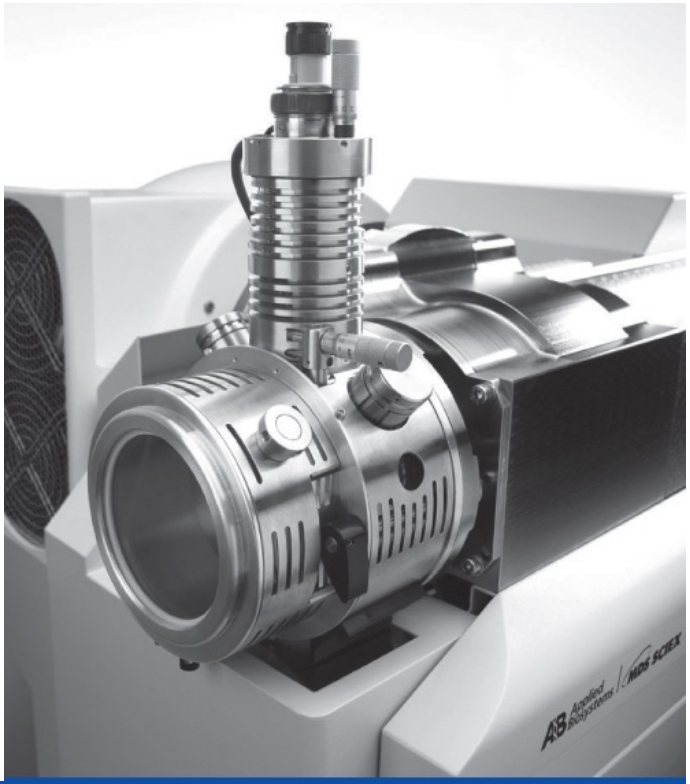


Applied Biosystems | MDS SCIEX

Introduction to the 4800 MALDI TOF/TOF™ Analyzer

Biology Proteomics Core Facility

Mass spectrometry:

- Principle
  - Measures molecular weight in ionic status according to mass/charge ratio (M/*z*)
  - Ion source: substance (peptides)
  - Ion separator: separation of ions in gas phase
  - Ion detector: very sensitive

- Methods of Ionization
  - Electro-Spray-Ionization(ESI) : ESI-MS, LC-ESI MS
  - Matrix-Assisted Laser Desorption and Ionization: MALDI-MS, LC-MALDI MS

- Ion Separator
  - Time-of-flight tube / quadruples / ion trap/

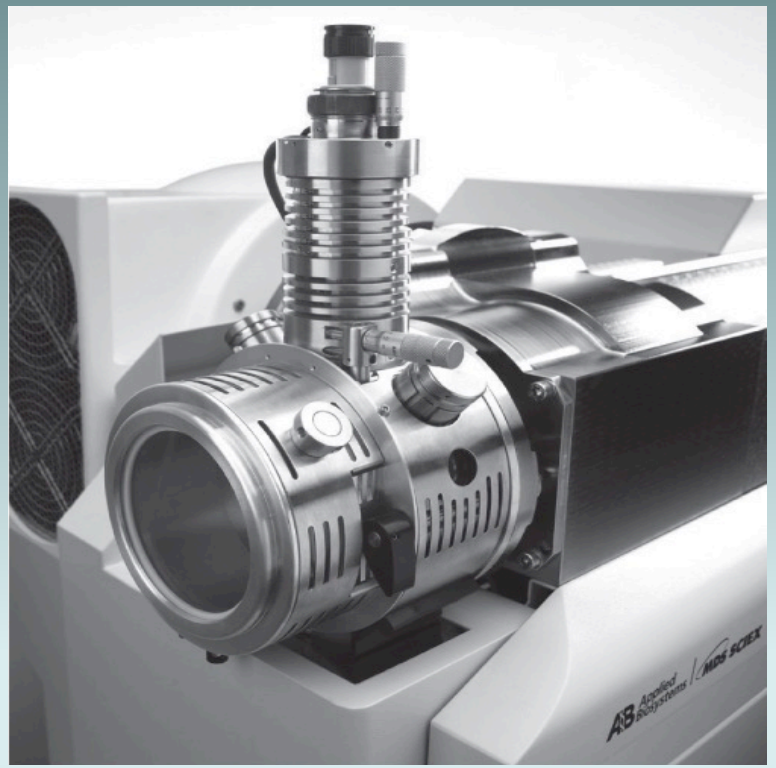
- Methods of Detection
  - Linear Detection
  - Reflective Detection

- Degree of MS dimension
  - MS-Tof (time of flight)
  - MS/MS-Tof/Tof (tandem MS)

