72.0436	Chromatogram
+++	Oxamyl Fragm 90	2313500	C 3 H 8 N 1 O 2	[M+H]+	-0.00	0.7	0.1	6.1	15322	2601	3.73	3.73	90.0550	90.0549	Chromatogram
++	Penconazole	6624606	C 13 H 16 Cl 2 N 3	[M+H]+	-0.00	0.8	0.2	61.9	10727	2320	10.81	10.81	284.0716	284.0714	Chromatogram
+++	Procymidone	3280908	C 13 H 12 Cl 2 N 1 O 2	[M+H]+	-0.01	2.3	0.6	18.8	7169	1482	10.20	10.21	284.0240	284.0233	Chromatogram
++	Procymidone (NH4)	3280908	C 13 H 15 Cl 2 N 2 O 2	[M+H]+	-0.01	4.5	1.3	19.9	25823	5285	10.20	10.21	301.0505	301.0492	Chromatogram
+++	Pyrimethanil	5311200	C 12 H 14 N 3	[M+H]+	0.00	0.6	0.1	28.0	29088	5231	9.43	9.43	200.1182	200.1181	Chromatogram
+++	Triadimenol	5521903	C 14 H 19 Cl 1 N 3 O 2	[M+H]+	0.00	0.9	0.3	29.8	13470	1945	9.91	9.91	296.1160	296.1158	Chromatogram
+++	Triadimenol (Na)	5521903	C 14 H 18 Cl 1 N 3 Na 1 O 2	[I]1+	0.00	1.2	0.4	22.7	9821	1742	9.91	9.91	318.0980	318.0976	Chromatogram
+++	Trifloxystrobin	14151707	C 20 H 20 F 3 N 2 O 4	[M+H]+	0.01	0.9	0.4	34.0	2087	562	11.59	11.58	409.1370	409.1366	Chromatogram

Endive (čekanka):

Found	Compound Name	Reg.No.	Mol.Formula	PMI	d RT [min]	Err [ppm]	Err [mDa]	mSigma	Area	Intens.	RT,exp.[min]	RT,meas.[min]	m/z,calc.	m/z,meas.	Algorithm
+++	Boscalid	18842506	C 18 H 13 Cl 2 N 2 O 1	[M+H]+	-0.00	1.5	0.5	4.0	30512	6121	9.38	9.38	343.0399	343.0394	Chromatogram
++	Chlorpyriphos	292102	C 9 H 12 Cl 3 N 1 O 3 P 1 S 1	[M+H]+	-0.02	1.8	-0.6	59.4	2061	420	12.66	12.68	349.9336	349.9342	Chromatogram
++	Imidacloprid	13826103	C 9 H 11 Cl 1 N 5 O 2	[M+H]+	-0.01	3.6	0.9	16.2	48616	9731	4.71	4.72	256.0596	256.0587	Chromatogram
++	Linuron	33002	C 9 H 11 Cl 2 N 2 O 2	[M+H]+	-0.01	3.4	0.8	19.6	10477	2034	9.27	9.28	249.0192	249.0184	Chromatogram
---	Metalaxyl	5783701	C 15 H 22 N 1 O 4	[M+H]+	-0.02	0.8	-0.2	233.0	23509	4496	8.18	8.20	280.1543	280.1546	Chromatogram
+++	Propamocarb	2457905	C 9 H 21 N 2 O 2	[M+H]+	-0.02	1.8	0.3	0.4	9618110	781054	3.40	3.42	189.1598	189.1594	Chromatogram
+++	Pyraclostrobin	17501300	C 19 H 19 Cl 1 N 3 O 4	[M+H]+	0.01	2.0	0.8	28.3	16424	3448	11.16	11.15	388.1059	388.1051	Chromatogram

Pomelo:

Found	Compound Name	Reg.No.	Mol.Formula	PMI	d RT [min]	Err [ppm]	Err [mDa]	mSigma	Area	Intens.	RT,exp.[min]	RT,meas.[min]	m/z,calc.	m/z,meas.	Algorithm
++	Chlorpyriphos	292102	C 9 H 12 Cl 3 N 1 O 3 P 1 S 1	[M+H]+	0.00	3.5	1.2	33.3	8610	2272	12.66	12.66	349.9336	349.9323	Chromatogram
---	Cyprodinil	12155202	C 14 H 16 N 3	[M+H]+	-0.06	2.5	-0.6	212.2	1004	179	11.02	11.08	226.1339	226.1344	Chromatogram
+++	Methidathion	95008	C 6 H 12 N 2 O 4 P 1 S 3	[M+H]+	-0.01	1.1	0.3	3.5	31208	6495	8.67	8.68	302.9691	302.9688	Chromatogram
+++	Methidathion (NH4)	95008	C 6 H 15 N 3 O 4 P 1 S 3	[M+H]+	-0.01	0.2	0.1	5.5	27494	5569	8.67	8.68	319.9957	319.9956	Chromatogram
++	Methidathion Fragm 145	95008	C 4 H 5 N 2 O 2 S 1	[M+H]+	-0.01	3.6	0.5	12.1	12030	2393	8.67	8.68	145.0066	145.0061	Chromatogram
+++	Prochloraz	6774705	C 15 H 17 Cl 3 N 3 O 2	[M+H]+	-0.00	1.2	0.4	7.9	56576	11148	11.23	11.23	376.0381	376.0376	Chromatogram
+++	Triazophos	2401708	C 12 H 17 N 3 O 3 P 1 S 1	[M+H]+	0.01	0.1	-0.0	4.5	38161	8261	9.89	9.88	314.0723	314.0723	Chromatogram

Overview of the talk

- Who is Bruker and who is **Bruker Daltonics?**
- Types of MS detectors for coupling with HPLC, UHPLC and nanoHPLC: **ESI, APCI, nanoESI or even MALDI?**
- **Applications in metabolomics and food control:**
  - **HPLC-ESI-QTOF and -UHR-TOF (maXis)**
  - ID of unknowns, impurities
  - Multitarget screening and quantitation using hrEIC
- **Applications in proteomics:**
  - **PRIME – combination of nanoLC-ESI (amaZon/impact)** and **nanoLC-MALDI** (ultraflextreme MALDI-TOF/TOF) for ID of proteins
  - Top Down proteomics

Proteins and peptides

Separation of Proteins and Peptides

- Liquid Chromatography - HPLC, CapLC, NanoLC, 2D NanoLC etc.
- Capillary Electrophoresis - CE
- 1D and 2D Gel Electrophoresis
- Affinity Chromatography (eg. magnetic beads – HIC, IEX, IMAC, Prot-G, etc.)

Characterization, Identification and Quantitation of Proteins and Peptides, Localization of PTMs, Top-Down and Bottom-up proteomics

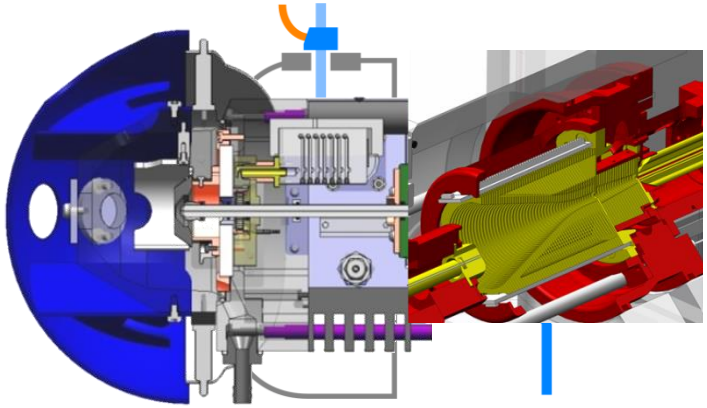
MASS SPECTROMETRY!!!

