MALDI-TOF/TOF mass spectrometry

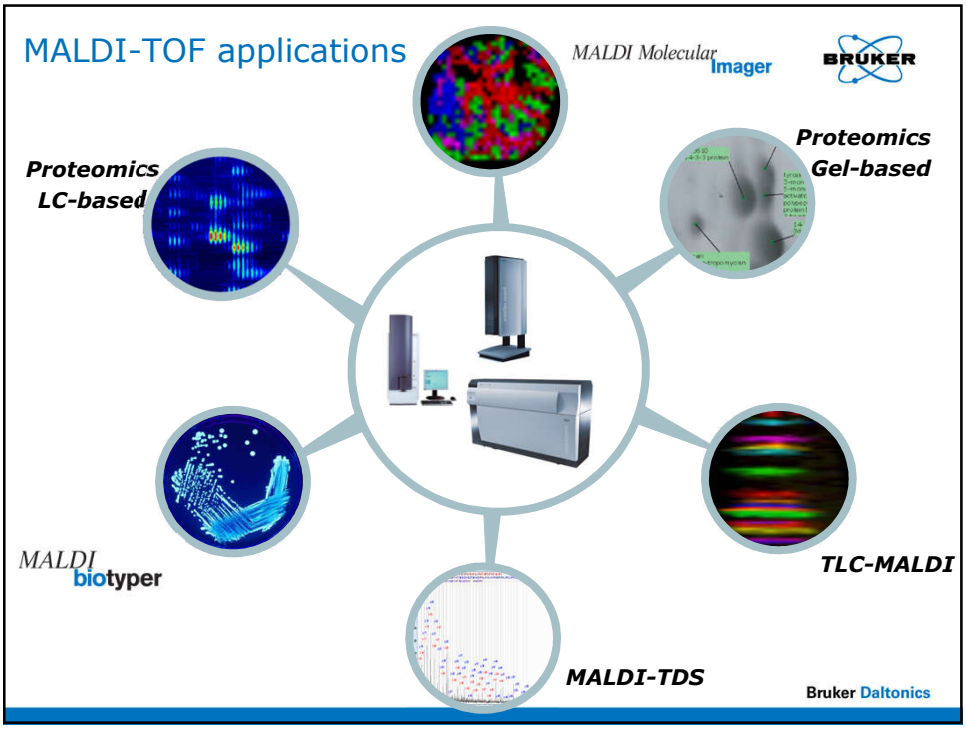
An Introduction

Contact : ms.support.us@bruker.com
Phone: 978-663-3660 Ext 5

Bruker MALDI-TOF(/TOF) Portfolio:

- microflex LT : Bench-top systems, 60 Hz nitrogen laser,
- autoflex speed: Floor standing, various configuration available (lin, ref, TOF/TOF), 2KHz for MS, 200Hz for MSMS;
- ultrafleXtreme: Top-of-the-line instrument, 2000 Hz MS, 1000 Hz for MSMS,

MALDI-TOF applications



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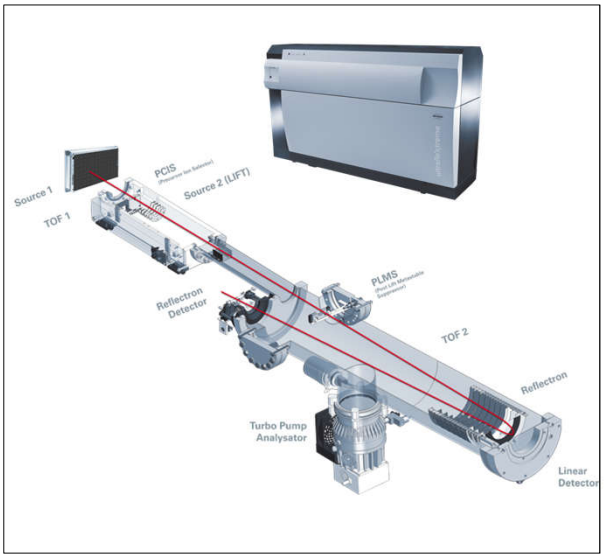
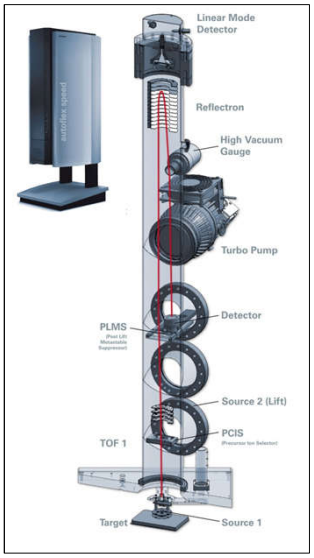
Diagrams of autoflex speed and ultrafleXtreme MALDI-TOF/TOF MS systems





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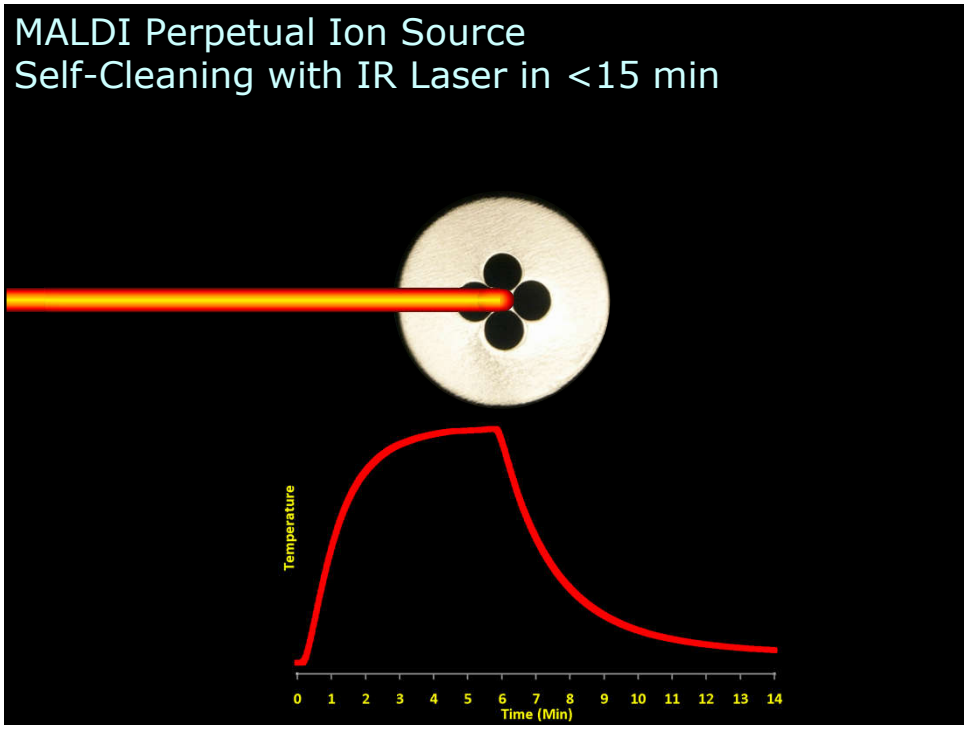
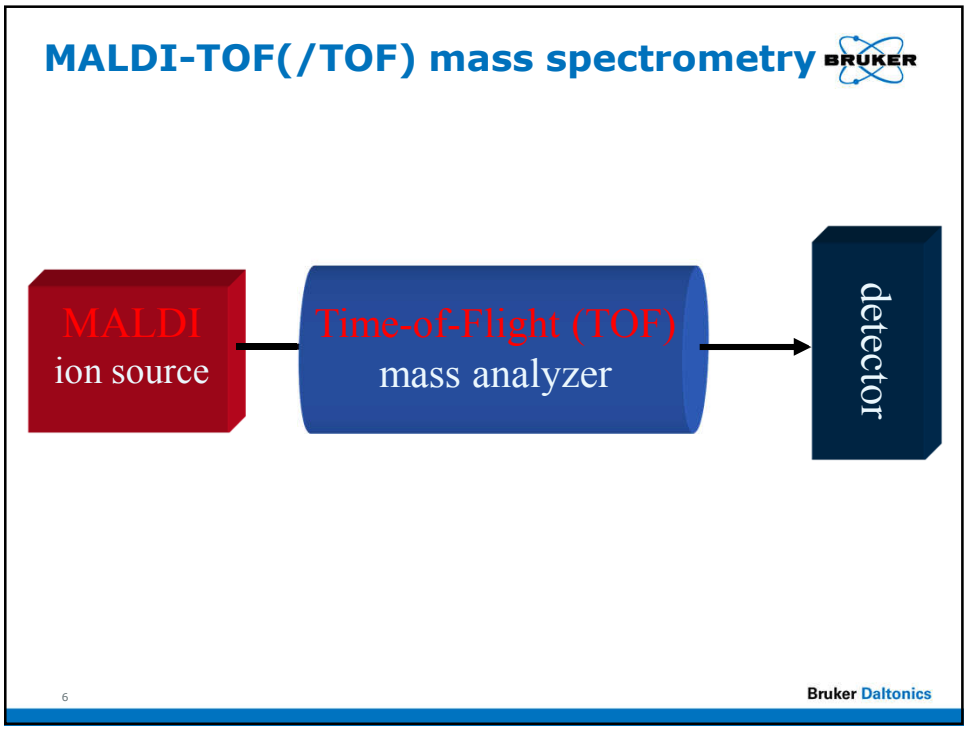

MALDI Perpetual Ion Source
Self-Cleaning with IR Laser in <15 min
Temperature
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Time (Min)


MALDI-TOF(/TOF) mass spectrometry
BRUKER
MALDI
ion source
Time-of-Flight (TOF)
mass analyzer
detector
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MALDI

MALDI stands for …

Matrix Assisted Laser Desorption / Ionization

invented by

Tanaka (Nobel prize awarded 2002), Hillenkamp and Karas

in the mid ´80s

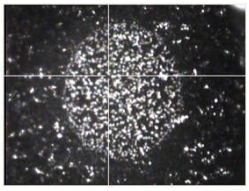
How does **MALDI** work?

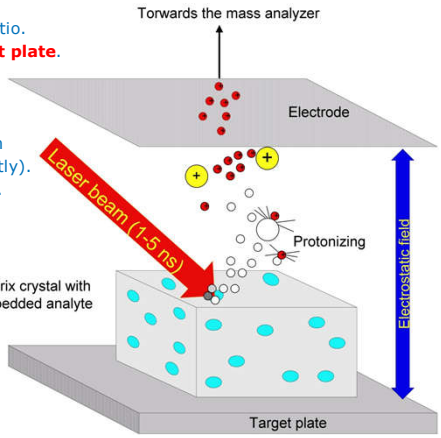
Required items:

- a **sample** to be analyzed
- a **matrix** substance
- a **laser**

MALDI preparation / MS analysis:

1.) **Mix sample and matrix** solutions in suitable ratio.
2.) Put a sub µl aliquot of this mixture on the **target plate**.
3.) Let the mixture **co-crystallize**.
4.) Insert the target plate in the MALDI mass spec (running under **high vacuum**).
5.) **Shoot with the laser** on the MALDI preparation to **generate ions** (**singly charged** predominantly).
6.) **Collect**, **analyze** and **detect** the resulting **ions**.





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How does **MALDI** work?

Fundamental papers on the principle of MALDI:

M. Karas, D. Bachmann, F. Hillenkamp

Analytical Chemistry, 57, 2935-2939 (1985)

K. Tanaka, H. Waiki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida

Rapid Communications in Mass Spectrometry, 2, 151-153 (1988)

R. C. Beavis, B. Chait, K.G. Standing

Rapid Communications in Mass Spectrometry, 3, 233-237 (1989)

M. Karas, M. Glückmann, J. Schäfer

Journal of Mass Spectrometry, 35, 1-12, (2000)

R. Zenobi and R. Knochenmuss

Mass Spectrometry Reviews, 17, 337-366 (1998)

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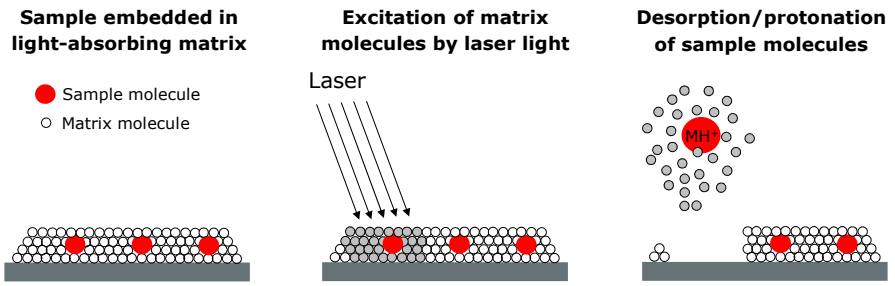
What role does the **MALDI matrix** play?

The matrix transfers the energy needed for ionization from the laser light to the sample molecules.