Methods of Ionization

MALDI

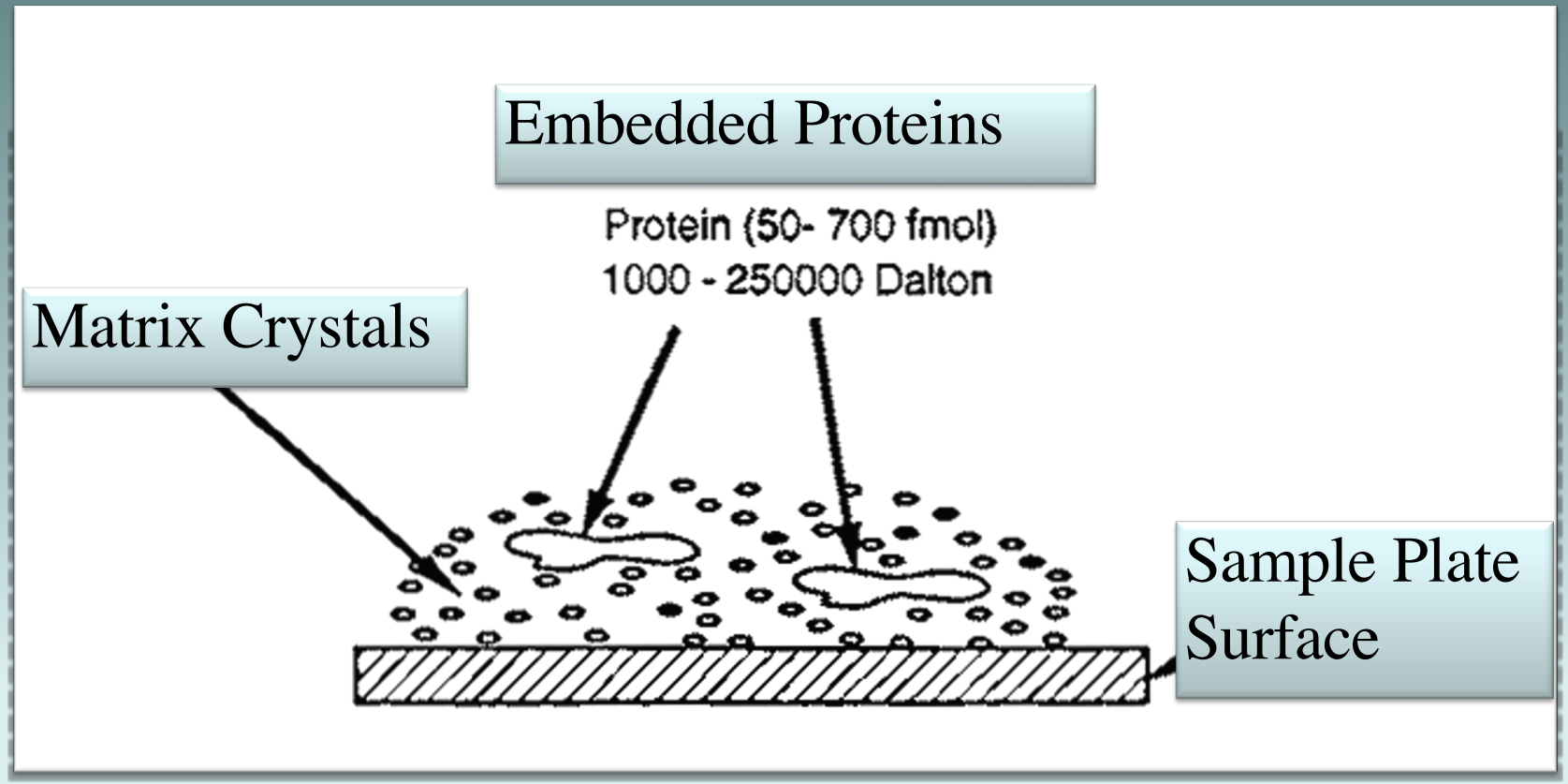
ESI



Matrix-Assisted Laser Desorption/Ionization (MALDI)

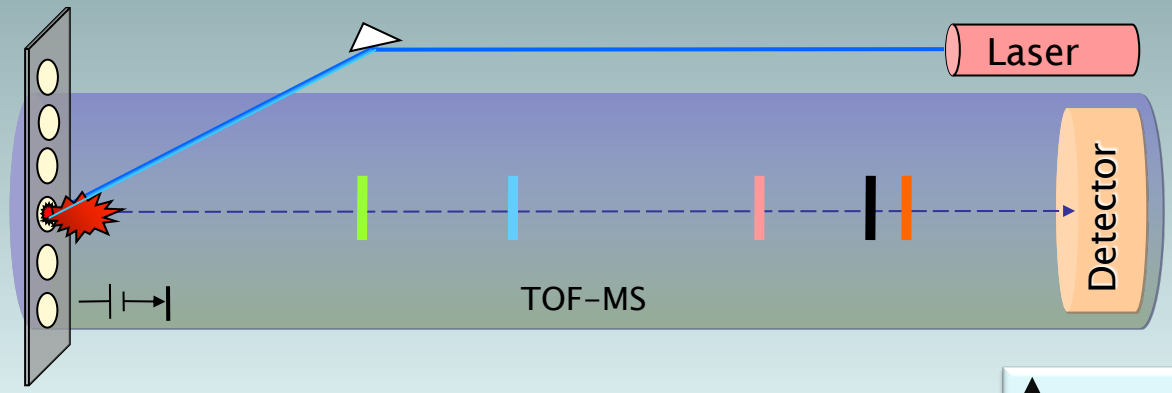
- Samples introduced to the MS on a passive probe
- Desorption/ionization method for non-volatile compounds
  - analyte embedded in a solid-state matrix crystal
- Matrix - energy absorbing compound responsible for converting laser energy to thermal energy, which facilitates the desorption/ionization process
- Discovered in late 1980s by groups in Germany and Japan
  - SHARES 2002 NOBEL PRIZE FOR APPLICATION OF LASER DESORPTION TO LARGE BIOMOLECULES

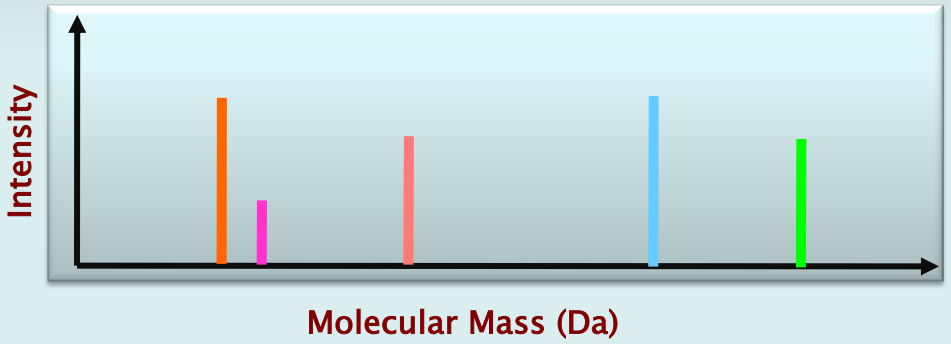
The MALDI-TOF Process



MALDI TOF-MS Detection

- Proteins are undergo Desorption/Ionization by Matrix and Laser
- Ionized proteins are detected and their mass accurately determined by Time-of-Flight Mass Spectrometry





Interpretation of Mass Spectra

- M/z: the mass-to-charge ratio

$$M/z = \frac{MW_{analyte} + \Sigma(MW_{cs})}{\Sigma \text{ Charge}}$$

cs = charging species

Ion Populations and Interpretation of Mass Spectra

- **Parent molecular ion:** $(M+H)^+$

  formed by adding a charged species (usually a proton)

- **Pseudomolecular ion:** $(M+2H)^{+2}$, $(M+Na)^+$, $(2M+H)^+$, $(2M-H+2Na)^+$

  formed by adding multiple protons; an ion formed by adding single or multiple non-proton charging species; an ion formed by clustering of parent molecular species; or a combination of the latter