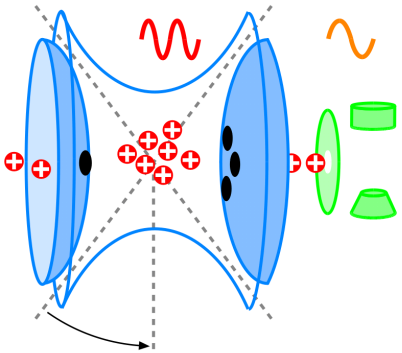
Mass Spectrometer

Ionization Source	Ion Optics/Isolation	Detector
Electrospray (ESI, NanoESI)	-	High Capacity Ion Trap (or LIT)
	Quadrupole (Q)	FT ICR
MALDI (SELDI)	Time of Flight (TOF)	Time of Flight (TOF)

ESI–based MS detection

nanoESI – nanoElectrospray

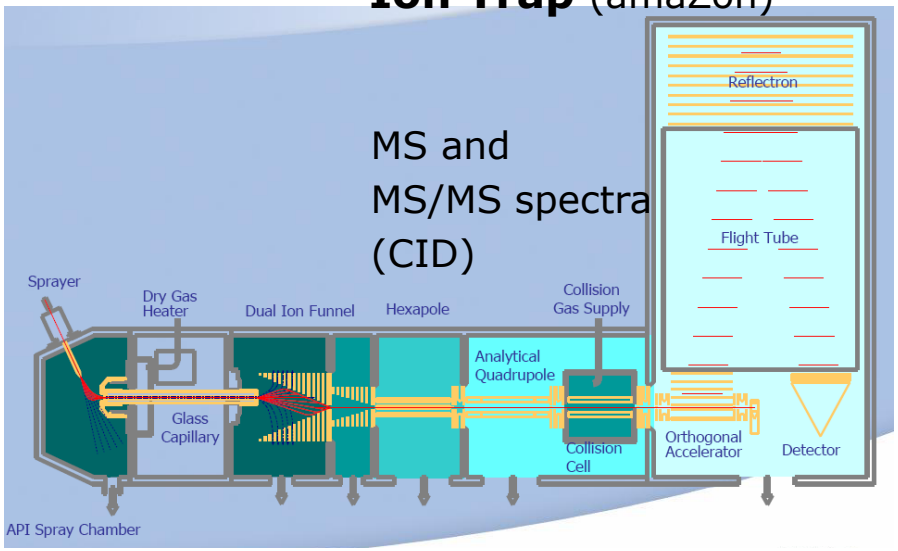




MS and
MS^n spectra
(CID/ETD)

Ion Trap (amaZon)

MS and
MS/MS spectra
(CID)



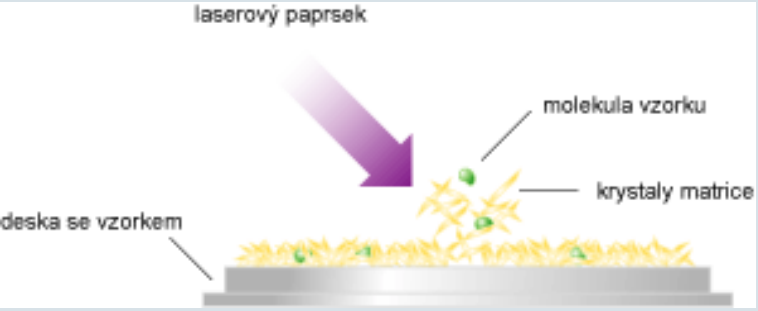
micrOTOF-Q II

maXis impact

qQ-TOF and UHR-TOF

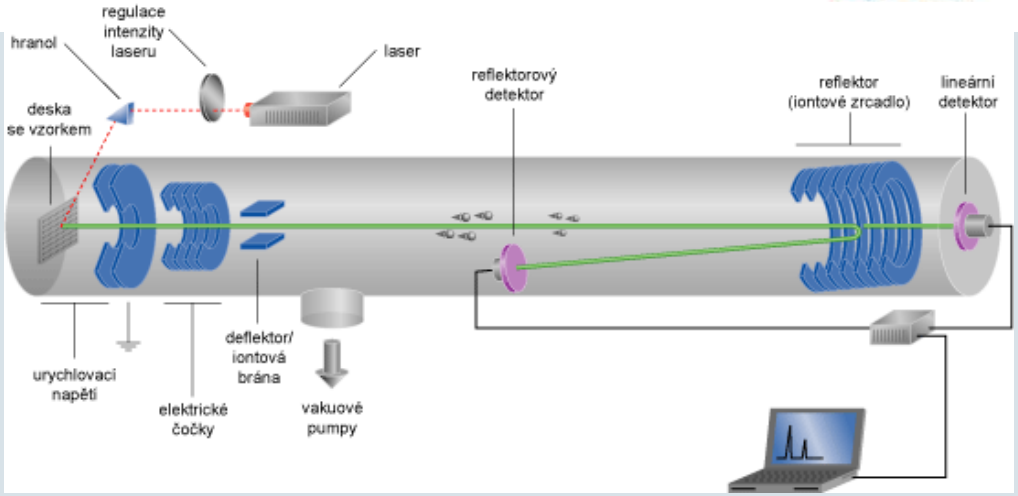
MALDI – based MS Detection

MALDI = Matrix Assisted
Laser Desorption-Ionization

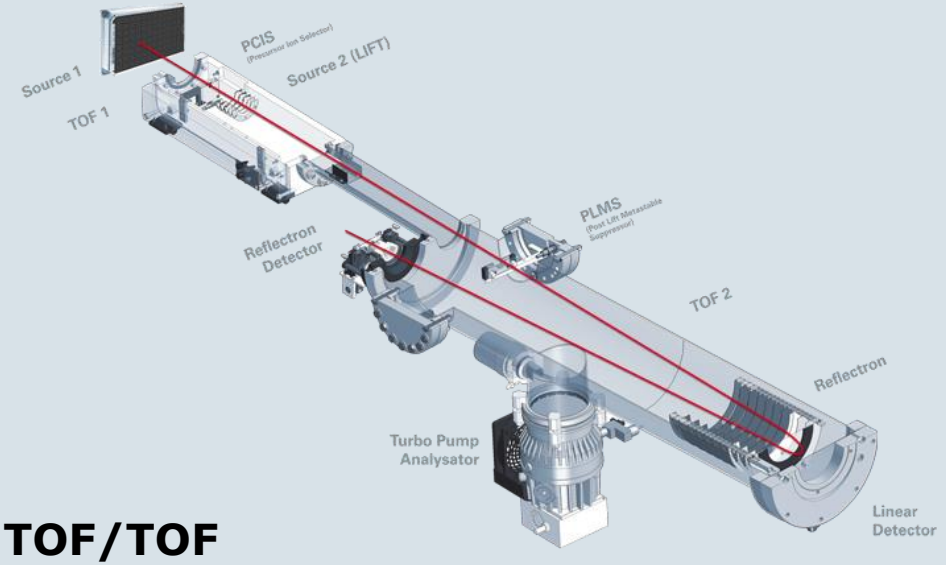


ultraflextreme

AutoflexSpeed



- Time of Flight (TOF)