Positive ionization mode:



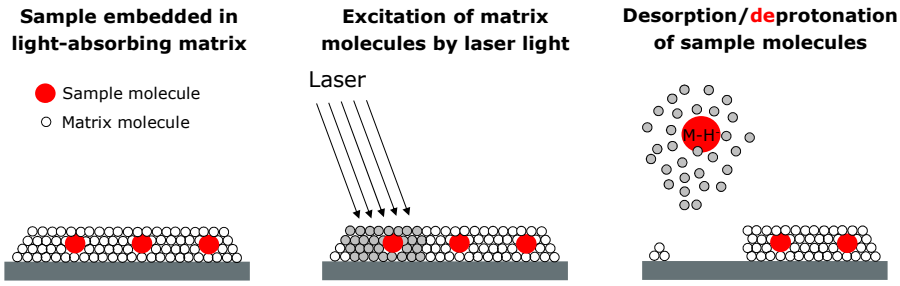
$$M \xrightarrow[\text{Matrix}]{\text{Laser energy}} [M+H]^+$$

Formation of alternative adducts depends on the presence of respective cations (either being ubiquitary present or actively added – depending on type of sample):
$[M+Na]^+$; $[M+K]^+$; $[M+Cu]^+$; $[M+Li]^+$; $[M+Ag]^+$

What role does the **MALDI matrix** play?

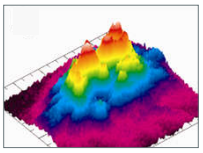
The matrix transfers the energy needed for ionization from the laser light to the sample molecules.

Negative ionization mode:

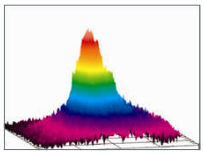


$$M \xrightarrow[\text{Matrix}]{\text{Laser energy}} [M-H]^-$$

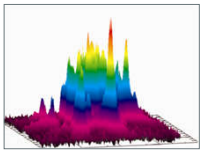
Types of laser commonly used in MALDI:



Nitrogen laser:
pro: well structured energy profile
contra: slow (maximum 60Hz)



Nd:YAG laser:
pro: fast (up to >1000Hz)
contra: Gaussian energy profile (non-structured)



Smartbeam/Smartbeam II (modified Nd:YAG laser):
pro: fast (up to 2000Hz)
pro: well structured energy profile

Reference: A. Holle, A. Haase, M. Kayser, J. Höhndorf, *Journal of Mass Spectrometry*, 41, 705-716 (2006)

Commonly used **MALDI matrix substances**:

Peptides:	4-Hydroxy-α-cyanocinnamic acid (**HCCA**)
Proteins:	2,5-Dihydroxyacetophenone (**DHAP**) Sinapinic acid (**SA**) 2,5-Dihydroxybenzoic acid (**DHB**)
Glycans:	2,5-Dihydroxybenzoic acid (**DHB**)
Nucleic acids:	3-Hydroxypicolinic acid (**HPA**) 2,4,6-Trihydroxyacetophenone (**THAP**)

Why different matrices for different types of sample?

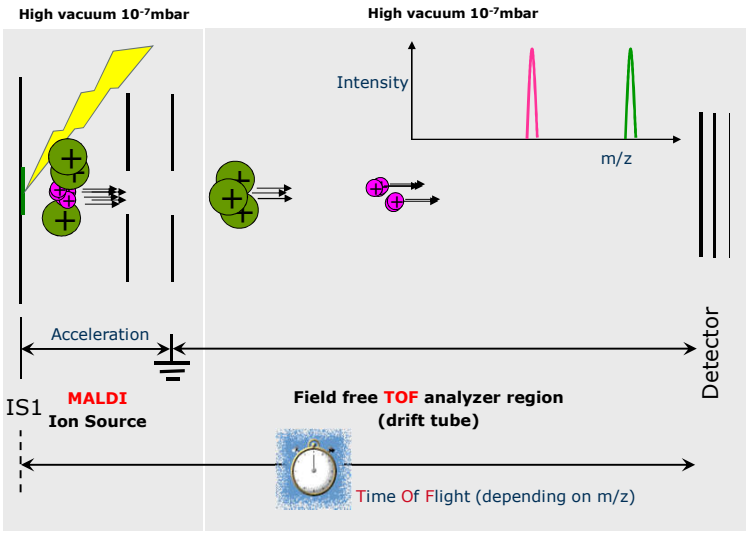
It´s all about

- the **amount of energy needed to ionize** a particular sample compound
  (individual matrices show specific „energy threshold")
- the **stability** of a particular sample compound
  (too „hot" matrix may lead to non-desired fragmentation of sample compounds)

MALDI-TOF
(Time of Flight)

MALDI-TOF

Basic principle of MALDI-Time-of-Flight based mass analysis:



MALDI-TOF

$$E_{pot} = zeU$$

$$E_{kin} = 1/2mv^2$$

$$zeU = 1/2mv^2$$

$$v = \sqrt{2zeU/m}$$

$$t = L\sqrt{m/2zeU}$$

This defines the potential energy at which all ions start from the MALDI target.

This equation defines the kinetic energy of ions after acceleration into the flight tube.

In the process of ion acceleration, energy is preserved but turns from potential into kinetic energy.

Transforming this equation shows the dependency of velocity of moving ions on their m/z value.

With the linear velocity v being defined as $L_{drift\ tube}/t_{flight}$, the dependency of the ions´ flight time t_{flight} from m/z becomes obvious.

To put the basic MALDI-TOF separation principle into simple words:
The larger its m/z, the slower an ion will fly, the longer the measured flight time will be.

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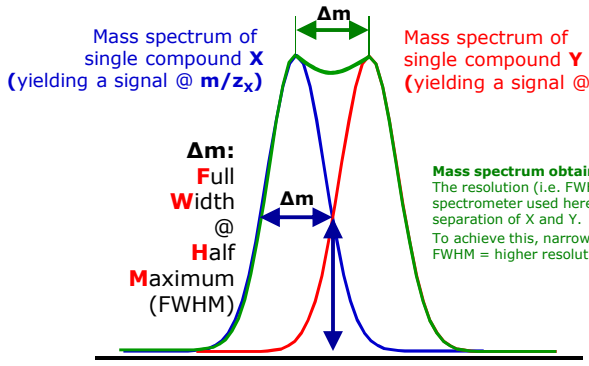
MALDI-TOF: Resolution

Resolution R defines, how well two peaks are separated from each other:

$$R = m/\Delta m$$

Δm is usually derived from a mass peak´s full width at half maximum (FWHM), as shown below for the exemplaric analysis of a mixture of two compounds X and Y:



Mass spectrum obtained from mixture [X+Y]:
The resolution (i.e. FWHM) provided by the mass spectrometer used here does not allow baseline separation of X and Y.
To achieve this, narrower peaks (i.e. smaller FWHM = higher resolution) would be required.

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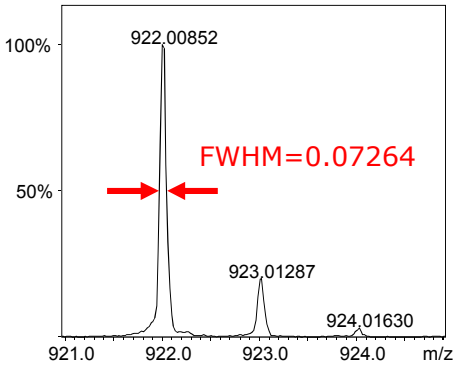
MALDI-TOF: Resolution

Example spectrum containing 3 singly charged $[M+H]^+$ signals at m/z 922Da, 923Da and 924Da:



Resolution = **m/Δm**
= 922.00852/0.07264
= **12693**

The resolution here is obviously more than sufficient to achieve baseline separation of the three compounds contained in the mixture.

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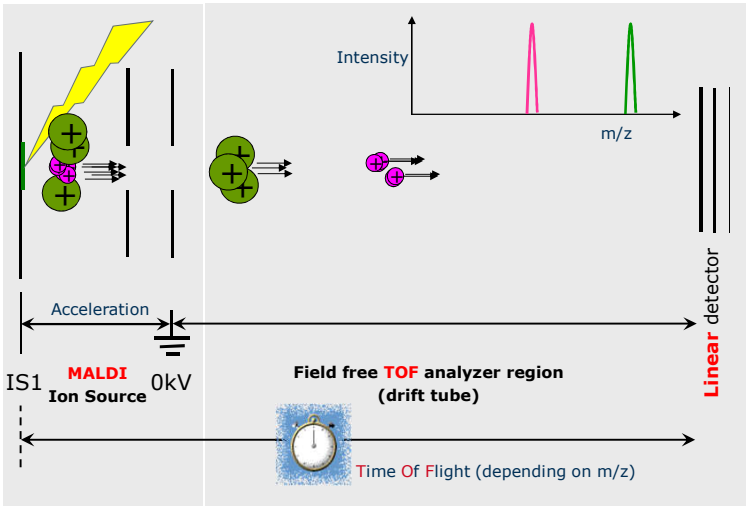
MALDI-TOF: Linear mode



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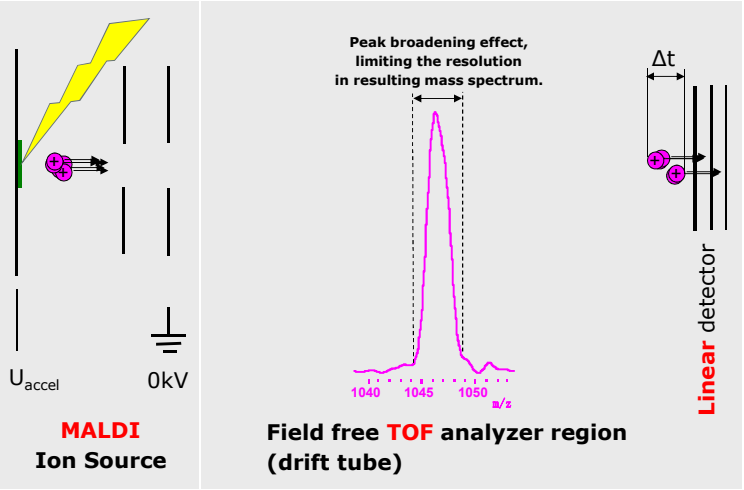
MALDI-TOF: Linear mode

Observation:

Ions of identical mass arrive at the detector at slightly different time points, causing peak broadening (i.e. limiting the resolution)

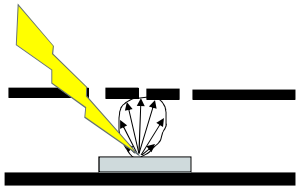


MALDI-TOF: Linear mode

Limited in resolution due to spatial spread and energy spread

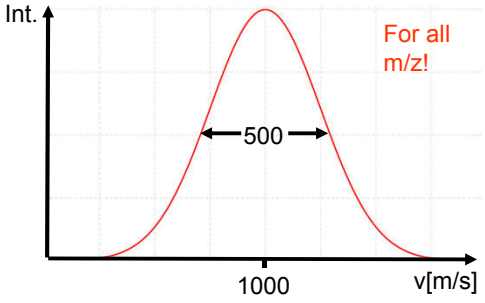
Spatial spread:

- initial movement of ions towards different directions
- ions are desorbed from different z-coordinates due to heterogeneity in size of matrix crystals



Initial energy (=speed) spread:

- heterogeneous secondary reactions (ion-ion; ion-neutral)



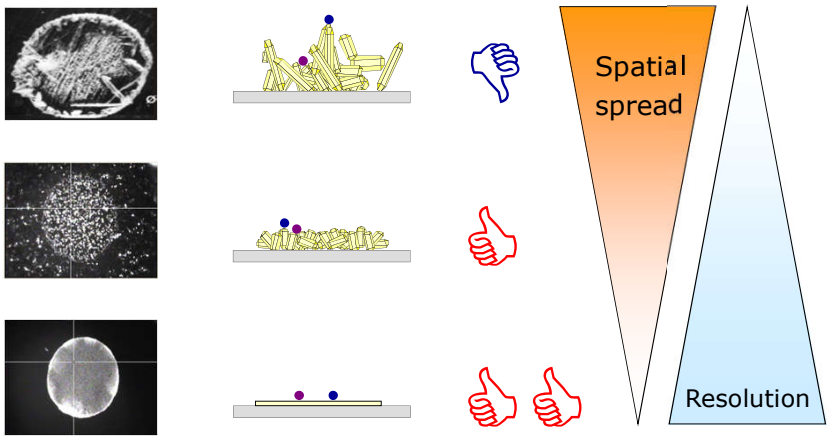
Reference:

W. Ens, Y. Mao, F. Mayer, K.G. Standing,
Rapid Communications In Mass Spectrometry, 5, 117-123 (1991)

MALDI-TOF
How to improve resolution???

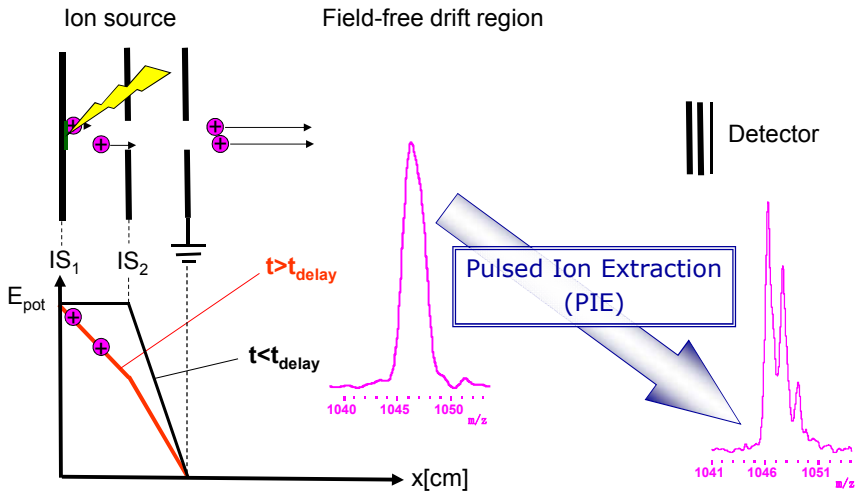
- Optimized **matrix preparation homogeneity** (minimizing spatial spread)
- **Pulsed ion extraction** for efficient ion focusing (minimizing initial energy spread)
- Further ion focusing by means of a **reflector** TOF setup (minimizing remaining energy spread)

MALDI-TOF
How to improve resolution???
- **Optimized matrix preparation homogeneity**



MALDI-TOF: How to improve resolution???