How to apply Mass Spectra to Protein ID

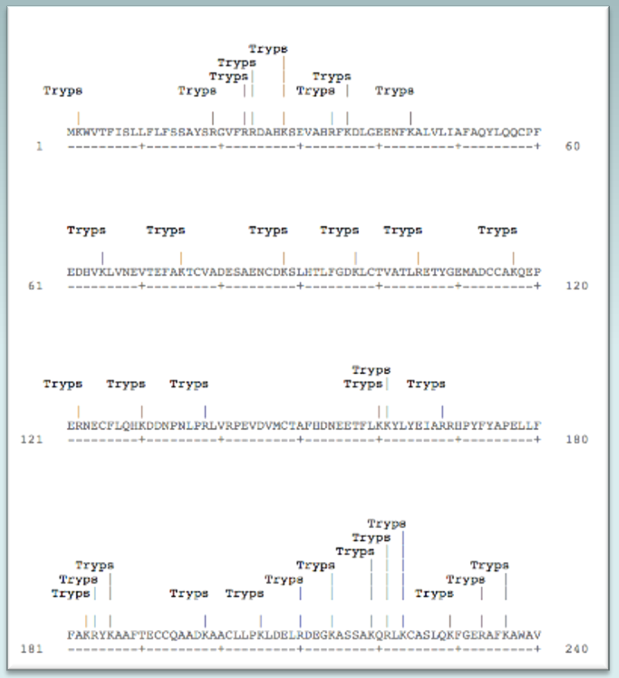
: Peptide Finger Printing

(Serum albumin)

Serum albumin precursor

The sequence to investigate:

```
        10         20         30         40         50         60
MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA FAQYLQQCPF

        70         80         90        100        110        120
EDHVKLVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP

       130        140        150        160        170        180
ERNECFLQHK DDNPNLPRLV RPEVDVMCTA FHDNEETFLK KYLYEIARRH PYFYAPELLF

       190        200        210        220        230        240
FAKRYKAAFT ECCQAADKAA CLLPKLDELR DEGKASSAKQ RLKCASLQKF GERAFKAWAV

       250        260        270        280        290        300
ARLSQRFPKA EFAEVSKLVT DLTKVHTECC HGDLLECADD RADLAKYICE NQDSISSKLK

       310        320        330        340        350        360
ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF LGMFLYEYAR

       370        380        390        400        410        420
RHPDYSVVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE FKPLVEEPQN LIKQNCELFE

       430        440        450        460        470        480
QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV

       490        500        510        520        530        540
LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL

       550        560        570        580        590        600
SEKERQIKKQ TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV

AASQAALGL
```

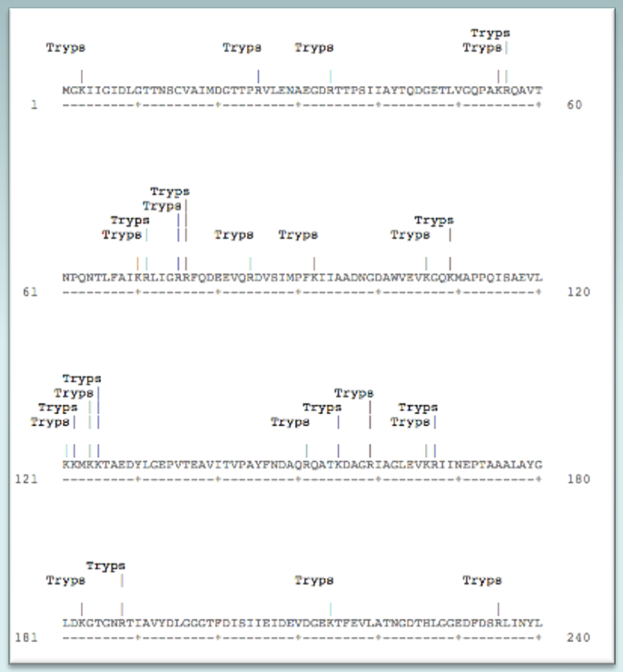


Heat shock protein, 70)

Chaperone protein DnaK (HSP70) (Heat shock 70 kDa protein) (Heat sho

The sequence to investigate:

```
        10         20         30         40         50         60
MGKIIGIDLG TTNSCVAIMD GTTPRVLENA EGDRTTPSII AYTQDGETLV GQPAKRQAVT

        70         80         90        100        110        120
NPQNTLFAIK RLIGRRFQDE EVQRDVSIMP FKIIAADNGD AWVEVKGQKM APPQISAEVL

       130        140        150        160        170        180
KKMKKTAEDY LGEPVTEAVI TVPAYFNDAQ RQATKDAGRI AGLEVKRIIN EPTAAALAYG

       190        200        210        220        230        240
LDKGTGNRTI AVYDLGGGTF DISIIEIDEV DGEKTFEVLA TNGDTHLGGE DFDSRLINYL

       250        260        270        280        290        300
VEEFKKDQGI DLRNDPLAMQ RLKEAAEKAK IELSSAQQTD VNLPYITADA TGPKHMNIKV

       310        320        330        340        350        360
TRAKLESLVE DLVNRSIEPL KVALQDAGLS VSDIDDVILV GGQTRMPMVQ KKVAEFFGKE

       370        380        390        400        410        420
PRKDVNPDEA VAIGAAVQGG VLTGDVKDVL LLDVTPLSLG IETMGGVMTT LIAKNTTIPT

       430        440        450        460        470        480
KHSQVFSTAE DNQSAVTIHV LQGERKRAAD NKSLGQFNLD GINPAPRGMP QIEVTFDIDA

       490        500        510        520        530        540
DGILHVSAKD KNSGKEQKIT IKASSGLNED EIQKMVRDAE ANAEADRKFE ELVQTRNQGD

       550        560        570        580        590        600
HLLHSTRKQV EEAGDKLPAD DKTAIESALT ALETALKGED KAAIEAKMQE LAQVSQKLME

       610        620        630
IAQQQHAQQQ TAGADASANN AKDDDVVDAE FEEVKDKK
```



(Serum albumin)