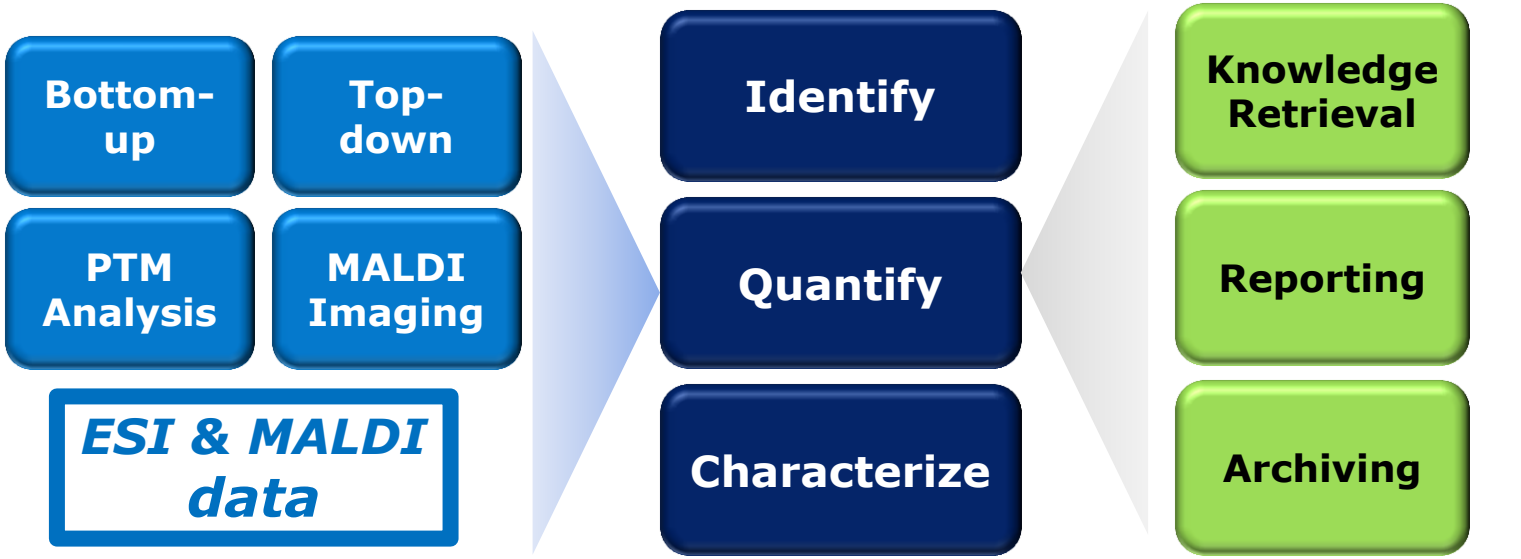
- TOF/TOF

Full Coverage of Technologies and Bioinformatics to Reveal the Proteome

The Proteome:

- far more complex than was ever expected
- highly dynamic in time, space and concentration
- highly variable due to modifications and mutations

requires complementary methods to generate reliable and complete information:



proteinscape

Bioinformatics

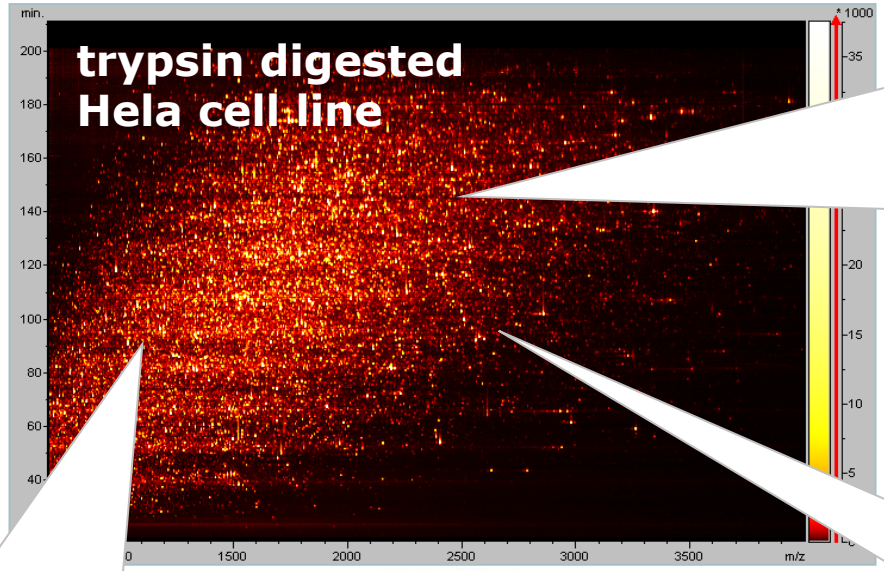
prime

Bottom-up Protein Identification

Any platform can make it !

Don't forget their other skills !

240 min gradient



maXis Impact:

3017

protein IDs

34583

25345 (6 µg)

Peptide ID's(2 µg)

ultrafleXtreme:

2840

protein IDs

11960

Peptide ID's(6µg)

amaZon Speed:

2544

protein IDs

22134

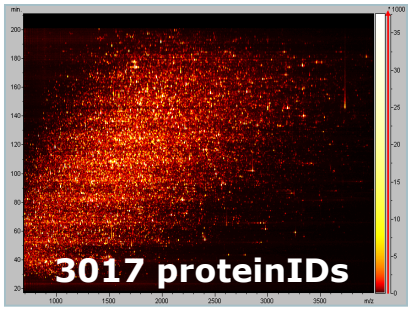
Peptide ID's (5 µg)

All protein numbers with FDR < 1%.

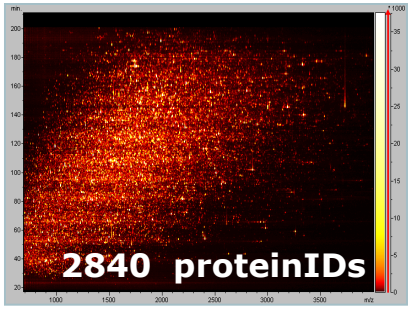
Bottom-up Protein Identification

Clear benefit provided by ESI/MALDI complementarity

maXis Impact



ultrafleXtreme



prime

Protein Extractor (proteinscape)

ESI+MALDI:

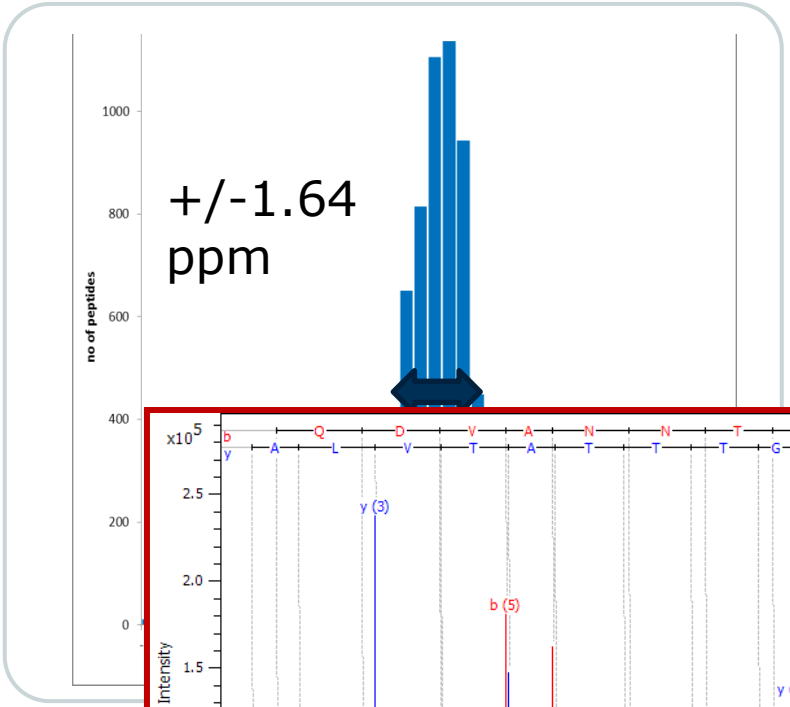
3526

non-redundant protein IDs

added ~20% by combining ESI and MALDI

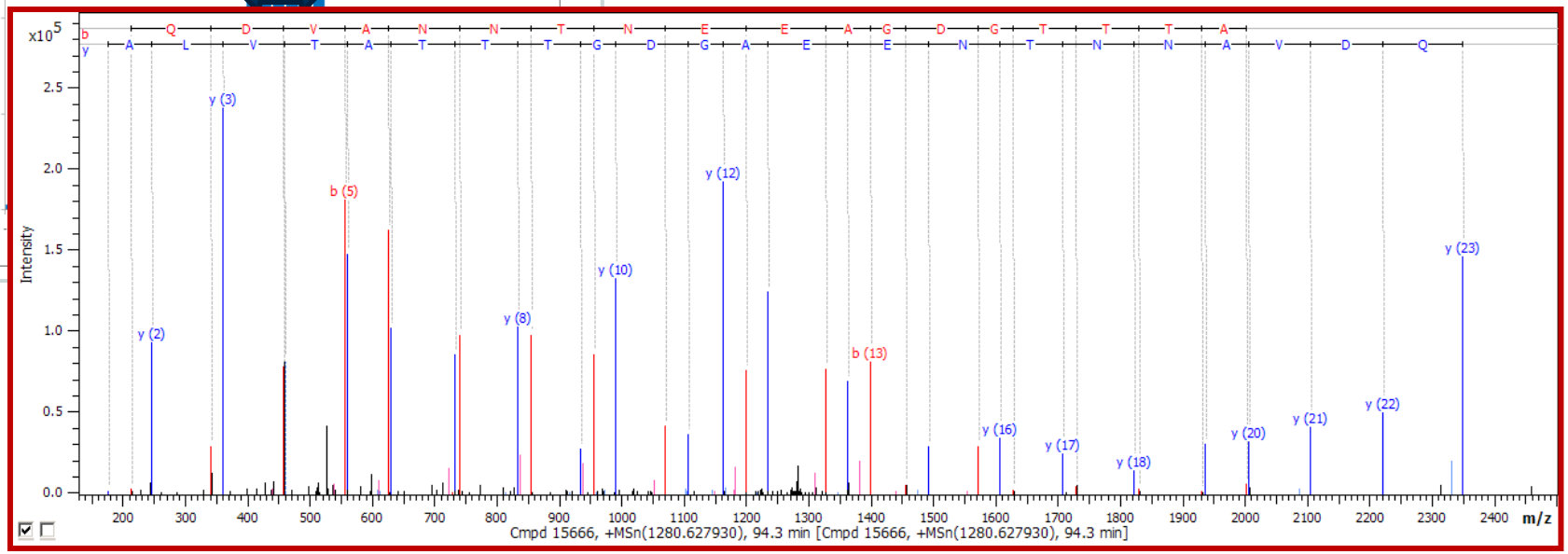
Takes 3 mouseclicks in proteinscape

maXis impact Performance

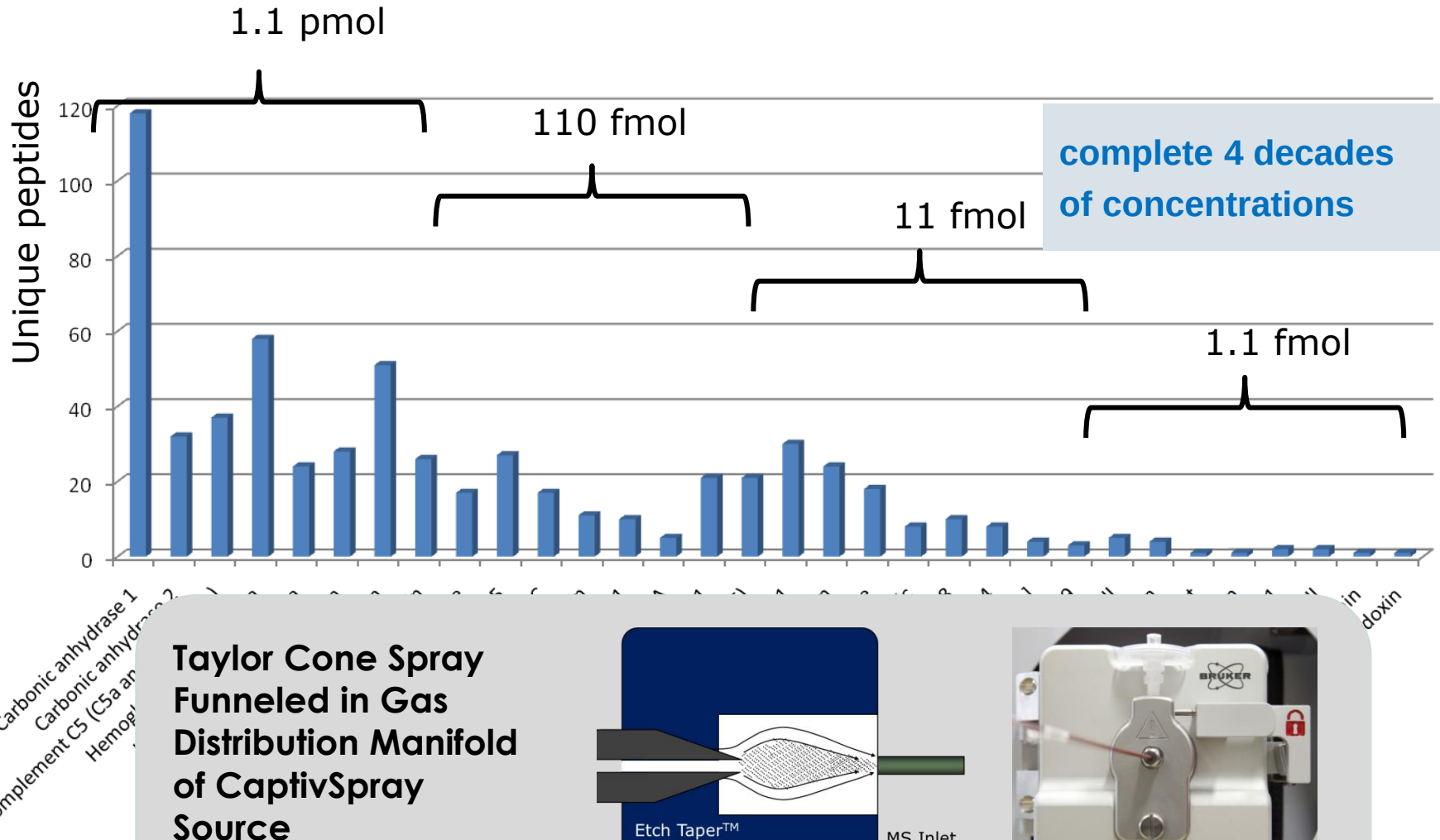


- **Average mass accuracy on 10,000 id'ed peptides**

- **Covering the entire m/z range for MS/MS at 20 Hz speed**



Dynamic range of the maXis impact



complete 4 decades of concentrations

Taylor Cone Spray Funneled in Gas Distribution Manifold of CaptivSpray Source

UPS-2 d

Bruker Daltonics

amaZon speed – scan modes and resolution

- **XtremeScan with 52.000 u/sec for real resolution of 2+ ions**
- **Full usability for MS/MS applications**