- **Pulsed ion extraction** for efficient ion focusing in the MALDI ion source



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How can I understand how PIE works?

PIE stands for **P**ulsed **I**on **E**xtraction. This means that the ions are not extracted as classically in a continuous extraction field, but are created in a zero extraction field, and then extracted after a certain delay time in a pulsed fashion.
This technique has two advantages: 1. The process is softer, as the ions are not accelerated through the dense plume of matrix molecules right after desorption, and 2. by choosing the right parameters a time focusing can be achieved. This together results in better resolution and better sensitivity, when using the correct parameters. On the other hand, wrong parameters can decrease resolution and sensitivity. Therefore the acquisition software greater than 3.0 allows for saving complete parameter sets. It is recommended to operate the system using parameter sets. Normally during installation some relevant sets have been stored on your system.

Method File Name	*IS1*	*IS2*	*Lens*	*Delay*
RP_3147	25	21.70	9.0	70ns
RP_6000	25	21.65	9.0	130ns
RP_8000	25	21.70	9.0	170ns

Method File Name	*IS1*	*IS2*	*Lens*	*Delay*
RP_1000	19	16.90	9.0	60ns
RP_2000	19	16.85	9.0	80ns
RP_3000	19	16.90	9.0	210ns
RP_6000	19	16.60	9.0	230ns
RP_12K	19	16.35	9.0	360ns
RP_17K	19	16.15	9.0	420ns

- Example only,

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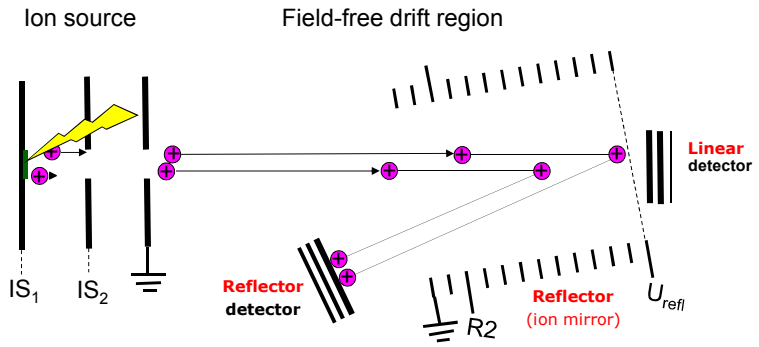
MALDI-TOF

How to improve resolution???

- Further ion focusing by means of a **reflector** TOF setup



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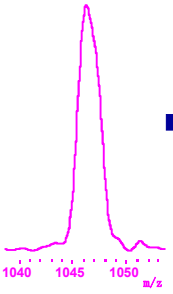
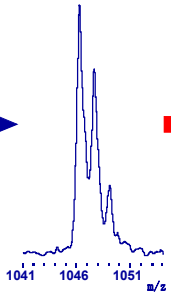
MALDI-TOF

How to improve resolution???

MALDI-TOF, linear

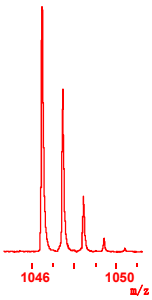


MALDI-TOF, linear + PIE



PIE compensates
- initial energy spread
- initial spatial spread

MALDI-TOF, reflector + PIE



Reflector compensates
- remaining energy spread

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MALDI-TOF

Linear vs. reflector mode

FAQ:

If MALDI-TOF performed in reflector mode gives so much better resolution - why then use linear mode at all???

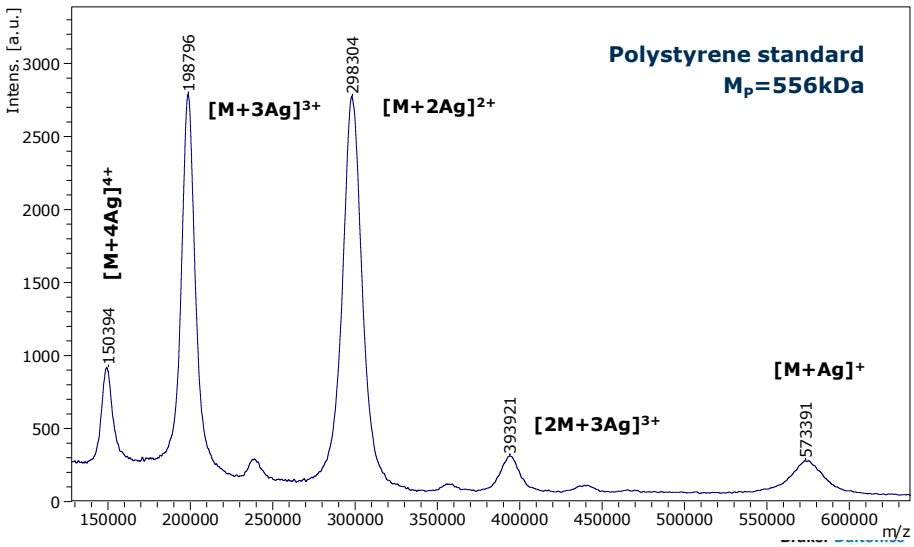
Answer:

Linear mode is used whenever analytes are not stable enough to survive the energetic stress which is inherent to passing the reflector (ions are deccelerated/re-accelerated in the reflector by a high kV electric field within nanoseconds!!!).

Especially larger sized molecules, e.g. intact proteins, show limited stability when passing the reflector field, and may undergo serious fragmentation, which results in either badly resolved spectra (peak fronting due to non-resolved fragments) and/or drastic loss in sensitivity (low mass fragments will miss the reflector detector).

ultrafleXtreme:

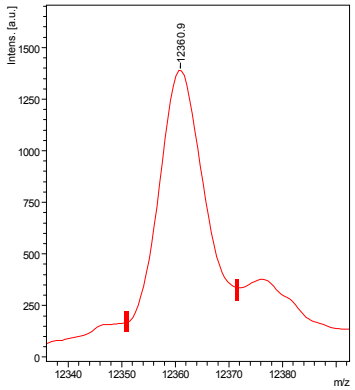
On-axis linear detector: Outstanding performance in HMW measurement



MALDI-TOF

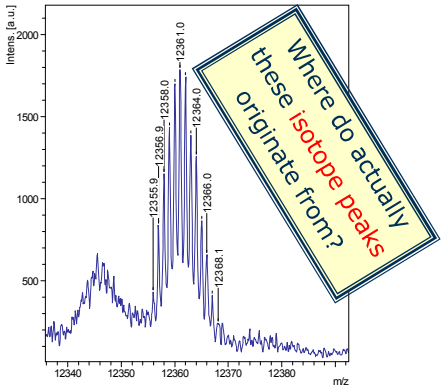
Linear vs. reflector mode: Cytochrome C (MW_{avg}=12360Da)

Linear mode: Low resolution
R=1,500



Spectrum shows one broad peak representing the envelope of the non-resolved isotope peaks.

Reflector mode: High resolution
R=30,000

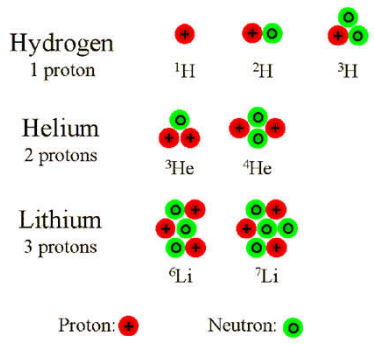


Spectrum shows all the isotope peaks well separated from each other.

MALDI-TOF

Where do these isotope peaks originate from?

Most elements are found in nature in form of different so called isotopes. Isotopes of an element have nuclei with the same number of protons (the same atomic number) but different numbers of neutrons.



However, both, protons and neutrons contribute to the weight of an atom, which explains the difference in weight of isotopes.

MALDI-TOF

Where do these isotope peaks originate from?

Isotope	Mass	[%] Abundance
1-H	1.007825	99.985
2-H (Deuterium)	2.014000	0.015
12-C	12.00000	98.90
13-C	13.00336	1.10
14-N	14.00307	99.63
15-N	15.00011	0.37
16-O	15.99491	99.76
18-O	17.99916	0.20
19-F	18.99840	100
23-Na	22.98977	100
31-P	30.97376	100
32-S	31.97207	95.03
34-S	33.96787	4.22
35-Cl	34.96885	76.77
37-Cl	36.96590	31.98
39-K	38.96371	93.26
79-Br	78.91834	50.69
81-Br	80.91629	49.31

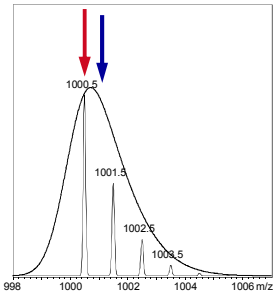
Elements that are found in nature in form of only one single isotope, are called monoisotopic elements.

MALDI-TOF

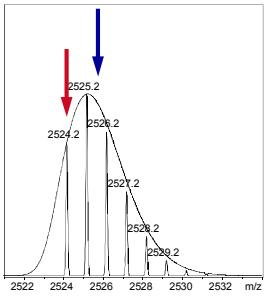
Where do these isotope peaks originate from?

For a given molecule, the individual isotopes of all the elements contained in it finally yield a characteristic intensity distribution of isotopic masses. This is shown below by means of the isotopically resolved mass spectra of 3 compounds being different in size:

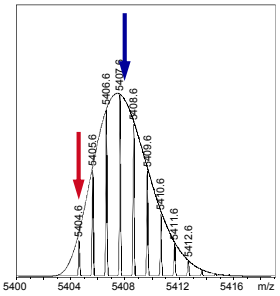
Element composition: $C_{41}H_{69}N_{13}O_{14}S$
Monoisotopic mass $[M+H]^+$: 1000.4880
Average mass $[M+H]^+$: 1001.1409



Element composition: $C_{112}H_{164}N_{29}O_{34}S_2$
Monoisotopic mass $[M+H]^+$: 2524.1510
Average mass $[M+H]^+$: 2525.8196



Element composition: $C_{253}H_{363}N_{55}O_{75}S$
Monoisotopic mass $[M+H]^+$: 5404.6075
Average mass $[M+H]^+$: 5407.9984



The **monoisotopic mass** is the sum of the masses of all the atoms present in a molecule using the mass of the most abundant isotope for each element.
The **average mass** of a molecule is the sum of elemental masses using the average weighted over all stable isotopes of each element contained in the molecule.

Calibration error, ppm calculation;

The calibration error (mass deviation) can be calculated by using the theoretical (m_{theo})and measured ($m_{measured}$)mass of the unknown substance :

$$Error_{ppm} = \frac{m_{measured} - m_{theo}}{m_{theo}} * 10^6$$

To calculate the average calibration error over four measurements (m1, m2, m3 and m4) following formula needs to be used

$$D_{avg} = \frac{|m_{theo} - m_1| + |m_{theo} - m_2| + |m_{theo} - m_3| + |m_{theo} - m_4|}{4 * m_{theo}} * 10^6$$

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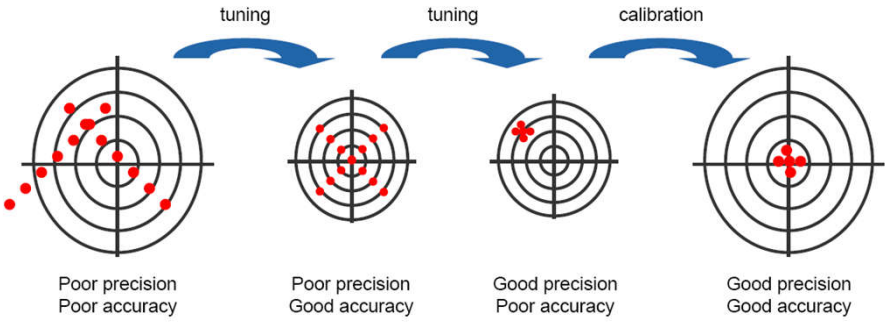
MALDI-TOF: Calibration

Precision: Variation of values obtained from repetitive measurements performed under identical conditions (*random error*)

Accuracy: Deviation of a measured value from the reference value (*systematic error*)



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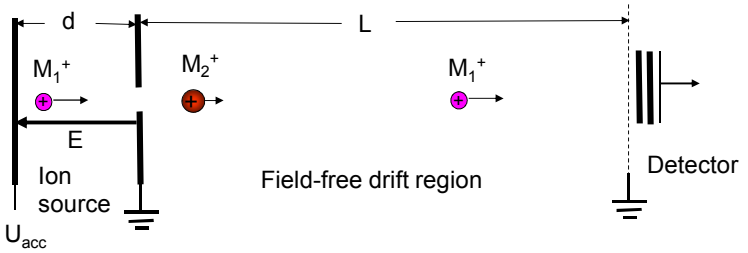
MALDI-TOF: Calibration



$$t_{of} = t_{delay} + t_{acc} + t_{drift} \quad (1)$$

t_{acc} can be calculated as followed. The force to an charge q in an electric field E is

$$F = E\,q = M\,a \quad (2)$$

MALDI-TOF: Calibration

Where a is the mass dependent acceleration. It is related to the time t_{acc} and the distance d between target and grounded acceleration plate.

$$d = a/2\ t_{acc}^2 \quad (3)$$

Replacing in (2) the electric field E = U/d, the charge q = z e, the number of charges z times the elementary charge e, and from (3) $a=2d/t_{acc}^2$ than the time-of-flight through the acceleration region is

$$t_{acc} = d\ \sqrt{(2m/Uze)} \quad (4)$$

MALDI-TOF: Calibration

For the calculation of t_{drift} we have to do the following considerations. All ions start from the target at the same potential energy

$$E_{pot} = qU = zeU \qquad (5)$$

Where q is the charge of an ion which z number of charges e. e is elementary charge and U is the high voltage applied to the target. Because of the conservation of energy, after acceleration the potential energy is tranferred into kinetic energy

$$E_{kin} = 1/2\ m\ v2 \qquad (6)$$

From the constant movement in an field-free region we know that the speed v is the distance L divided by the time t_{drift}. Replacing v in (6) by s/t_{drift} we get (as already shown earlier)

$$t_{drift} = L\ \sqrt{(m/2zeU)} \qquad (7)$$

MALDI-TOF: Calibration

Combining of equation (4) – (7) the total time-of-flight is

$$t_{of} = t_{delay} + t_{acc} + t_{drift} = t_{delay} + d\ \sqrt{(2m/Uze)} + L\ \sqrt{(m/2zeU)}$$

TOF equation!

$$t = C_0 + C_1\sqrt{m}$$ **(Linear Mode)**

$$t = C_0 + C_1\sqrt{m} + C_2 m$$ **(Quadratic or cubic enhanced)**

Advanced reading: Calibration: Quadratic, linear and one-point calibration;

The determination of C_0 and C_1 is carried out by means of two signals of known standard masses m_1 and m_2.