• Chain Serum albumin at positions 25 - 609 [Theoretical pI: 5.67 / Mw (average mass): 66472.21 / Mw (monoisotopic mass): 66428.93]

mass	position	#MC	modifications		peptide sequence
4089.8799	301-337	1			ECCEKPLLEKSHCIAEVEND EMPADLPSLAADFVESK
4037.8935	509-543	1	PHOS: 513 SUCC: 543	4117.8735 4137.9635	RPCFSALEVDETYVPKEFNA ETFTFHADICTLSEK
3626.8086	397-426	1			VFDEFKPLVEEPQNLIKQNC ELFEQLGEYK
3563.8606	45-75	1			ALVLIAFAQYLQQCPFEDHV KLVNEVTEFAK
3514.6729	131-160	1			DDNPNLPRLVRPEVDVMCTA FHDNEETFLK
3407.6108	384-413	1			CCAAADPHECYAKVFDEFKP LVEEPQNLIK
3365.6874	37-65	1			DLGEENFKALVLIAFAQYLQ QCPFEDHVK
3362.5224	311-341	1			SHCIAEVENDEMPADLPSLA ADFVESKDVCK
3059.4998	470-496	1			MPCAEDYLSVVLNQLCVLHE KTPVSDR
2917.3229	311-337	0			SHCIAEVENDEMPADLPSLA ADFVESK
2859.3474	500-524	1	PHOS: 513	2939.3274	CCTESLVNRRPCFSALEVDE TYVPK
2721.3374	139-161	1			LVRPEVDVMCTAFHDNEETF LKK
2686.2269	258-281	1			LVTDLTKVHTECCHGDLLEC ADDR
2593.2425	139-160	0			LVRPEVDVMCTAFHDNEETF LK
2560.2720	469-490	1			RMPCAEDYLSVVLNQLCVLH EK
2542.2758	414-434	1			QNCELFEQLGEYKFQNALLV R
2515.1326	66-88	1	PHOS: 82	2595.1126	LVNEVTEFAKTCVADESAEN CDK
2488.1449	525-545	1	SUCC: 543	2588.2149	EFNAETFTFHADICTLSEKE R
2433.2635	45-65	0			ALVLIAFAQYLQQCPFEDHV K
2414.0533	265-286	1			VHTECCHGDLLECADDRADL AK
2404.1709	470-490	0			MPCAEDYLSVVLNQLCVLHE K
2383.0540	76-97	1	PHOS: 82, 89	2543.0140	TCVADESAENCDKSLHTLFG DK
2347.0039	376-396	1			TYETTLEKCCAAADPHECYA K
2300.1056	342-360	1			NYAEAKDVFLGMFLYEYAR
2203.0012	525-543	0	SUCC: 543	2303.0712	EFNAETFTFHADICTLSEK
2177.9698	98-117	1			LCTVATLRETYGEMADCCAK
2045.0953	397-413	0			VFDEFKPLVEEPQNLIK
1959.7881	106-122	1			ETYGEMADCCAKQEPER
1953.9231	187-205	1			AAFTECCQAADKAACLLPK
1939.9079	123-138	1			NECFLQHKDDNPNLPR
1924.0862	439-456	1	PHOS: 443, 444,446	2164.0262	VPQVSTPTLVEVSRNLGK
1915.7731	265-281	0			VHTECCHGDLLECADDR
1898.9951	169-183	1			RHPYFYAPELLFFAK

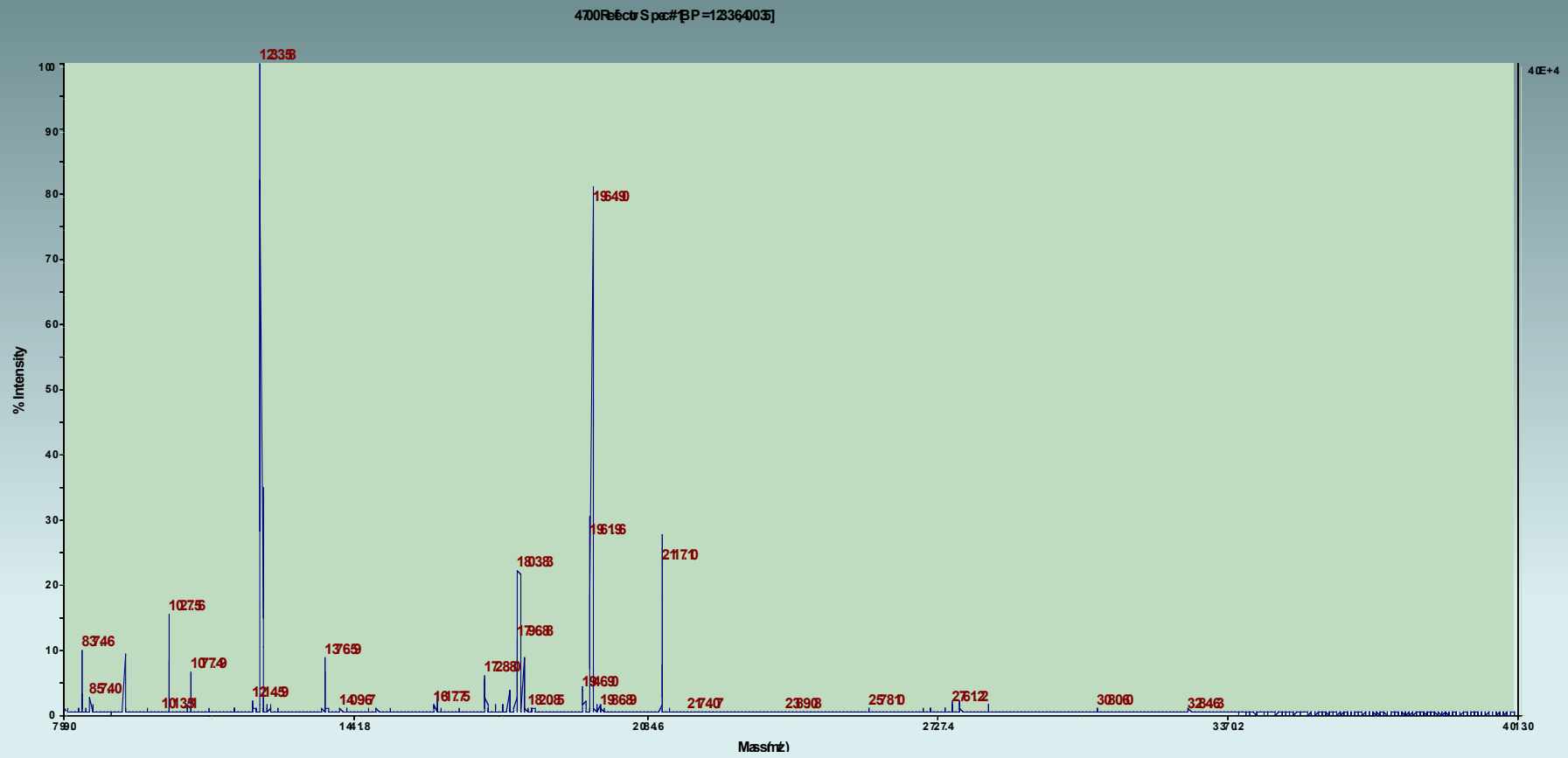
Heat shock protein, 70)