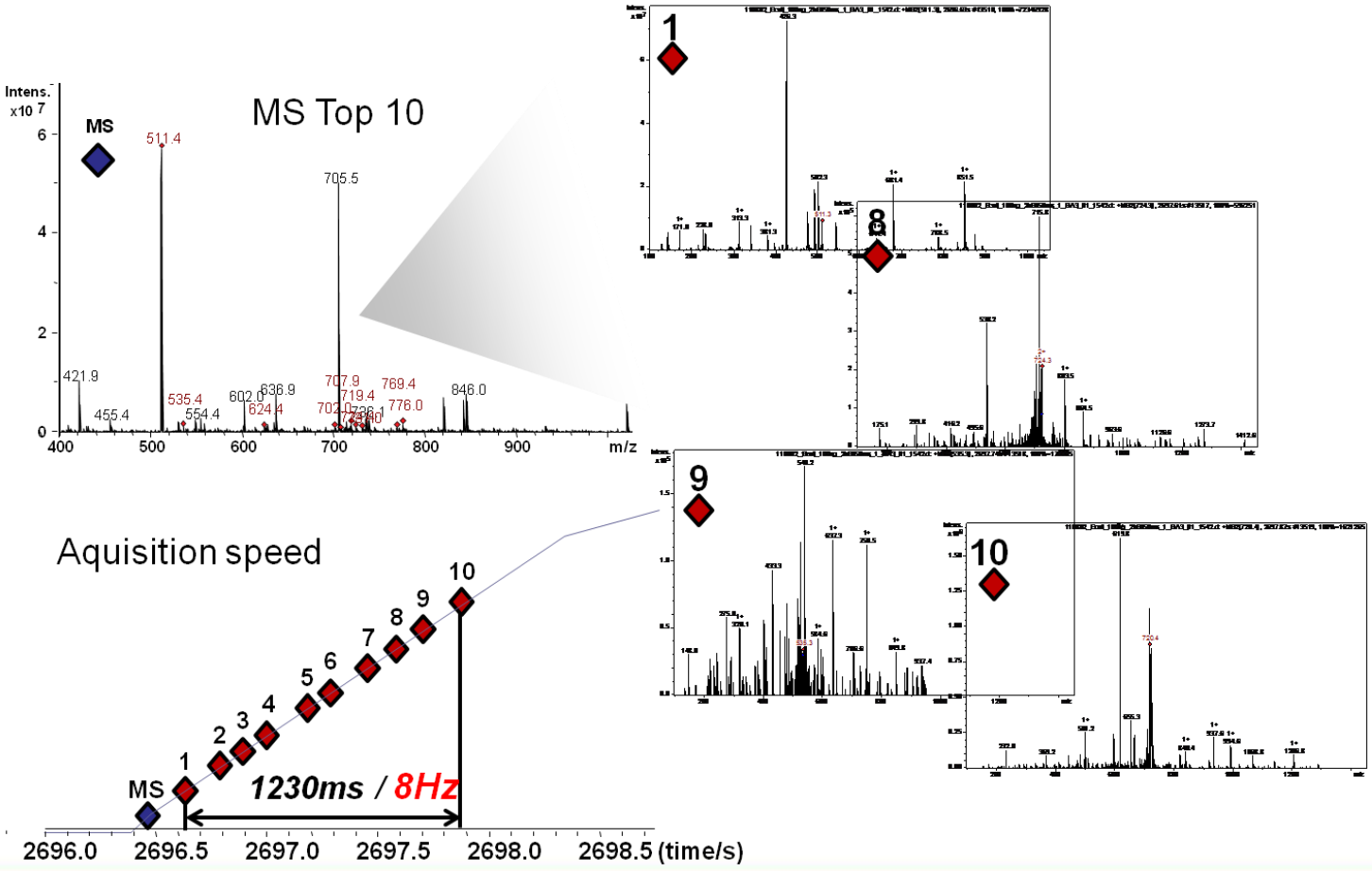
Scan Mode	Resolution	u/sec	m/z
XtremeScan	2 + ions	52.000	3000
UltraScan	3 + ions	32.000	3000
Enhanced Resolution	4 + ions	8.100	3000
Maximum Resolution	8 + ions	5.200	3000
Extended Mass Range		27.000	6000
Peptide Scan MS MS/MS	 4+ ions 2+ ions	 8100 52.000	 3000 3000

Scan mode	FWHM
XtremeScan	< 0.50
UltraScan	< 0.40
Enhanced Resolution	< 0.30
Maximum Resolution	< 0.10*

* For multiple charged ions

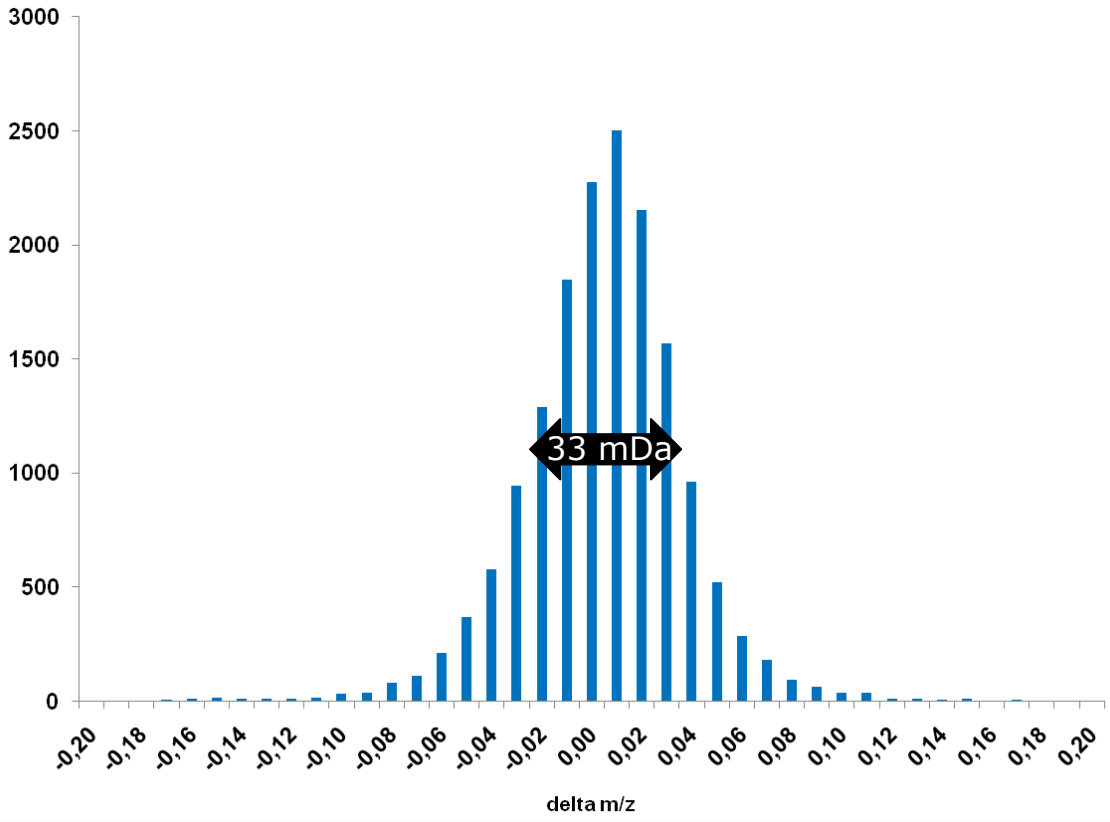
New analytical performance levels for – *8 Hz MS/MS speed*