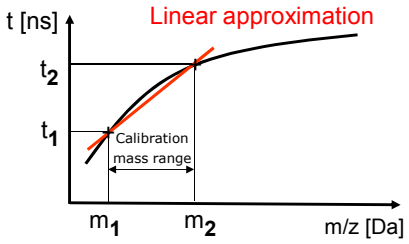
MALDI-TOF: Calibration

By measuring the time-of-flight t_1 and t_2 of two known masses m_1 and m_2 in a mass spectrum, C_0 and C_1 can be determined:

$$t_1 = C_0 + C_1\sqrt{m_1}$$

$$t_2 = C_0 + C_1\sqrt{m_2}$$

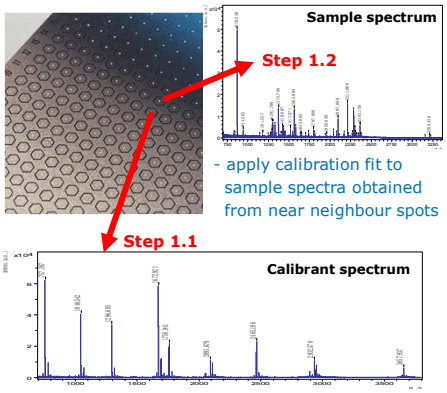
$\Rightarrow$ C_0, C_1



For a sufficiently narrow mass range, a linear approximation of t_{of} may be sufficient in terms of calibration accuracy (2 known calibrant masses are required here). However, for the calibration of an extended mass range, a quadratic polynom provides a much better description of the relationship between flight time and m/z. Minimum 4 known calibrant masses are required when applying a quadratic calibration function!

MALDI-TOF: Calibration strategies

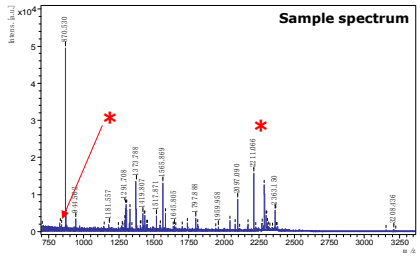
Step 1) External calibration



- apply calibration fit to sample spectra obtained from near neighbour spots

- calibrants of known mass cover mass range of interest
- m/z vs. flight time is fitted using a polynom of varying order (depending on size of mass range to be calibrated and number of available calibrant signals, resp.)

Step 2) Internal re-calibration (optionally)



* denotes compounds of known identity/mass
842.509 Da (trypsin artefact)
2211.104 Da (trypsin artefact)

Internal re-calibration allows for

- optimum mass accuracy due to compensation of spot-to-spot heterogeneities that typically cause mass errors after external calibration

MALDI-TOF: Calibration strategies

When to use which calibration polynom?

Compass for Flex series 1.3 or higher:

	Min. number of calibrant peaks	Calibration polynom to be used
external calibration:	2 4 6	linear quadratic cubic enhanced
internal re-calibration: (external pre-calibration: quadratic)	1 4 6	linear correction quadratic cubic enhanced
internal re-calibration: (external pre-calibration: cubic enhanced)	1 4	linear correction cubic enhanced

Note: For optimum mass accuracy, calibrants in general have to cover the entire mass range that is to be calibrated. Extrapolation of calibration functions will always have a negative effect on the resulting mass accuracy.

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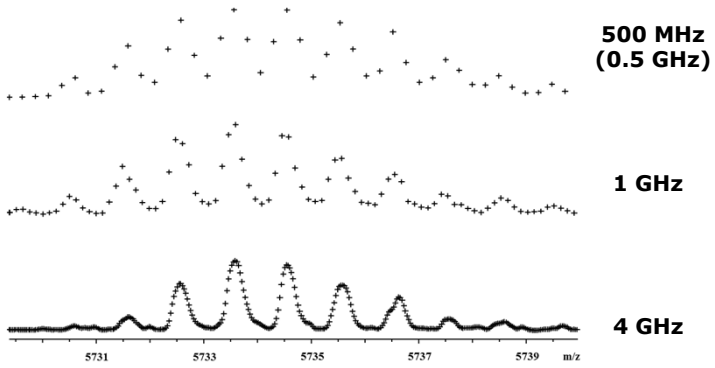
Mass Accuracy – Digitization Rate

How many data points are required to see peak features?

Bovine Insulin collected with 3 different digitization rates



Comparison of Peptide Mass Accuracy from 0.5, 1 and 2 GHz data

	0.5 GHz	1 GHz	2 GHz
Bradykinin	29 ppm	4.3 ppm	2.8 ppm
Angiotensin I	14.8 ppm	2.0 ppm	1.7 ppm

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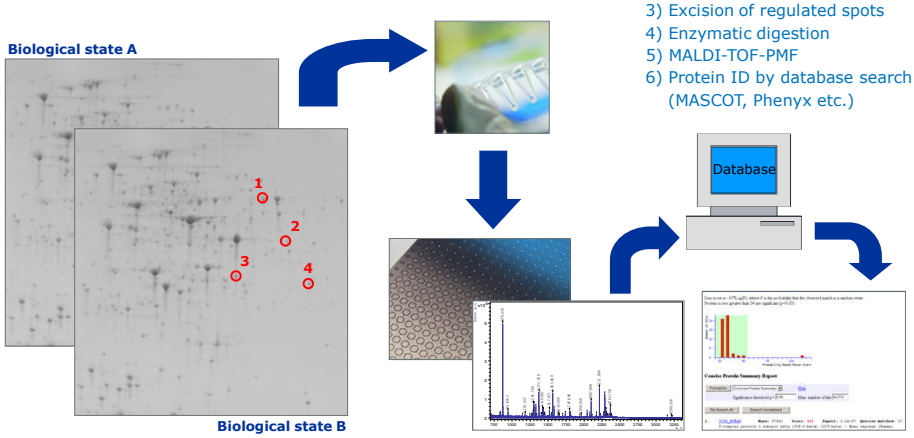
MALDI-TOF: Typical applications

Gel-based proteomics:

Protein ID by peptide mass fingerprinting (PMF)

- reflectron MALDI-TOF
- mass range: 700 … 4000Da

1) 2D gel separation of proteins
2) Comparative image analysis
3) Excision of regulated spots
4) Enzymatic digestion
5) MALDI-TOF-PMF
6) Protein ID by database search (MASCOT, Phenyx etc.)



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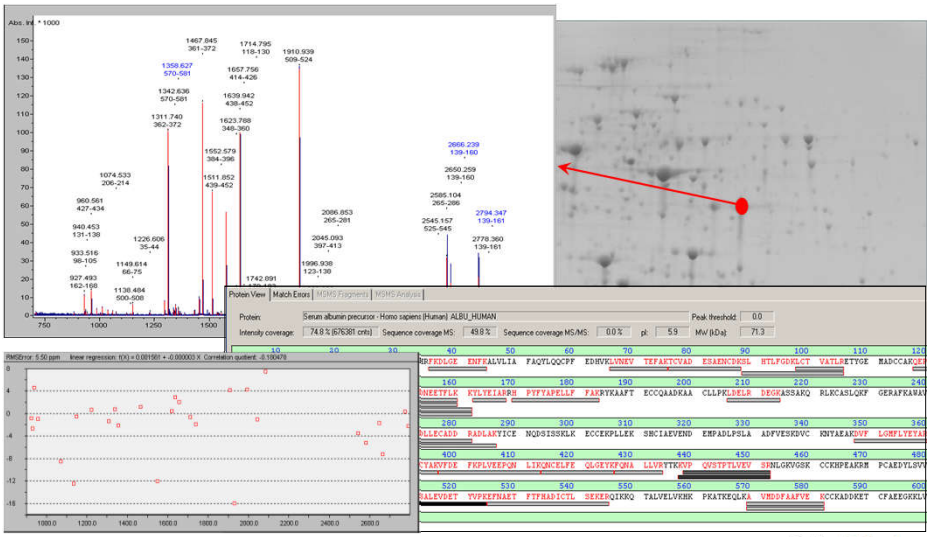
MALDI-TOF: Typical applications

Gel-based proteomics:

Protein ID by peptide mass fingerprinting (PMF)



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MALDI-TOF: Typical applications

Gel-based proteomics:

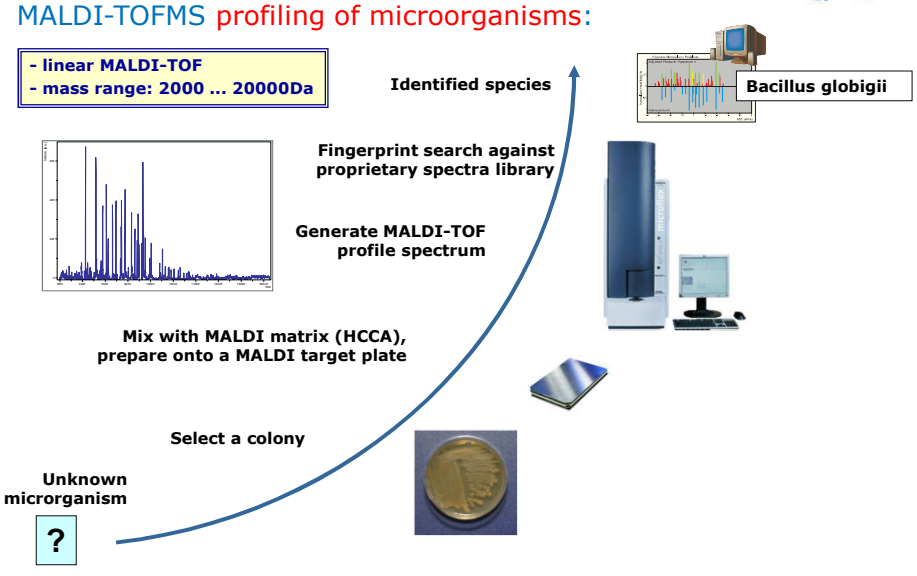
Protein ID by peptide mass fingerprinting (PMF)

Why is MALDI-TOF the method of choice here?

+ speed (1 gel spot = 3-15 seconds of measurement)
+ fully automatized measurement
+ REALLY high throughput
+ sensitivity (low amol range)
+ good mass accuracy and sufficiently high sequence coverage to yield highly significant results

MALDI-TOF: Typical applications

MALDI-TOFMS profiling of microorganisms:



MALDI-TOF: Typical applications

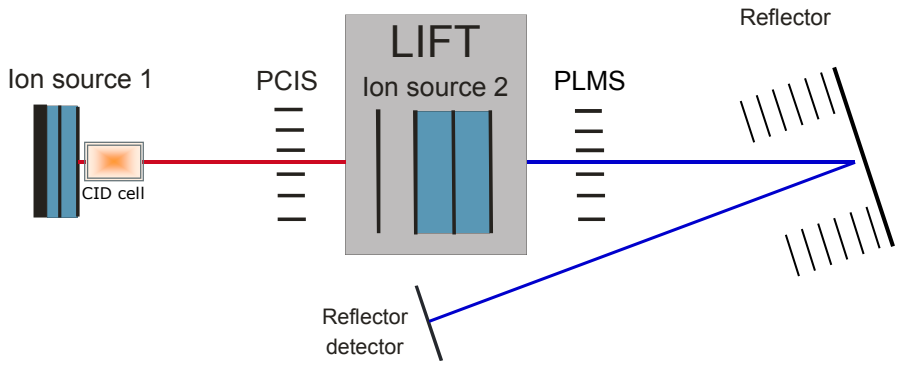
MALDI-TOFMS profiling of microorganisms:

+ analysis speed
  (5…15s per sample)
+ minimum sample prep
+ fully automated
+ highly significant results

MALDI-TOF/TOF

MALDI-TOF/TOF:

Principal scheme:



Ion path in TOF1 region (linear TOF)
Ion path in TOF2 region (reflector TOF)

Ion source 1 = MALDI ion source
Ion source 2 = LIFT re-acceleration cell
PCIS = Timed ion gate
PLMS = Post LIFT metastable suppressor

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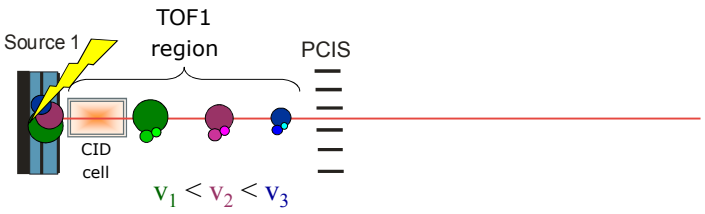
MALDI-TOF/TOF:

Analysis of a mixture containing 3 compounds (**green**,**red**, **blue**) being different in mass:



Molecular ions are separated in TOF1 according to their mass.
Part of the ions undergo fragmentation in TOF1 region induced by:
- metastable laser induced decay (**LID**)
- collision induced decay (**CID**)

Most important:
Fragments and their precursor ions travel at the same velocity.