• Chain Chaperone protein DnaK at positions 2 - 638 [Theoretical pI: 4.83 / Mw (average mass): 68983.76 / Mw (monoisotopic mass): 68941.44]

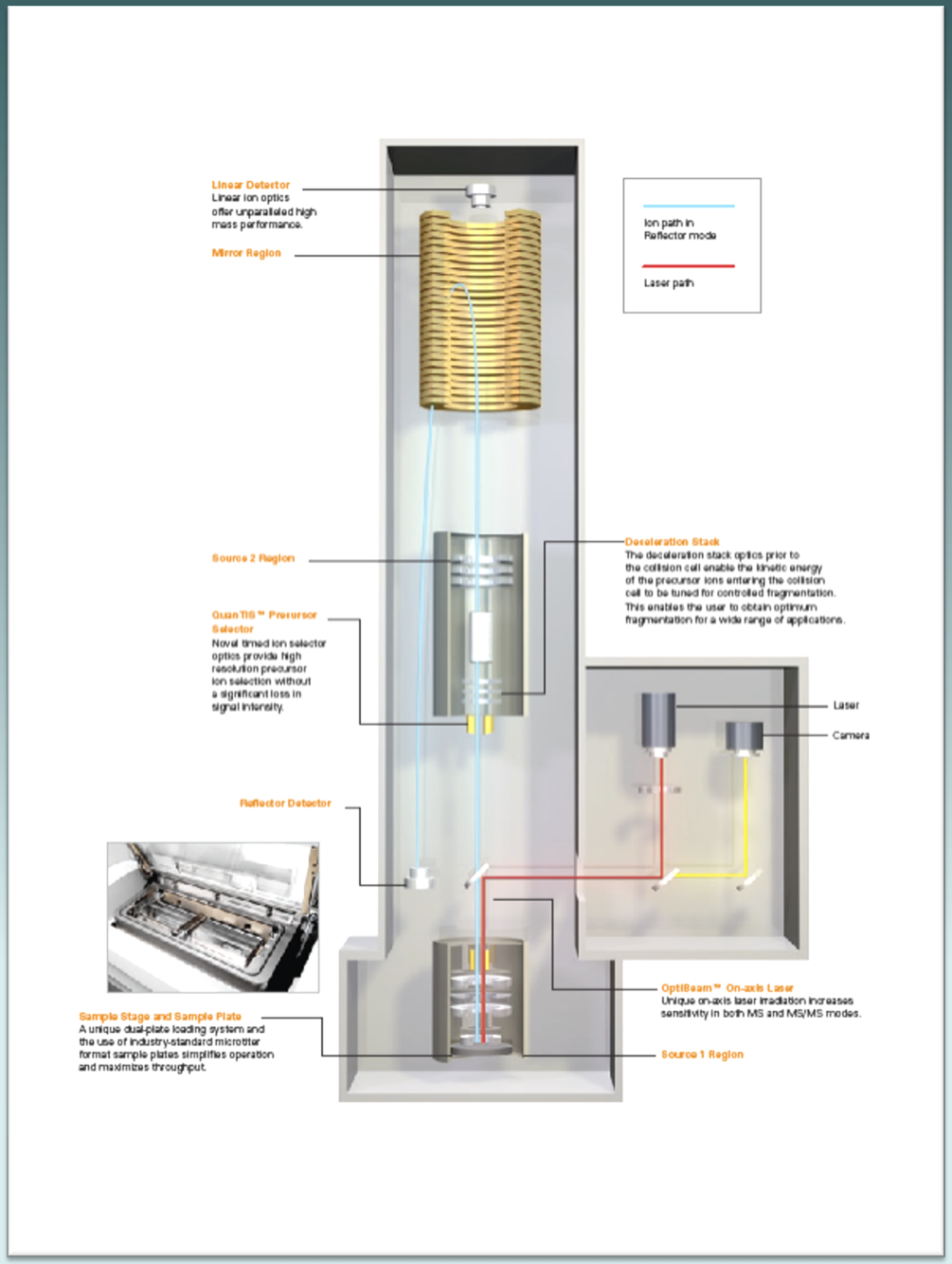
mass	position	#MC	modifications		peptide sequence
5076.7097	364-414	1			DVNPDEAVAIGAAVQGGVLT GDVKDVLLLDVTPLSLGIET MGGVMTTLIAK
5031.3904	189-235	1			TIAVYDLGGGTFDISIIEID EVDGEKTFEVLATNGDTHLG GEDFDSR
4114.8894	598-635	1			LMEIAQQQHAQQQTAGADAS ANNAKDDDVVDAEFEEVK
3935.9959	453-489	1			SLGQFNLDGINPAPRGMPQI EVTFDIDADGILHVSAK
3766.8235	588-622	1			MQELAQVSQKLMEIAQQQHA QQQTAGADASANNAK
3555.9627	388-421	1	ACET: 421	3598.0027	DVLLLDVTPLSLGIETMGGV MTTLIAKNTTIPTK
3529.7121	215-245	1	ACET: 245 SUCC: 245	3571.7521 3629.7821	TFEVLATNGDTHLGGEDFDS RLINYLVEEFK
3408.7142	415-445	1	ACET: 421	3450.7542	NTTIPTKHSQVFSTAEDNQS AVTIHVLQGER
3297.6273	126-155	1			TAEDYLGEPVTEAVITVPAY FNDAQRQATK
3254.6062	184-214	1			GTGNRTIAVYDLGGGTFDIS IIEIDEVDGEK
3231.5983	4-34	1			IIGIDLGTTNSCVAIMDGTT PRVLENAEGDR
3173.5960	26-55	1			VLENAEGDRTTPSIIAYTQD GETLVGQPAK
3155.6438	322-351	1			VALQDAGLSVSDIDDVILVG GQTRMPMVQK
3155.6041	271-299	1			IELSSAQQTDVNLPYITADA TGPKHMNIK
3108.6786	316-345	1			SIEPLKVALQDAGLSVSDID DVILVGGQTR
2997.4839	125-151	1			KTAEDYLGEPVTEAVITVPA YFNDAQR
2869.3890	126-151	0			TAEDYLGEPVTEAVITVPAY FNDAQR
2800.5449	388-414	0			DVLLLDVTPLSLGIETMGGV MTTLIAK
2781.3914	422-446	1			HSQVFSTAEDNQSAVTIHVL QGERK
2769.3716	189-214	0			TIAVYDLGGGTFDISIIEID EVDGEK
2731.4148	269-294	1			AKIELSSAQQTDVNLPYITA DATGPK
2653.2964	422-445	0			HSQVFSTAEDNQSAVTIHVL QGER
2624.2481	598-622	0			LMEIAQQQHAQQQTAGADAS ANNAK
2599.3072	468-491	1			GMPQIEVTFDIDADGILHVS AKDK
2532.2827	271-294	0			IELSSAQQTDVNLPYITADA TGPK
2441.2881	322-345	0			VALQDAGLSVSDIDDVILVG GQTR
2433.2476	2-25	1			GKIIGIDLGTTNSCVAIMDG TTPR
2423.2776	363-387	1			KDVNPDEAVAIGAAVQGGVL TGDVK
2418.2373	85-106	1			DVSIMPFKIIAADNGDAWVE VK
2356.1853	468-489	0			GMPQIEVTFDIDADGILHVS AK
2346.2299	35-56	1			TTPSIIAYTQDGETLVGQPA KR
2295.1826	364-387	0			DVNPDEAVAIGAAVQGGVLT GDVK