- **Aquisition speed in MS/MS leading to drastically improved duty cycle**



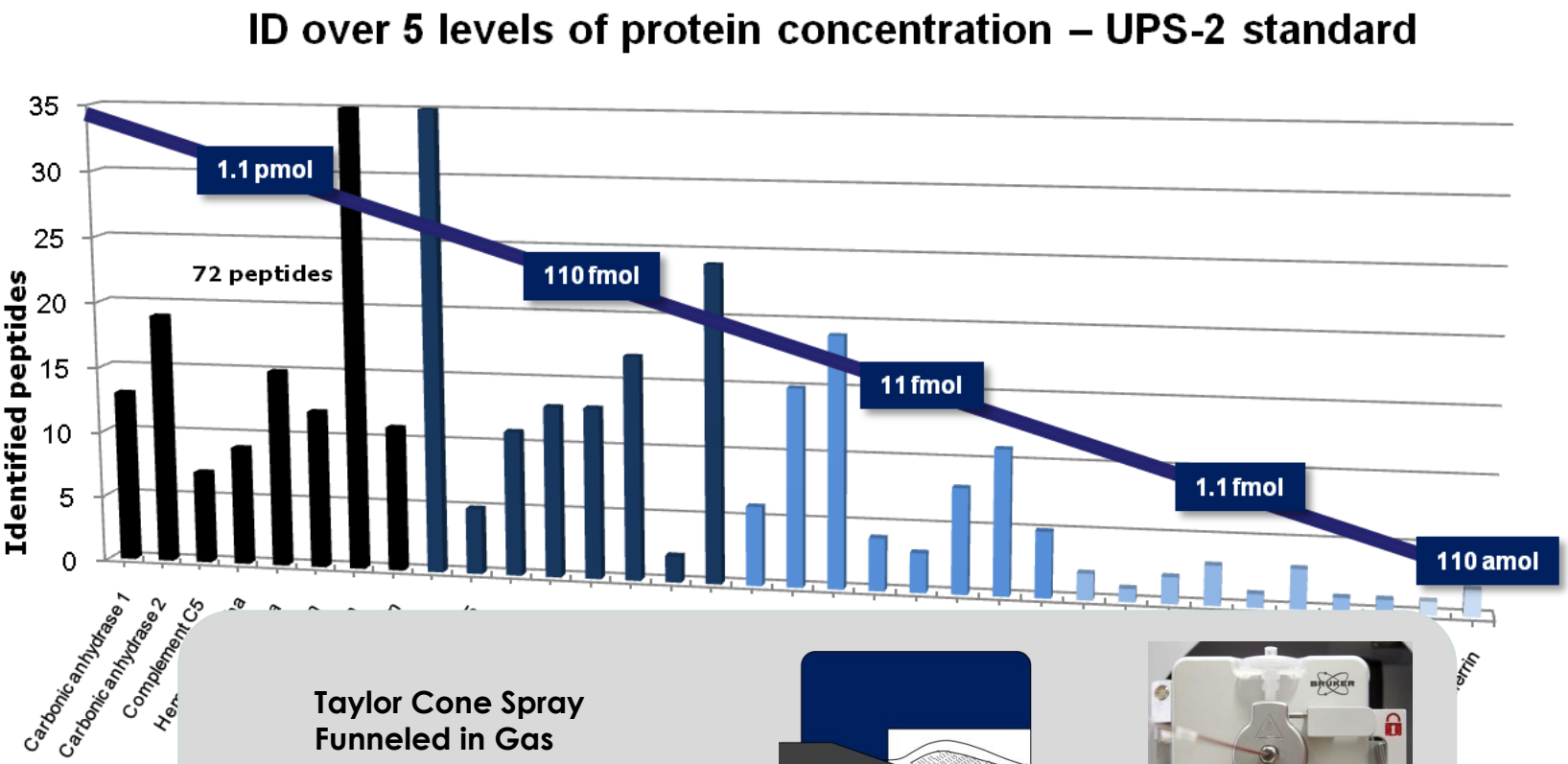
Averaged mass accuracy – amaZon speed

- **Typical mass accuracy for a proteomics data set: ~ 33 mDa in average**

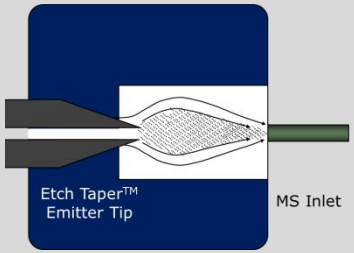


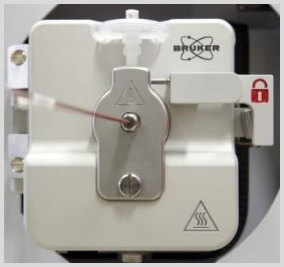
Covering a wide dynamic range with amaZon speed –UPS-2 standard

- **We are able to identify proteins covering 5 levels of concentration!**



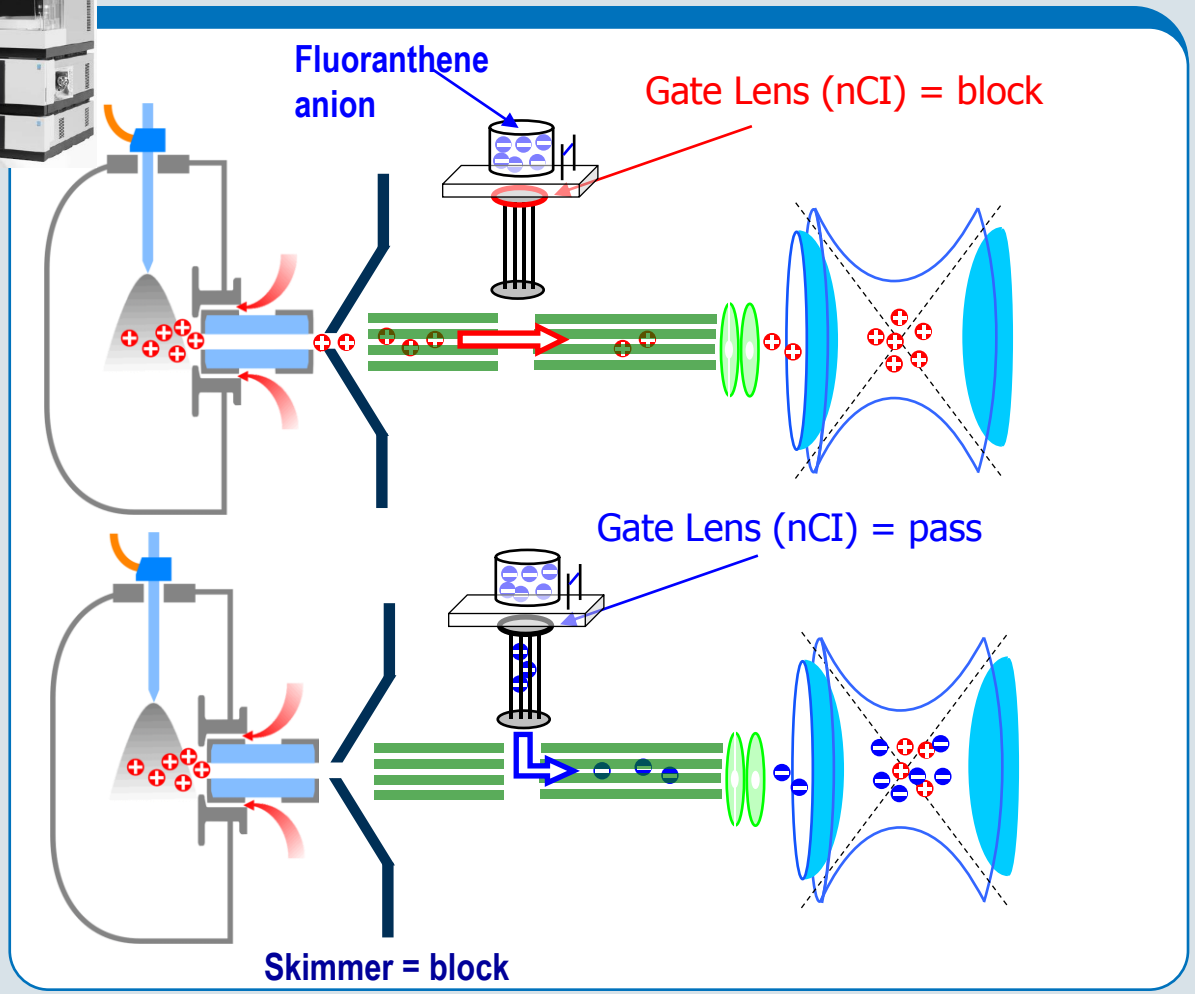
Taylor Cone Spray Funneled in Gas Distribution Manifold of CaptiveSpray Source