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MALDI-TOF/TOF tandem MS:
LID vs. **CID**

LID: Laser-Induced Dissociation

Most straightforward way to peptide backbone fragmentation (b,y-type ions).
Used for protein identification by means of peptide sequencing.

CID: Collision-Induced Dissociation (high energy)

Additional side chain cleavages. Higher relative intensity of internal fragments. Overall shift of average fragment size towards lower mass.

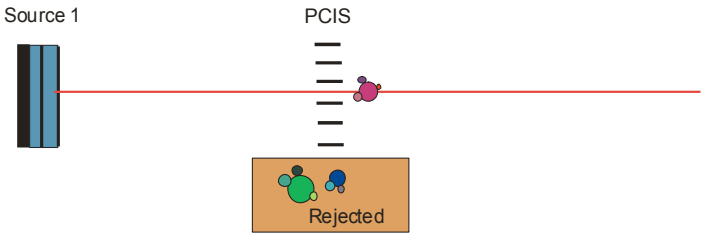
Used as an option in special applications, e.g.:

- *denovo sequencing* (enhanced immonium ions)
- *differentiation* of isobaric aminoacids *L and I* by respective side chain cleavages
- *detailed glycan analysis* (monomer linkage positions determined by products of specific crossring cleavages)

MALDI-TOF/TOF tandem MS:

Analysis of a mixture containing 3 compounds (**green**,**red**, **blue**) being different in mass:

Selecting the **red** precursor ion for fragment analysis by MS/MS:

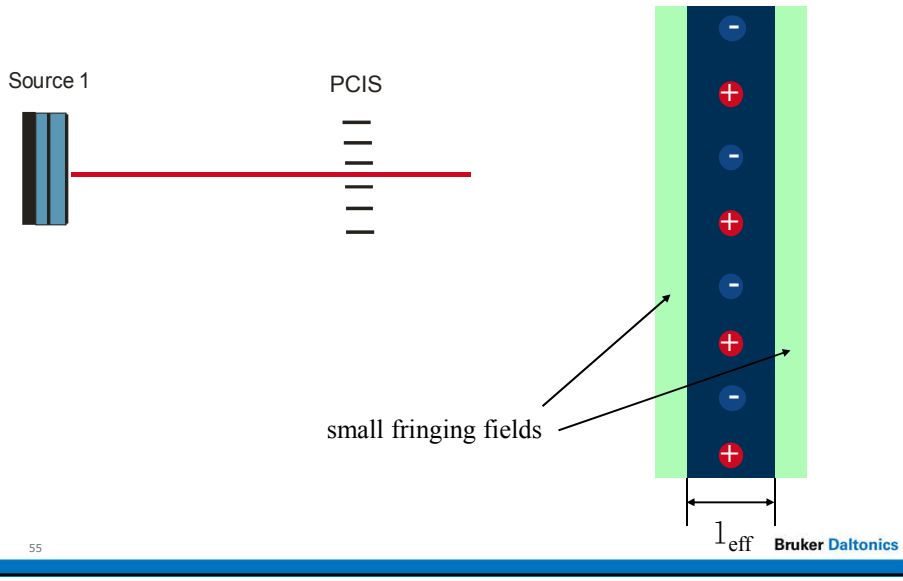


PCIS = Timed Ion Gate
(calibrated for time/mass correlation)

MALDI-TOF/TOF tandem MS:

PCIS = Timed Ion Gate

Bradboury-Niellson



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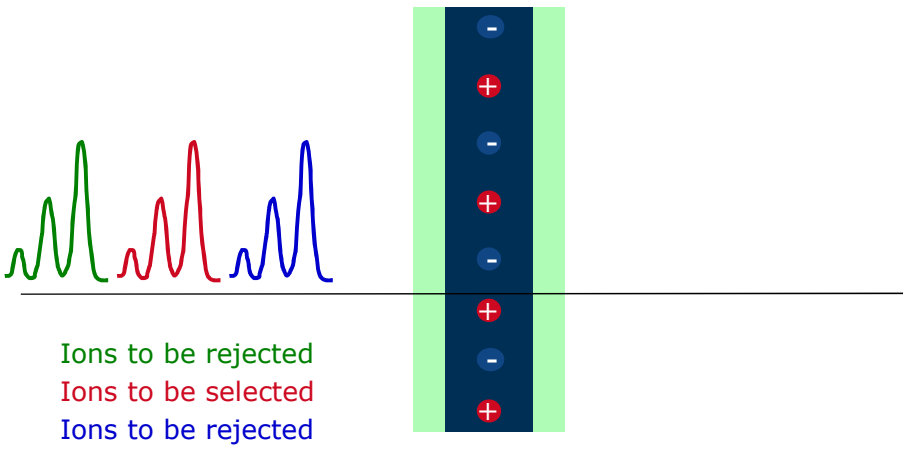
MALDI-TOF/TOF tandem MS:

PCIS = Timed Ion Gate



Ions to be rejected
Ions to be selected
Ions to be rejected

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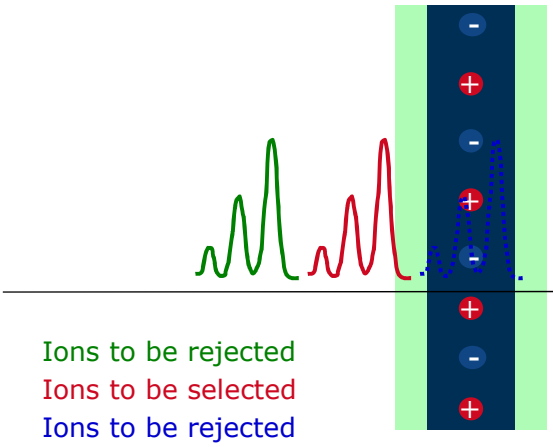
MALDI-TOF/TOF tandem MS:

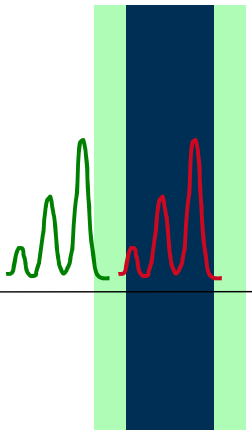
PCIS = Timed Ion Gate



Ions to be rejected
Ions to be selected
Ions to be rejected

MALDI-TOF/TOF tandem MS:

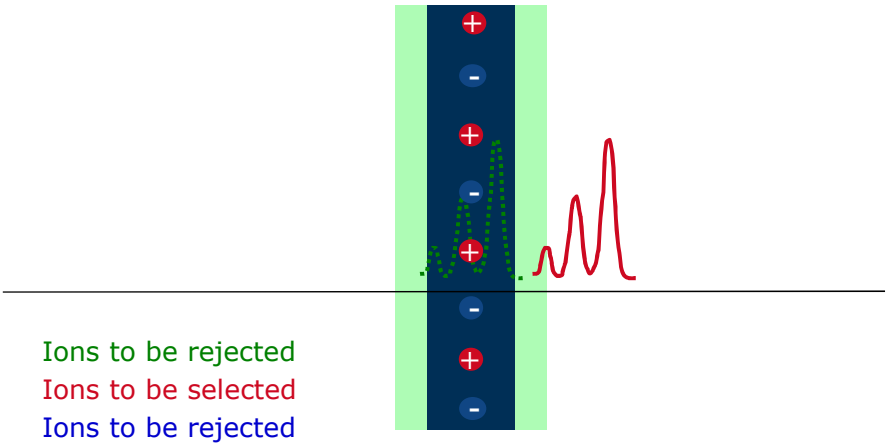
PCIS = Timed Ion Selector



Ions to be rejected
Ions to be selected
Ions to be rejected

MALDI-TOF/TOF tandem MS:

PCIS = Timed Ion Selector

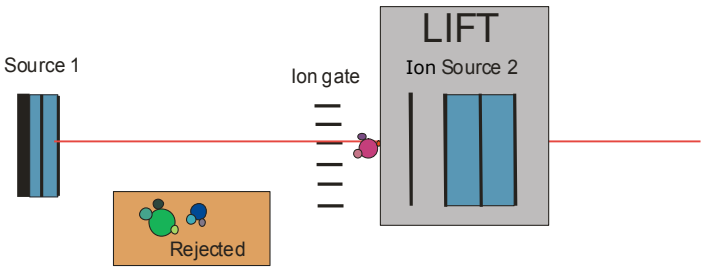


Ions to be rejected
Ions to be selected
Ions to be rejected

MALDI-TOF/TOF tandem MS:

Analysis of a mixture containing 3 compounds (**green**,**red**, **blue**) being different in mass:

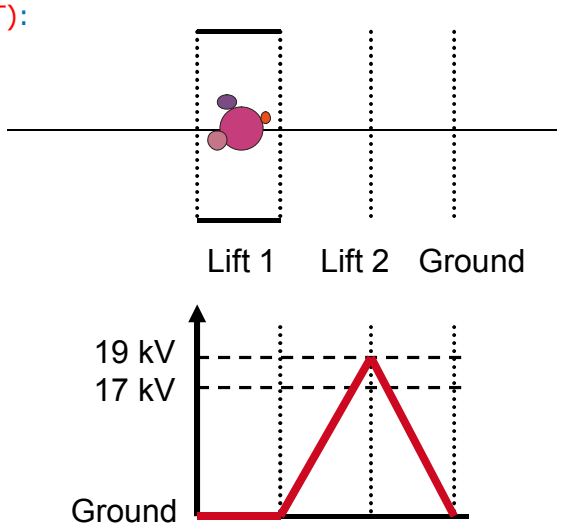
Red precursor ion selected for fragment analysis by MS/MS:



Ion Source 2 (LIFT):
Re-acceleration of fragments and remaining molecular ions.
Re-focusing (resolution!!!) of the ions.

MALDI-TOF/TOF tandem MS:

Reacceleration and focusing potentials applied in the Ion Source 2 (LIFT):



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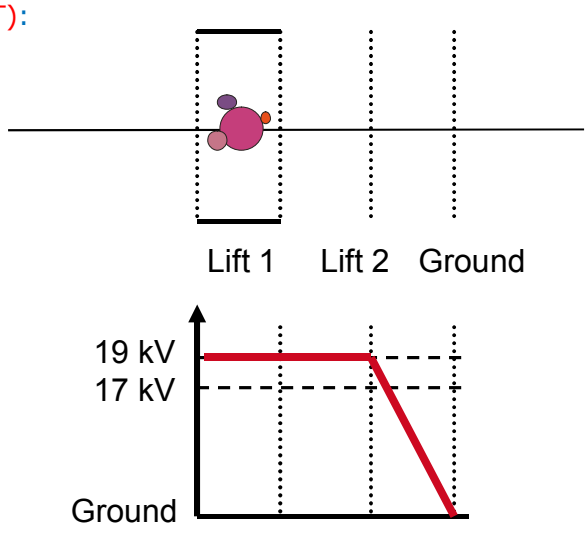
MALDI-TOF/TOF tandem MS:

Reacceleration and focusing potentials applied in the Ion Source 2 (LIFT):



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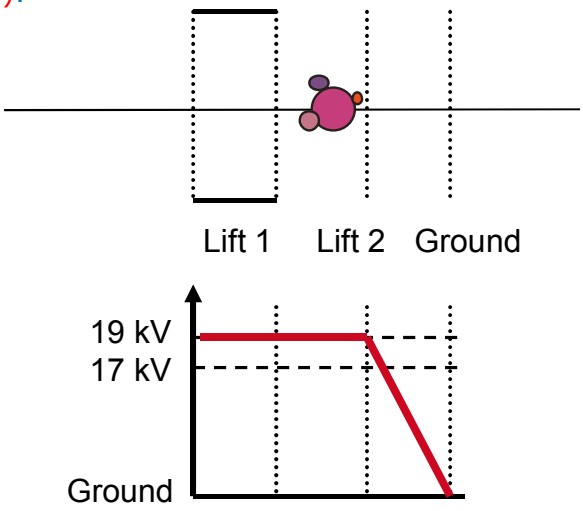
MALDI-TOF/TOF tandem MS:

Reacceleration and focusing potentials applied in the Ion Source 2 (LIFT):