How does the data look?

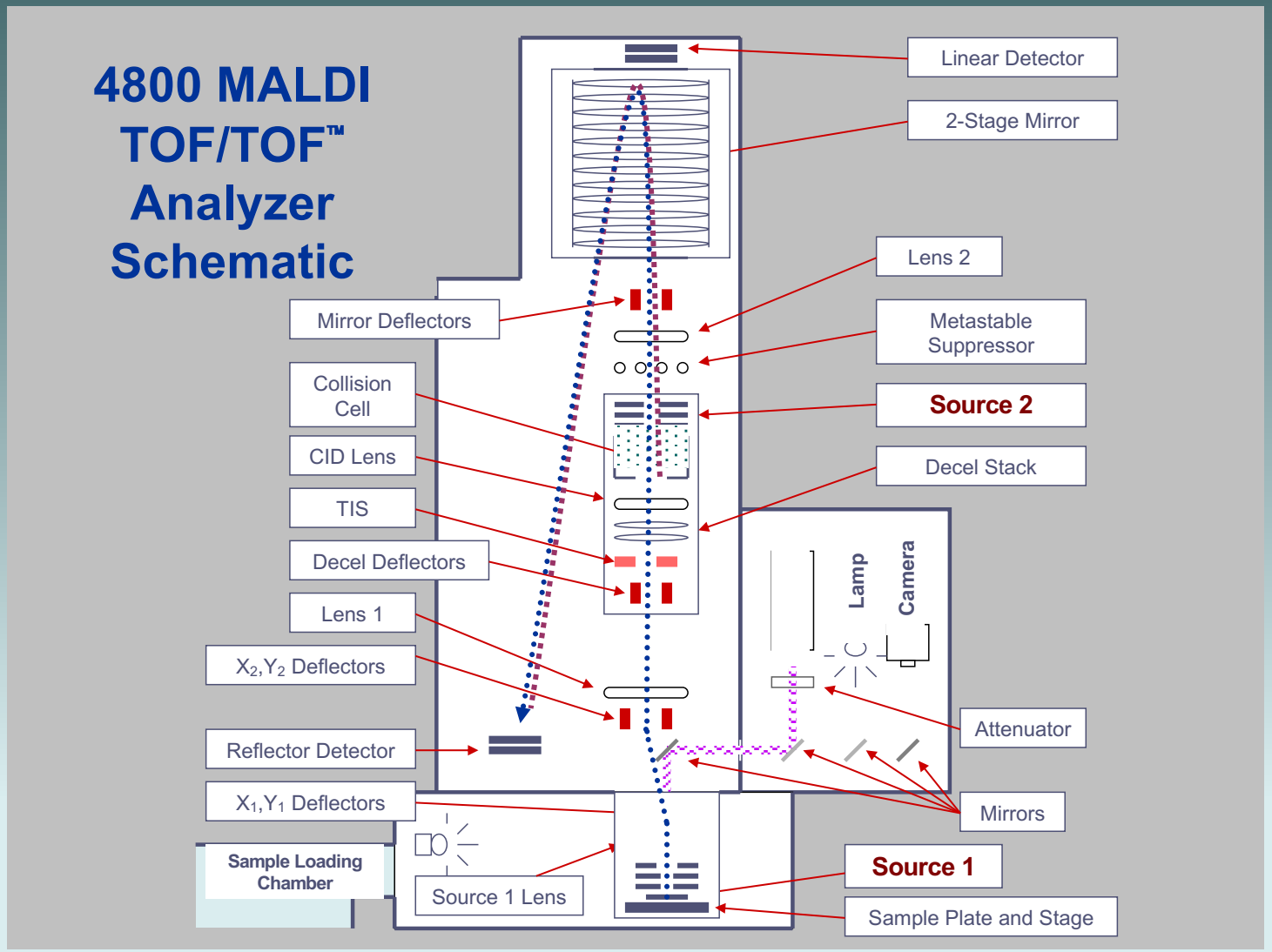
- **MS Profile of Trypsinized protein sample on MALDI-Tof**



How does Tandem MS/MS (Tof/Tof) work?