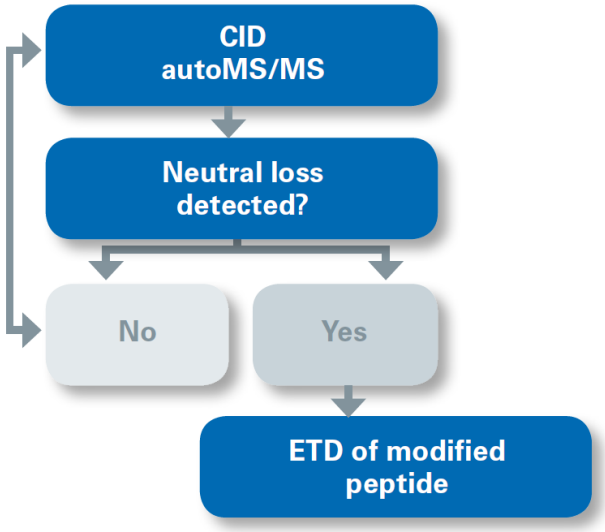
ETD in the amazon speed

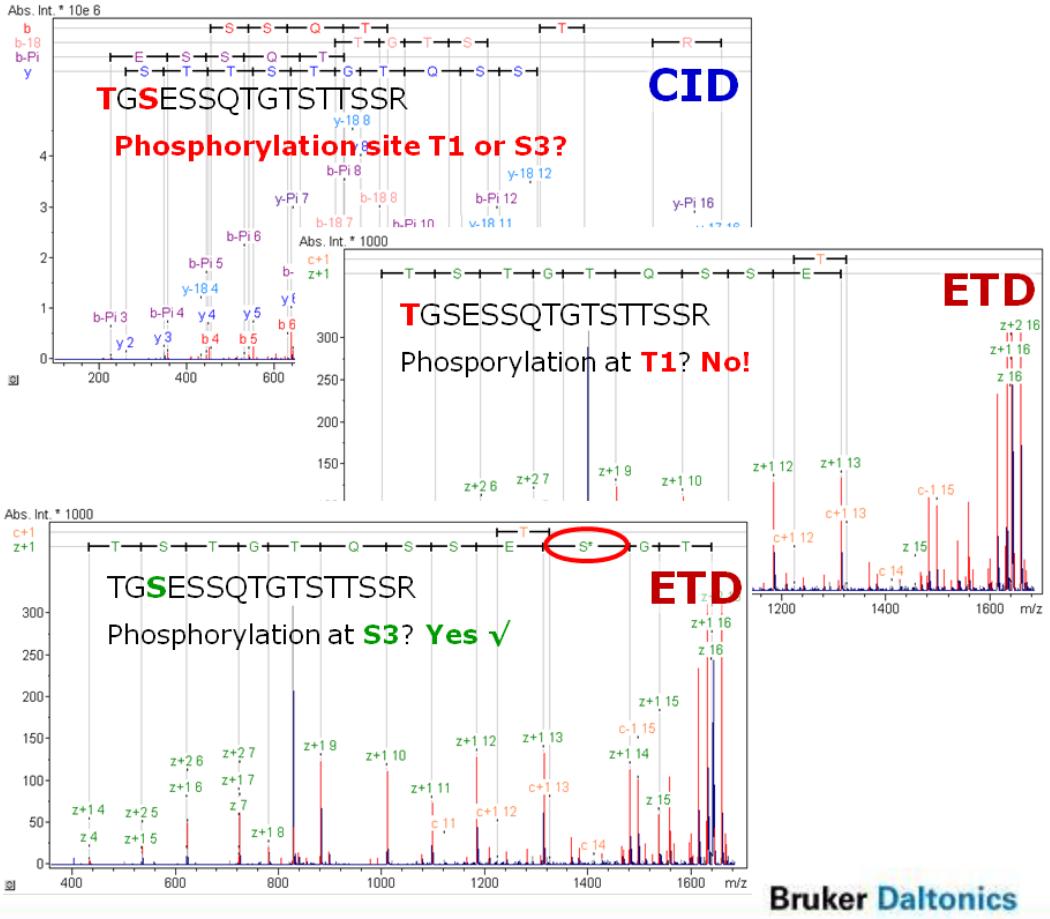
1. Electrospray ion accumulation
2. Precursor ion isolation
3. Reactant anion accumulation (nCI source)
4. ETD fragmentation
5. Scan



ETD for analysis of posttranslational modifications (PTMs)

- **ETD is the solution for the assignment of modification sites**
- **outstanding software tools – ProteinScape/biotools**





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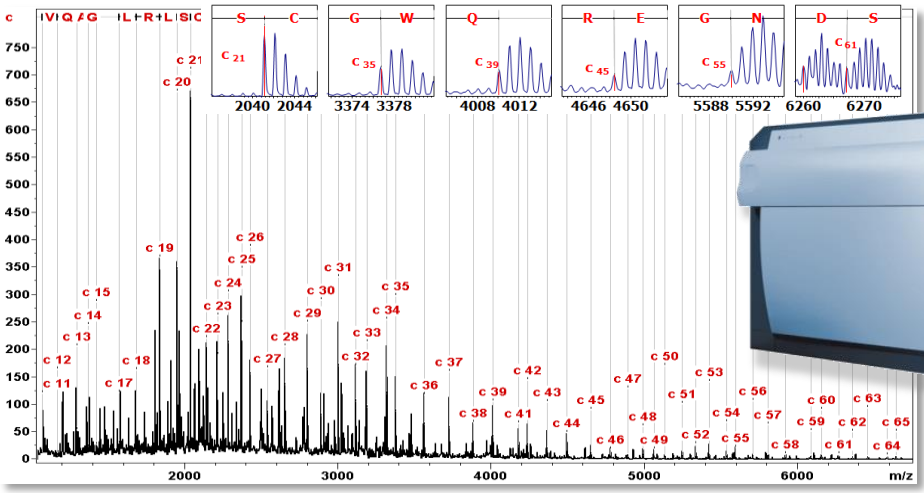
- **Applications in metabolomics and food control:**
  - **HPLC-ESI-QTOF and -UHR-TOF (maXis)**
  - ID of unknowns, impurities
  - Multitarget screening and quantitation using hrEIC
- **Applications in proteomics**:
  - **PRIME – combination of nanoLC-ESI (amaZon/impact)** and **nanoLC-MALDI** (ultraflextreme MALDI-TOF/TOF) for ID of proteins
  - **Top Down proteomics**

Top-down Characterization

Prime: *combine different MS technologies*



ultrafle*X*treme

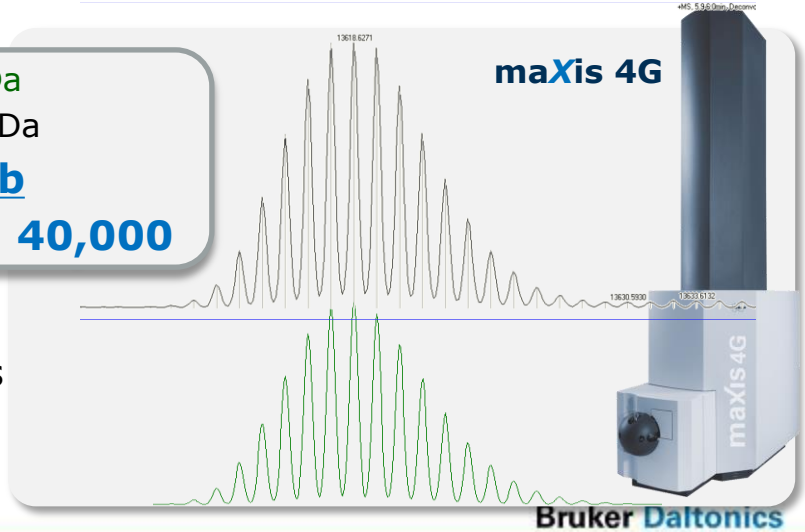
De-novo top-down sequencing of an intact 13.6 kDa Camelid antibody.