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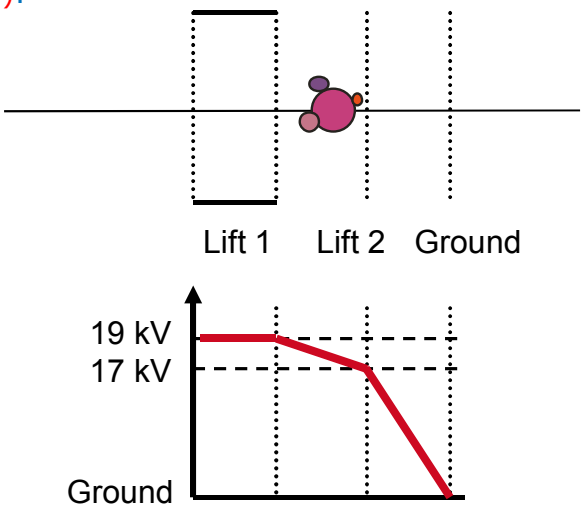
MALDI-TOF/TOF tandem MS:

Reacceleration and focusing potentials applied in the Ion Source 2 (LIFT):



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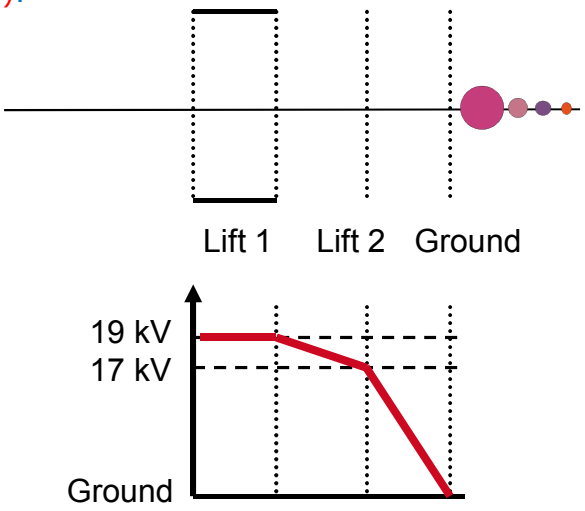
MALDI-TOF/TOF tandem MS:

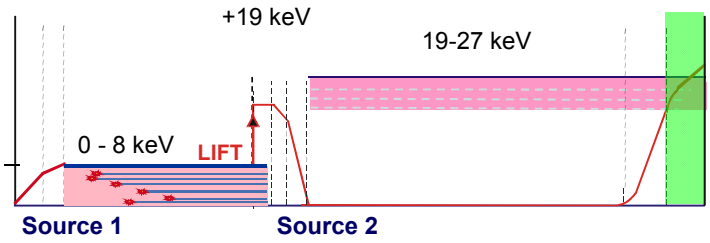
Reacceleration and focusing potentials applied in the Ion Source 2 (LIFT):



MALDI-TOF/TOF tandem MS:

FAQ: Why reacceleration of ions in Ion Source 2 (LIFT)?

Answer: To minimize the energy spread of the metastable fragments and, thus, to capture on the detector the complete fragment ion spectrum at one fixed reflector voltage.



A simple calculation example considering a precursor ion of $[M+H]^+$ = 1000Da:

Initial acceleration in source 1	= 8kV		
Precursor $[M+H]^+$	= 1000Da	$E_{kin\ precursor}$	= 8keV (**100%**)
Fragment f1 $[M+H]^+$	= 500Da	$E_{kin\ f1}$	= 4keV (**50%**)
Fragment f2 $[M+H]^+$	= 100Da	$E_{kin\ f2}$	= 0.8kV (**10%**)

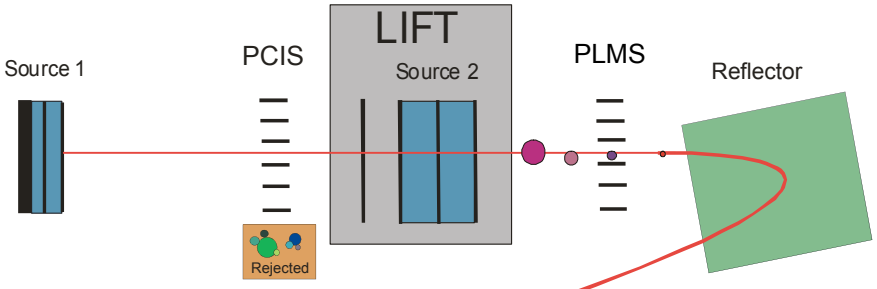
Reacceleration by 19kV narrows down this energy spread:

After reacceleration, even the smallest fragment ions have more than 70% of the energy of the precursor and, thus, will be captured on the detector.

$E_{kin\ precursor}$	= (8+19)keV = 27keV (**100%**)
$E_{kin\ f1}$	= (4+19)keV = 23keV (**85%**)
$E_{kin\ f2}$	= (0.8+19)kV = 19.8keV(**73%**)

MALDI-TOF/TOF tandem MS:

Analysis of a mixture containing 3 compounds (**green**,**red**, **blue**) being different in mass:



PLMS:

Post-LIFT Metastable Suppressor deflects remaining non-fragmented precursor ions to avoid undesired metastable decay in TOF2 region, which would yield ghost fragment peaks detected at wrong mass.

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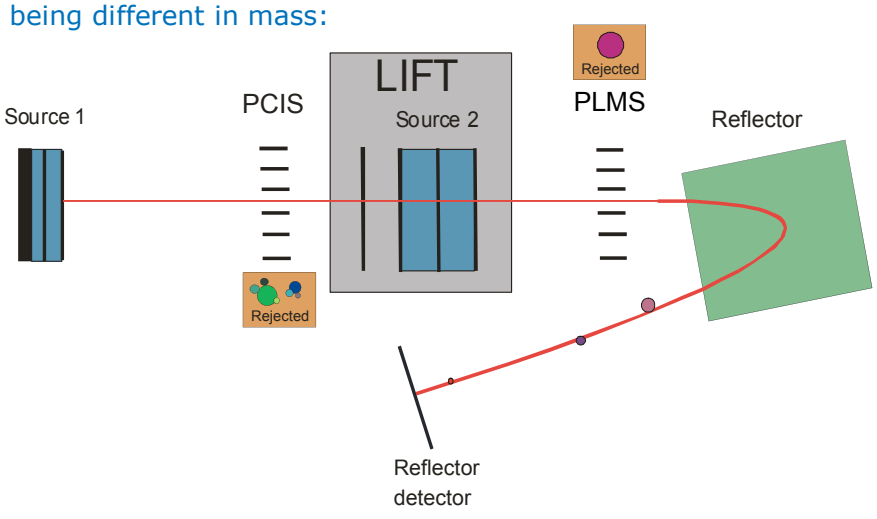
MALDI-TOF/TOF tandem MS:

Analysis of a mixture containing 3 compounds (**green**,**red**, **blue**) being different in mass:



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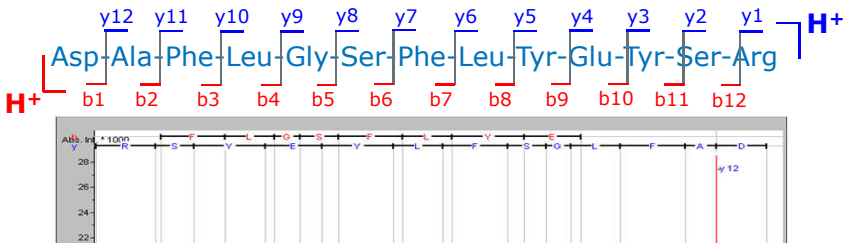
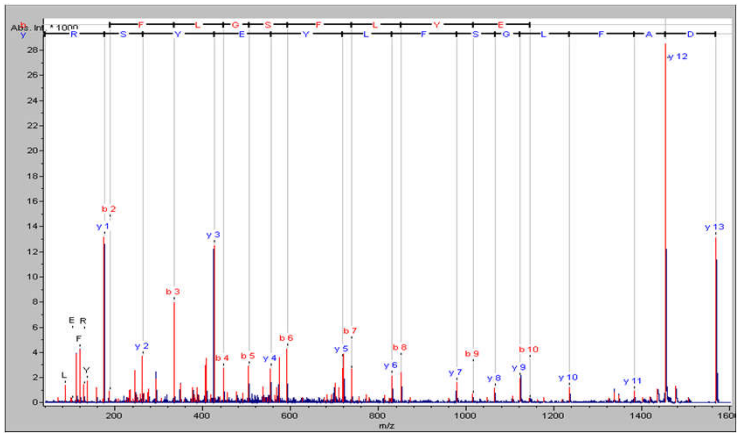
MALDI-TOF/TOF tandem MS:

Example MS/MS spectrum obtained from a peptide:





MALDI-TOF/TOF: Typical applications

Gel-based proteomics:

Protein ID based on PMF + datadependent MS/MS

1) Acquire PMF.
2) Run PMF database search.
3) Data dependent MS/MS acquisition

Successful protein ID by PMF

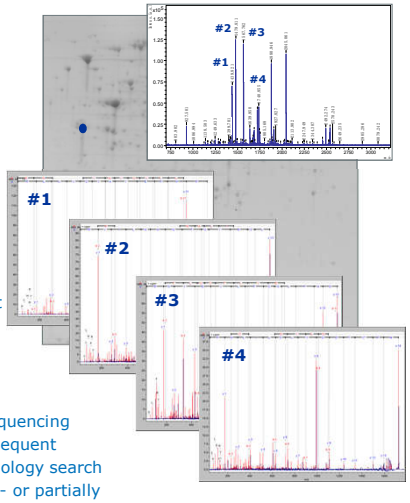
- acquire MS/MS of matching peptides for confirmation of protein ID
- acquire MS/MS of non-matching peptides (identify further proteins, unexpected modifications etc.)

PFF database search (combined MS/MS data)

Protein ID by PMF failed

- acquire MS/MS of most abundant peptides

DeNovo sequencing with subsequent MS BLAST homology search (in case of non- or partially sequenced taxa)



MALDI-TOF/TOF: Typical applications

Gel-based proteomics:

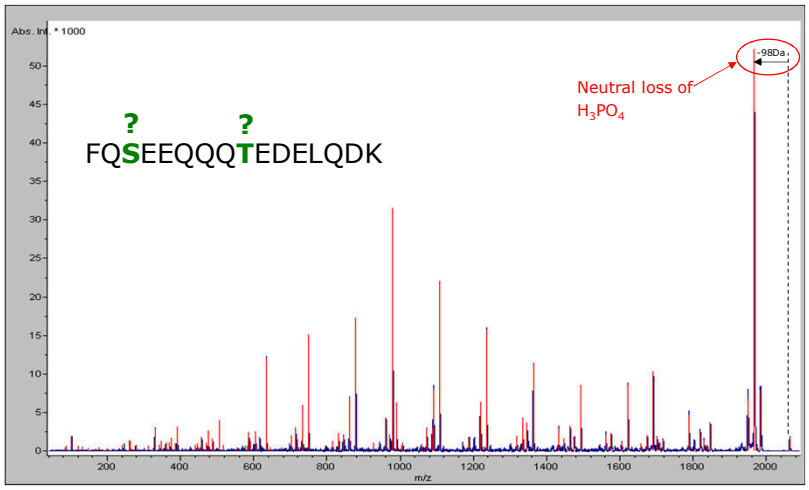
Protein ID based on PMF + datadependent MS/MS

Advantages of MALDI-TOF/TOF in gel-based proteomics:

+ speed (PMF + PFF analysis typically < 2min measuring time per gel spot)
+ amol sensitivity
+ automated, data dependent MS/MS workflow
+ improved significancy of combined (PMF+PFF) datasets compared to PMF only data

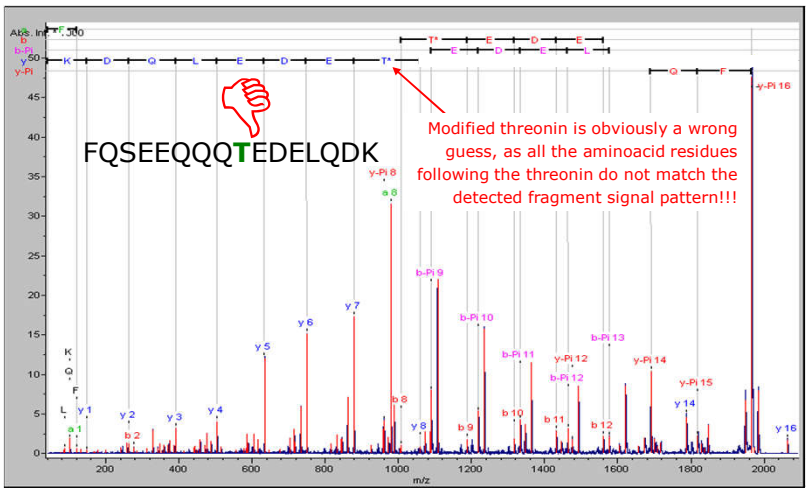
MALDI-TOF/TOF: Typical applications

Analysis of posttranslational modifications: Phosphorylation



MALDI-TOF/TOF: Typical applications

Analysis of posttranslational modifications: Phosphorylation



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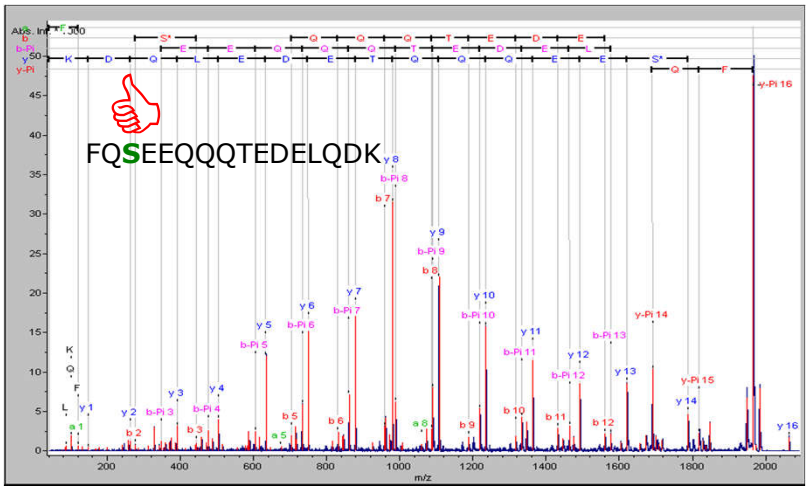
MALDI-TOF/TOF: Typical applications