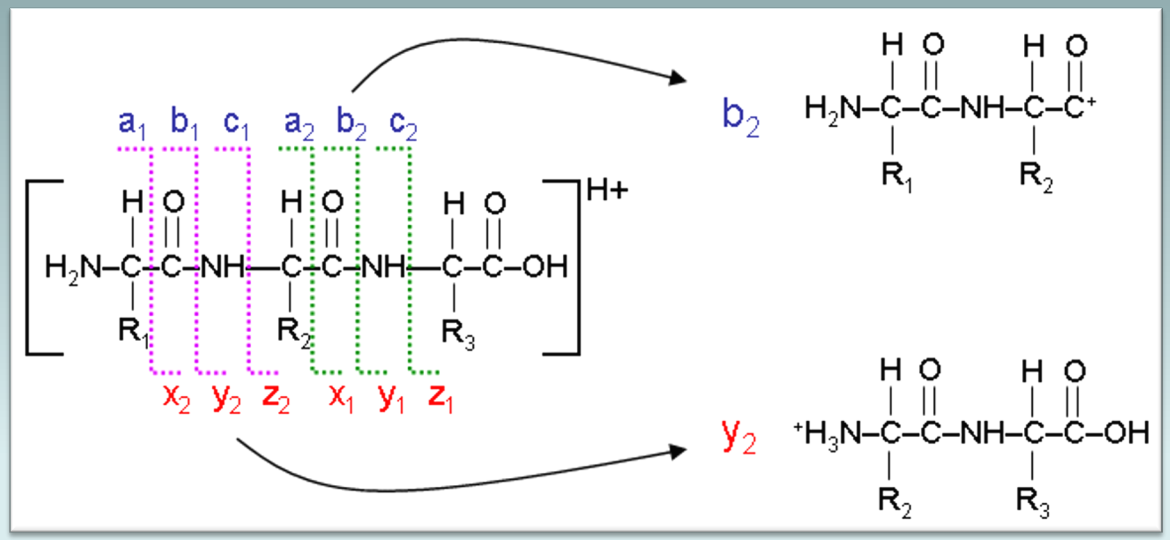


How does Tandem MS/MS (Tof/Tof) work?



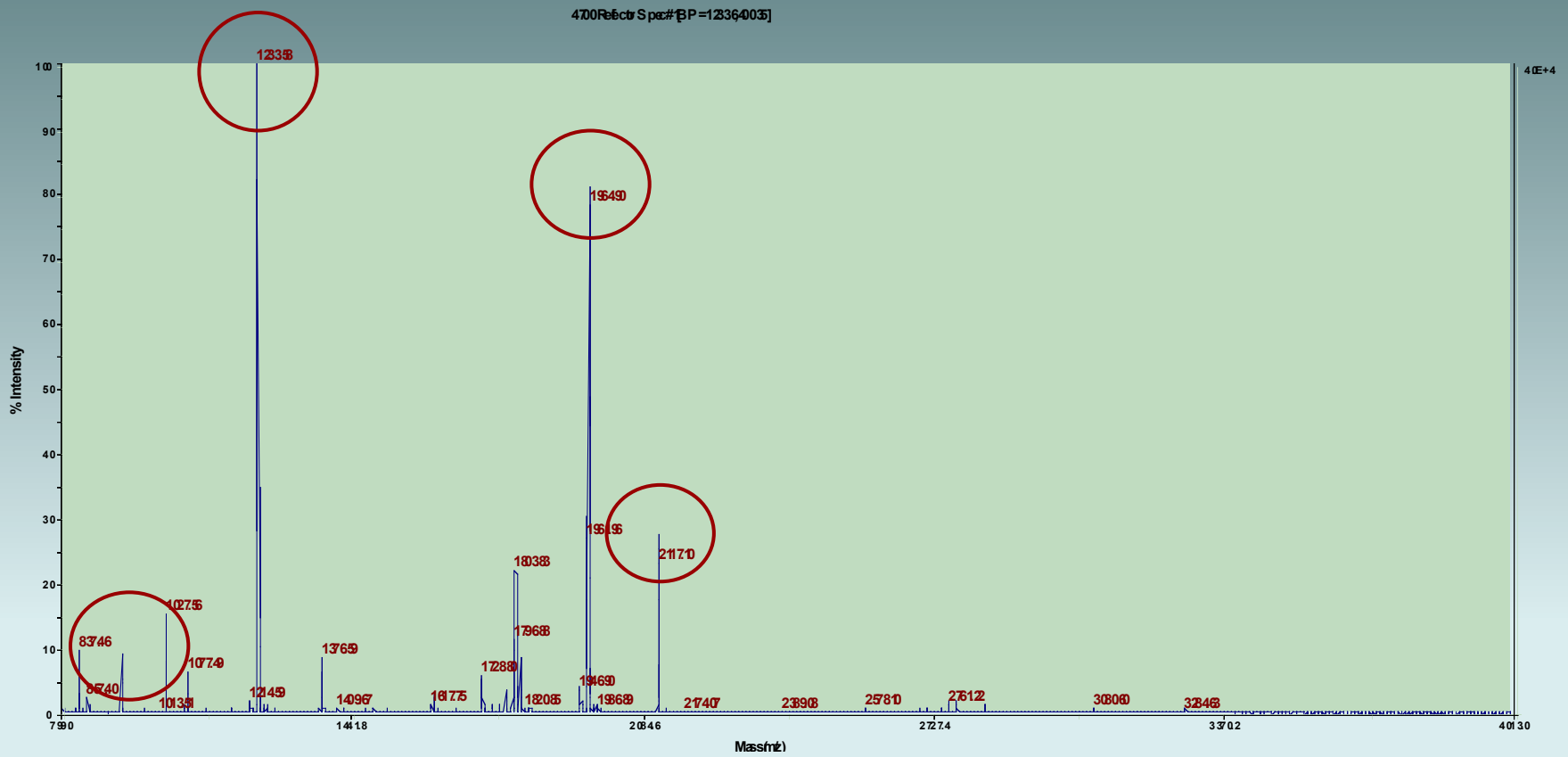
How does Tandem MS/MS (Tof/Tof) work?

- What is happening in the collision cell and after?
  - Random fragmentation by physical contact
  - 3 different types (CH-CO, CO-NH, NH-CH)
  - CO-NH breakage is most common
  - Mass differnce between adjacent b or y ion => indicative for the particular amino acid



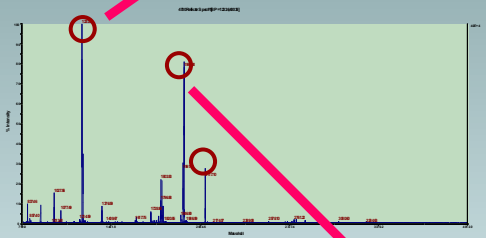
How does the data look?