Calc. MW: 13618.6263 Da

Meas. MW: 13618.6271 Da

Mass error: 60 ppb

Mass resolution > 40,000



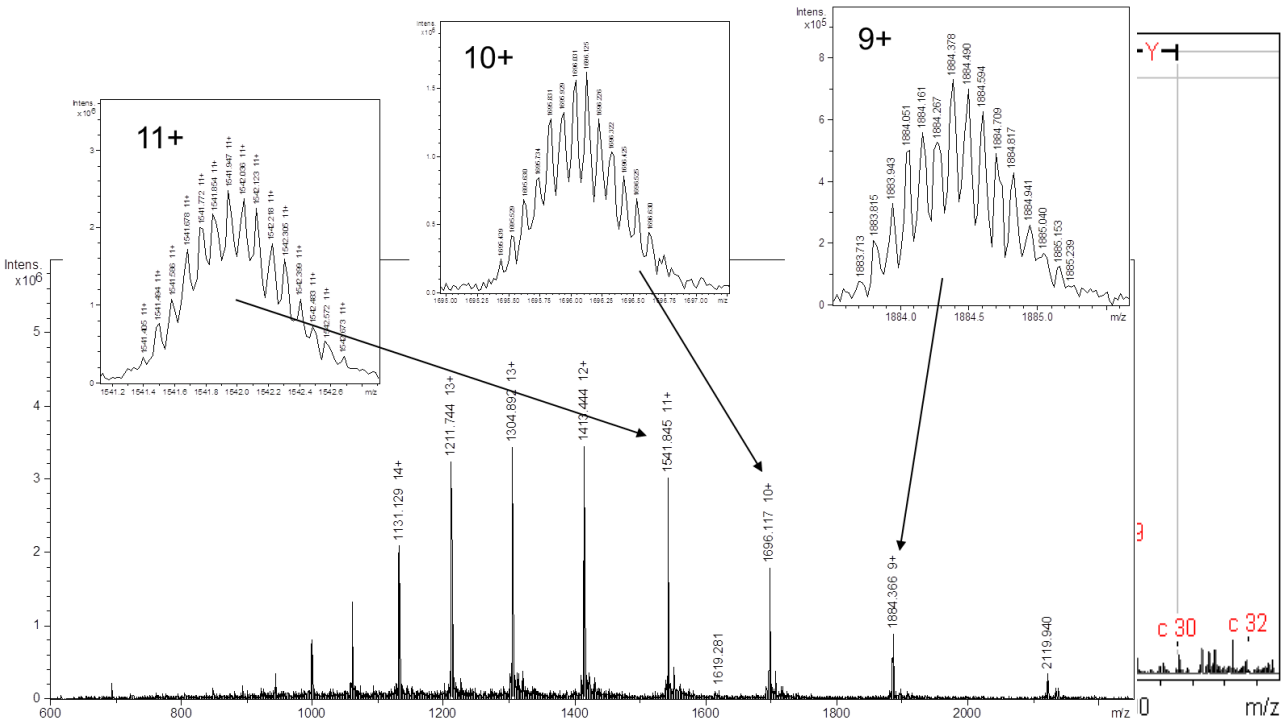
Confirmation of the expected sequence by exact protein mass and perfect isotope matching.

Top-down Characterization

Prime: *combine different MS technologies*

amaZon speed

```
1          10          20          3
MSYNL LGFLQ RSSNF QCQKL LWQLN GRLE
           70          80          9
EMLQN IFAIF RQDSS STGWN ETIVE NLLA
         130         140         15
HLKRY YGRIL HYLKA KEYSH CAWTI VRVE
```



ETD/PTR spectrum of intact β-Interferon (MW ca. 22.5 kDa) with N- and C-termini fully confirmed. The read-out extends up to the S-S crosslink at C31 - C141.

MALDI Molecular Tissue Imaging:

The spatially resolved molecular view into biology and disease

Prime: ***combines MALDI for discovery, ESI for top-down identification***

The MALDI Molecular Imager™ comprises the whole MALDI Imaging workflow.

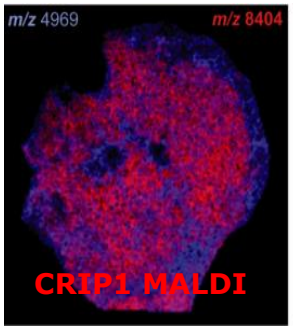
- **The ImagePrep- automated & reproducible matrix application**
- **mass spectrometers with optimized laser (smartbeam technology)**
- **Dedicated software for data acquisition and data visualization**
- **Sophisticated software for biostatistical analysis for expression profiling**

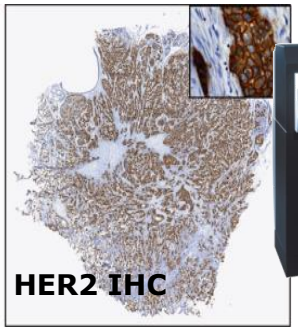
Plus ETD for top-down biomarker ID

MALDI Molecular Tissue Imaging:

The spatially resolved molecular view into biology and disease

Prime: ***combines MALDI for discovery, ESI for top-down identification***

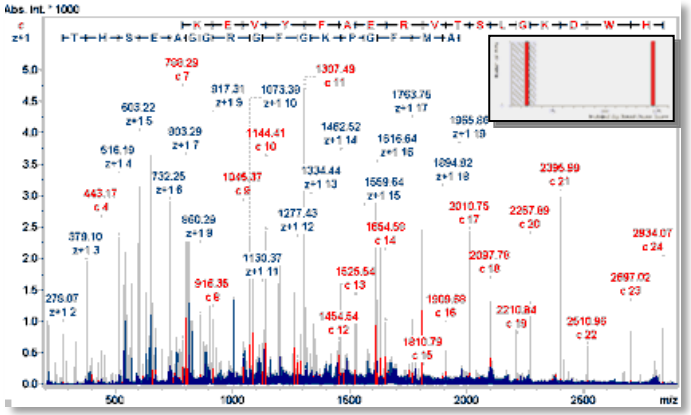


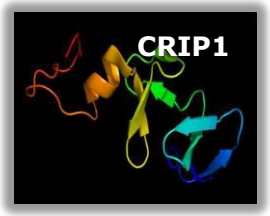


HPLC fractionation of intact proteins

HER 2+ Breast Cancer Tissue:
Discovery of a putative biomarker

Biomarker fractions analyzed by top-down ETD/PTR.
Identification of the biomarker





Full Coverage of Technologies and Bioinformatics to Reveal the Proteome

Bottom-up

Top-down

PTM Analysis

MALDI Imaging

ESI & MALDI data

Identify

Quantify

Characterize

Knowledge Retrieval

Reporting

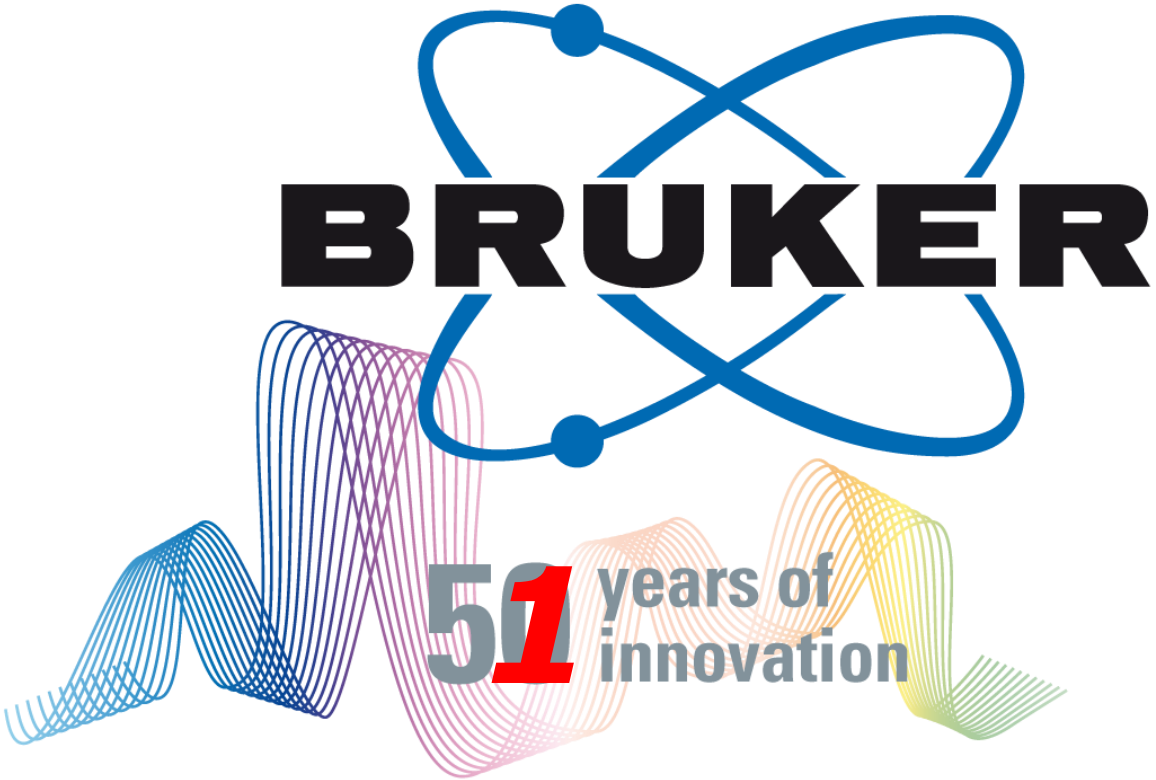
Archiving

proteinscape

Bioinformatics

prime

Děkuji za pozornost..



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