Analysis of posttranslational modifications: Phosphorylation



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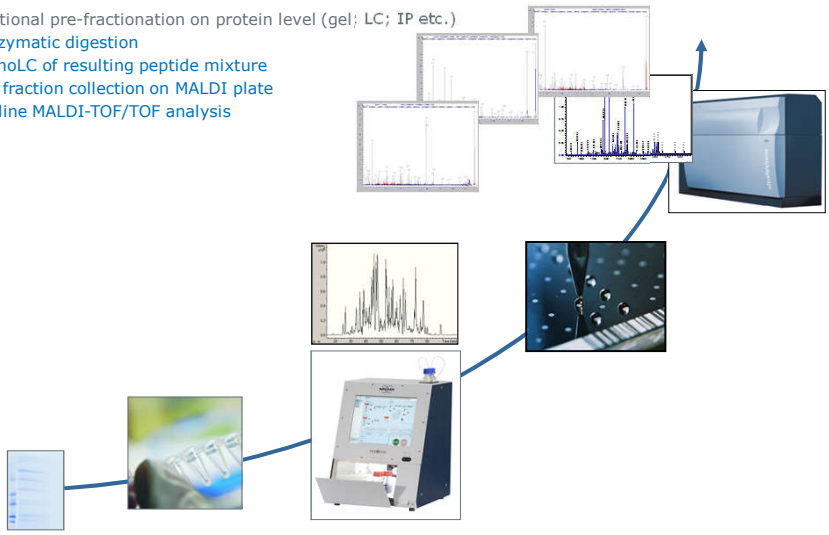
MALDI-TOF/TOF: Typical applications

LC-based proteomics: LC-MALDI analysis of complex samples

1) Optional pre-fractionation on protein level (gel; LC; IP etc.)
2) Enzymatic digestion
3) nanoLC of resulting peptide mixture
4) LC fraction collection on MALDI plate
5) offline MALDI-TOF/TOF analysis



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MALDI-TOF/TOF: Typical applications

LC-based proteomics: LC-MALDI analysis of complex samples

+ reduced ion suppression due to separation upfront to the mass spectrometer
+ no time constraints during data acquisition (no time pressure caused by LC speed)
+ increased depth of analysis (more protein IDs from complex mixtures; increased sequence coverage for individual proteins)
+ data dependent, non-redundant MS/MS precursor selection (esp. useful for non-isobaric SILE experiments)
+ LC separation is „frozen" on the MALDI plate and, thus, allows archiving of LC separated samples for later re-analysis
- a single LC-MS/MS experiment, when compared to online-ESI, is rather time consuming

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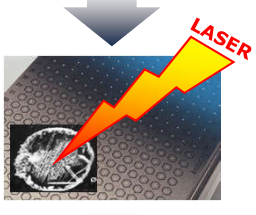
MALDI-TDS:

Top-down sequencing of intact proteins by means of MALDI-TOF/TOF

MALDI-TDS:

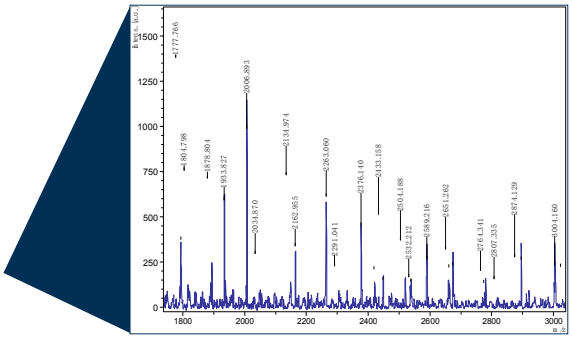
Top-down sequencing of intact proteins

+ MALDI matrix (1,5-DAN, sDHB)



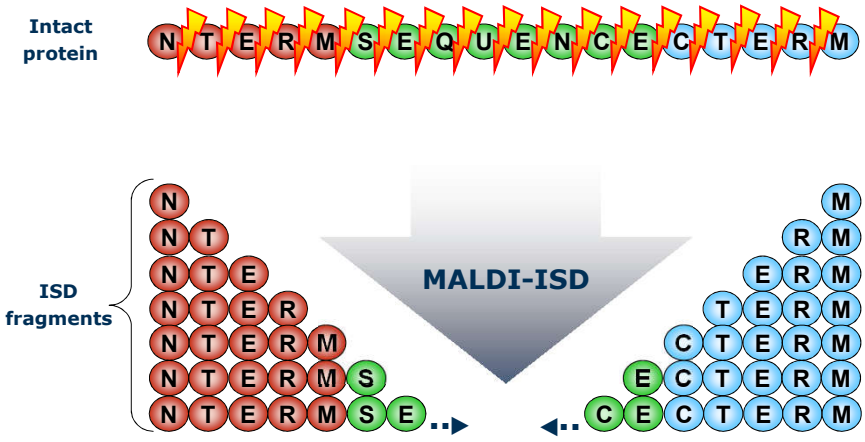
Bruker MALDI-TOF

In-Source Decay (ISD) upon MALDI process:



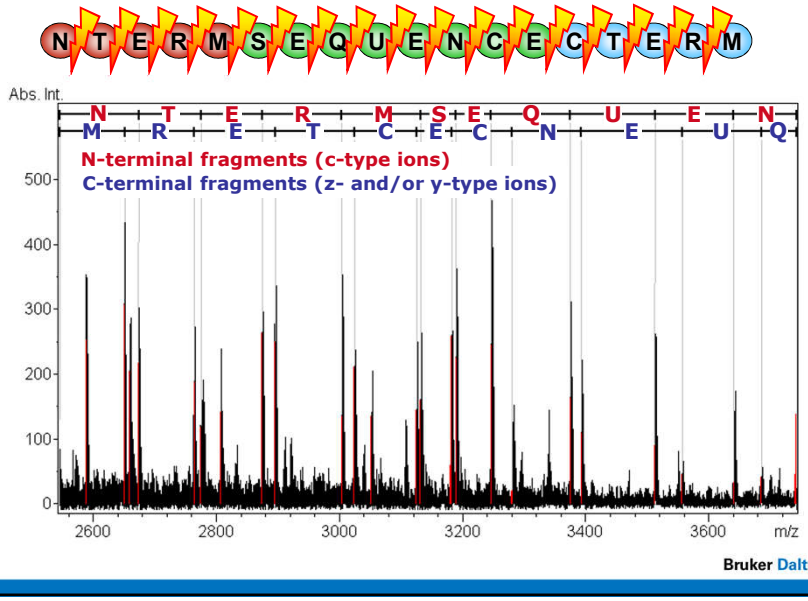
MALDI-TDS:

Top-down sequencing of intact proteins

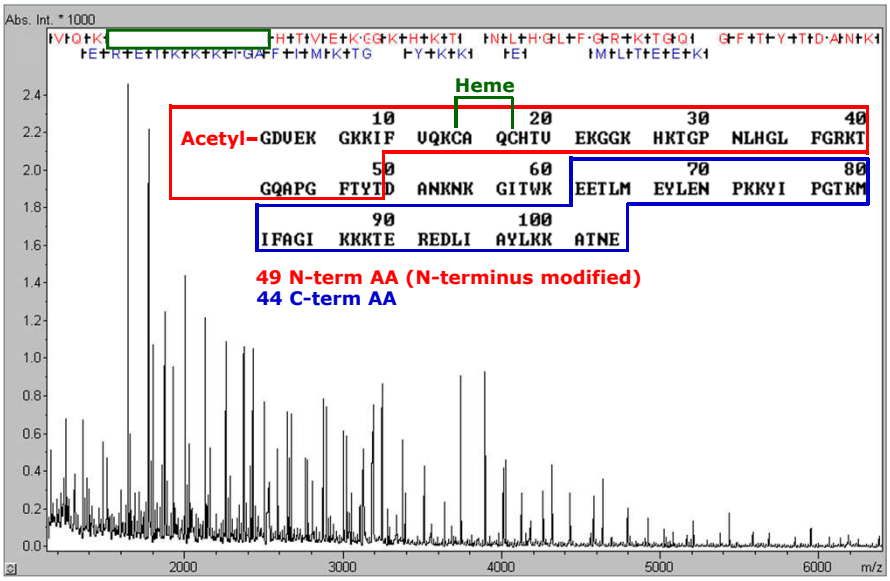


MALDI-TDS:

Top-down sequencing of intact proteins



MALDI-TDS:
MALDI-ISD-TOF spectrum of Cytochrome C (horse)

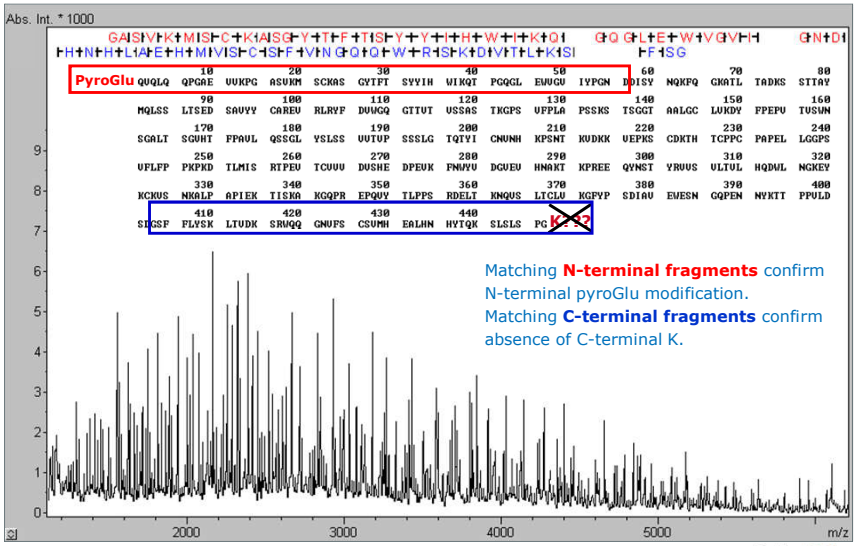


Spectrum acquired on **Bruker Ultraflex III TOF/TOF**

Bruker Daltonics

MALDI-TDS applications: Monoclonal antibodies

IgG heavy chain: Aim of analysis: - N-terminal pyroGlu???
- C-terminal lysine deleted???



Spectrum acquired on **Bruker Ultraflex III TOF/TOF**

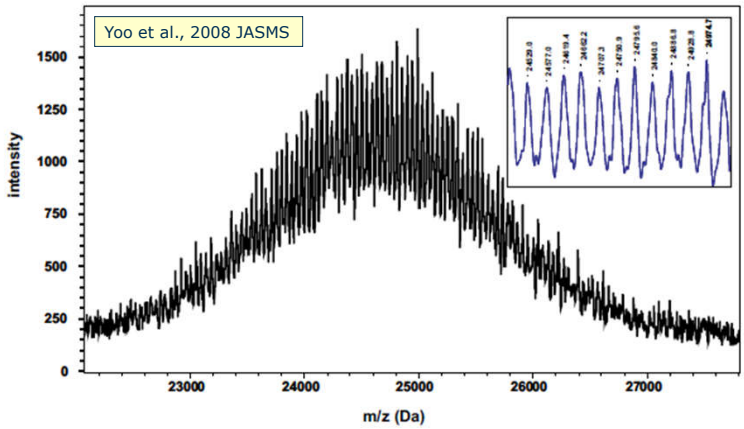
Bruker Daltonics

MALDI-TDS applications: PEGylated therapeutic peptides

Aim of analysis: Determination of PEGylation site

Peptide sequence: Acetyl-WVTHR*LAGLLSR*SGGVVK*KNFVPTDVGPXAF
PEG polymer: ~500mer (approx. 22kDa)

Linear MALDI-TOF spectrum showing MW distribution of the PEGylated peptide:



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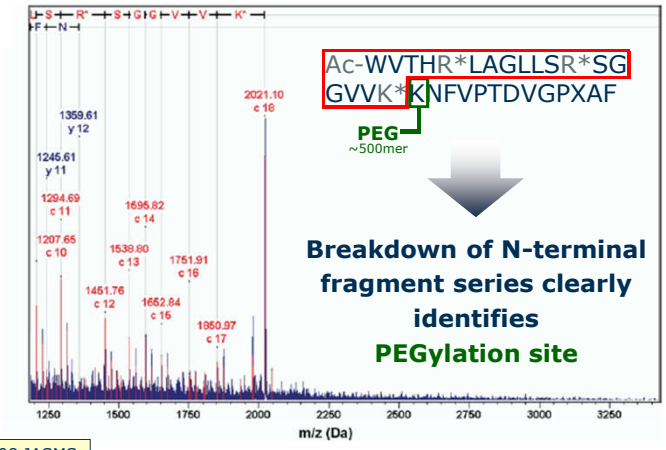
MALDI-TDS applications: PEGylated therapeutic peptides

Aim of analysis: Determination of PEGylation site

Peptide sequence: Acetyl-WVTHR*LAGLLSR*SGGVVK*KNFVPTDVGPXAF
PEG polymer: ~500mer (approx. 22kDa)

MALDI-ISD-TOF spectrum of the PEGylated peptide:



Yoo et al., 2008 JASMS

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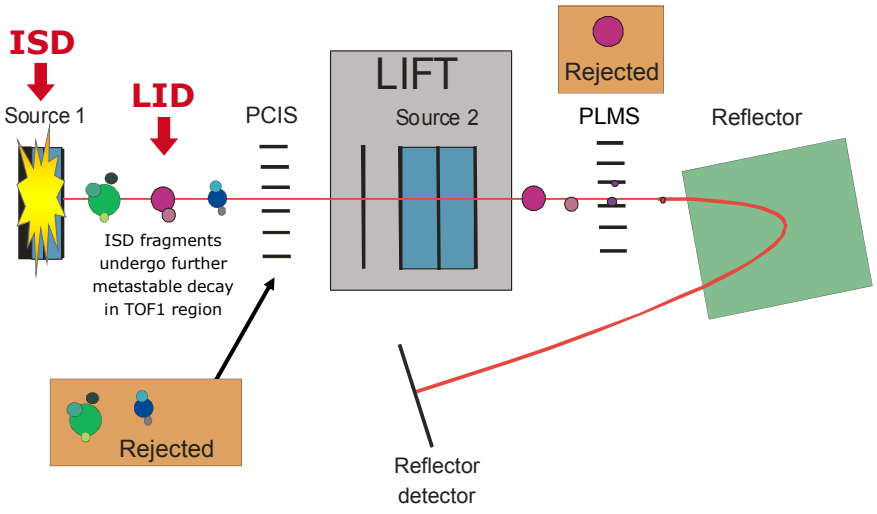
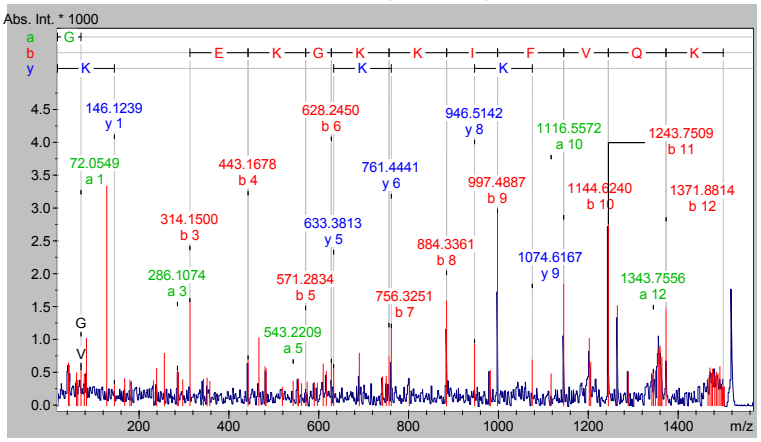
Advanced MALDI-TDS: T^3 sequencing: Further fragmentation (MS^3) of ISD fragments by LID

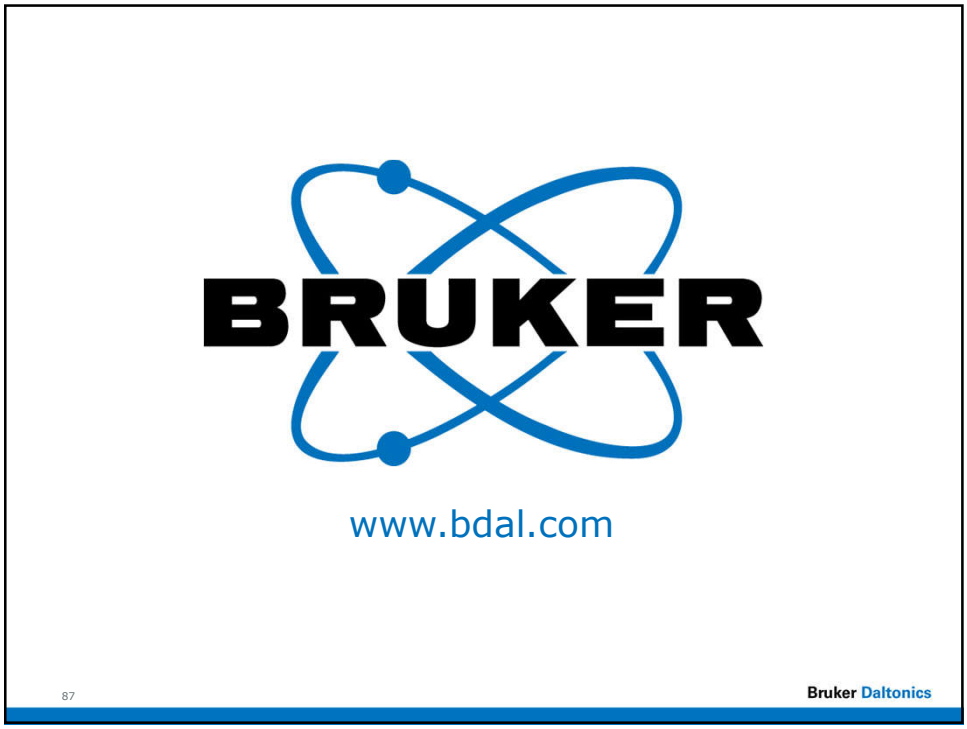


MALDI-T^3-TOF/TOF spectrum of ISD fragment c_{13} (m/z=1516.9Da) from Cytochrome C (horse)

Spectrum acquired on **Bruker ultrafleXtreme**



T^3 sequencing provides access to the very terminal aminoacid sequence, which is commonly not accessible by ISD (due to occurence of matrix clusters and other components occupying the low mass region in MS mode).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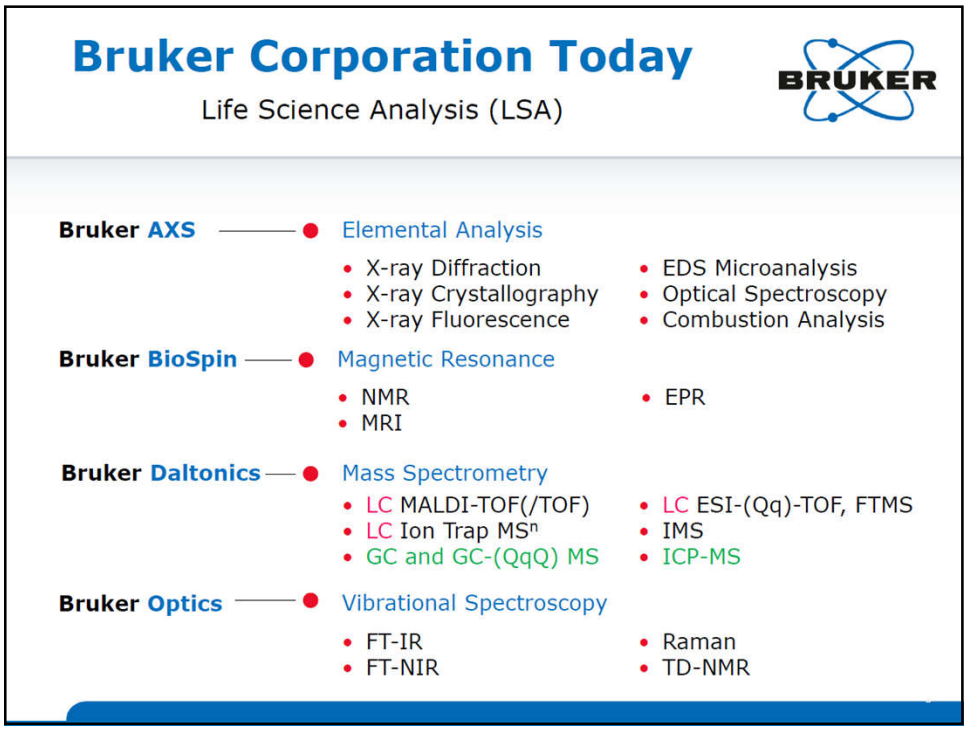
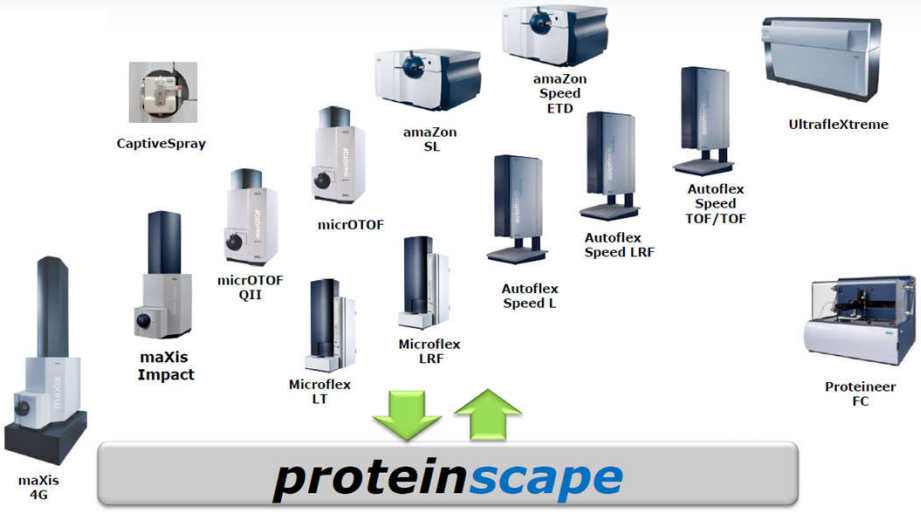
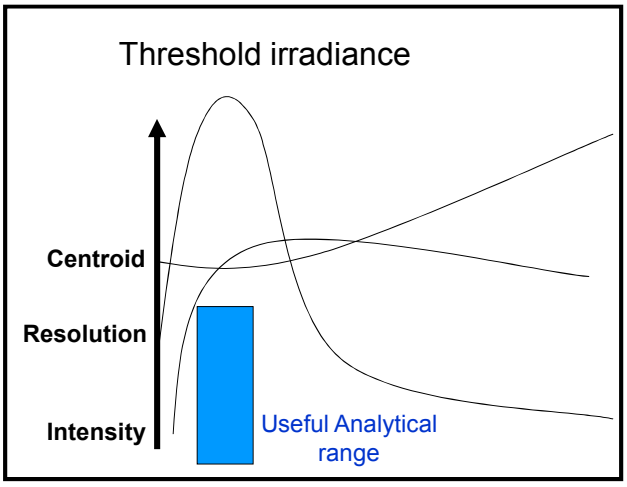

Bruker Corporation Today
Life Science Analysis (LSA)
BRUKER
Bruker AXS
Elemental Analysis
X-ray Diffraction
X-ray Crystallography
X-ray Fluorescence
EDS Microanalysis
Optical Spectroscopy
Combustion Analysis
Bruker BioSpin
Magnetic Resonance
NMR
MRI
EPR
Bruker Daltonics
Mass Spectrometry
LC MALDI-TOF(/TOF)
LC Ion Trap MSⁿ
GC and GC-(QqQ) MS
LC ESI-(Qq)-TOF, FTMS
IMS
ICP-MS
Bruker Optics
Vibrational Spectroscopy
FT-IR
FT-NIR
Raman
TD-NMR

PRIME

The Best of LC CSI-MS and LC MALDI-MS





Resolution, mass centroid, and intensity of a peak as a function of laser power.

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Reflector detector
The new reflector detector (auto-detector II) uses an electron multiplier instead of a multi channel plate (MCP). This electron multiplier has an shorter pulse width wich allows to achieve higher resolution.


Slide show

Linear detector
The new linear detector uses also an electron multiplier but with less resolution then the previous MCP. The advantage here is the higher sensitivity and no saturation effects.

The detector can be tilted on two axes (tip and turn). To control the stepper motors the board UTLMC (attached to the lift plate) was replaced by the board UTSCB.

Because the electron multipliers need more operarting voltage (up to 4000 V) than the MCPs (and also no gating is possible) the DSG module is replaced by two -5 kV power supplies.

Learn more about differences between electron multiplier and MCP

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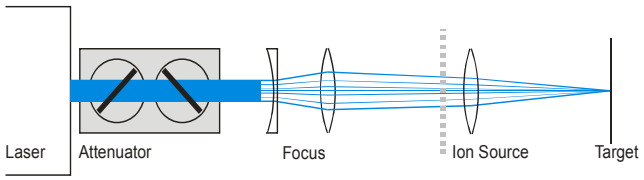
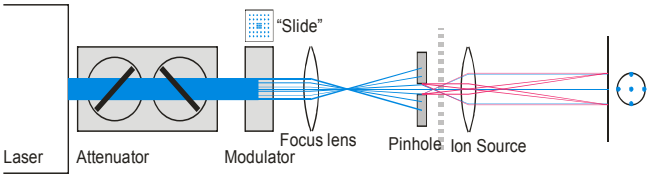
Traditional laser beam path



smartbeam™ laser beam path



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