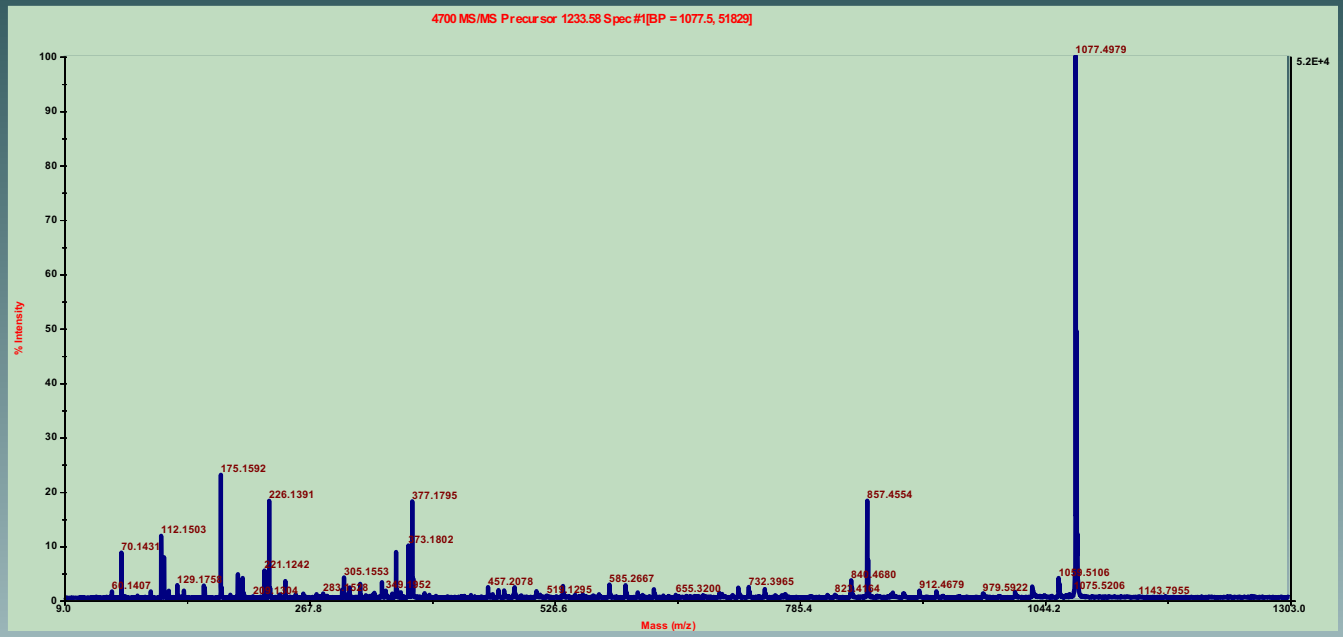
- **MS Profile of Trypsinized protein sample on MALDI-Tof**



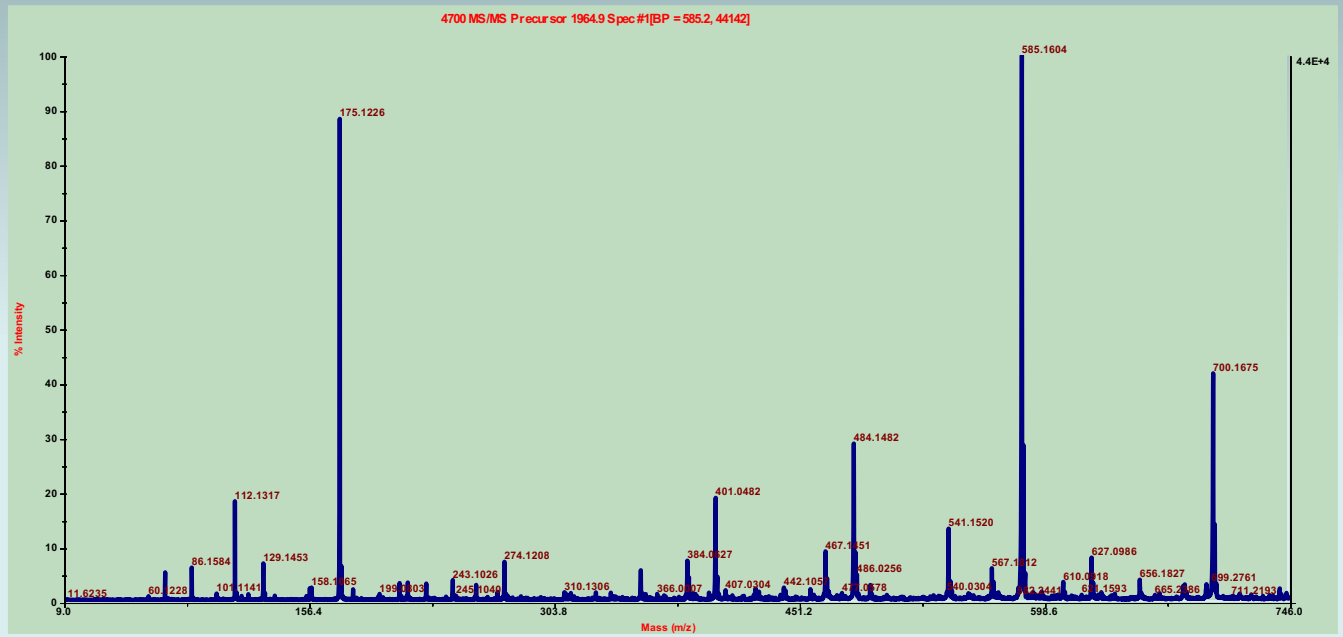
•MS/MS (Tof/Tof) Profiles of fragmented peptides precursor

Precursor, 1233.5 Da



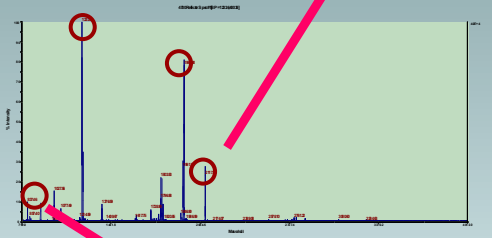


Precursor, 1964.9 Da

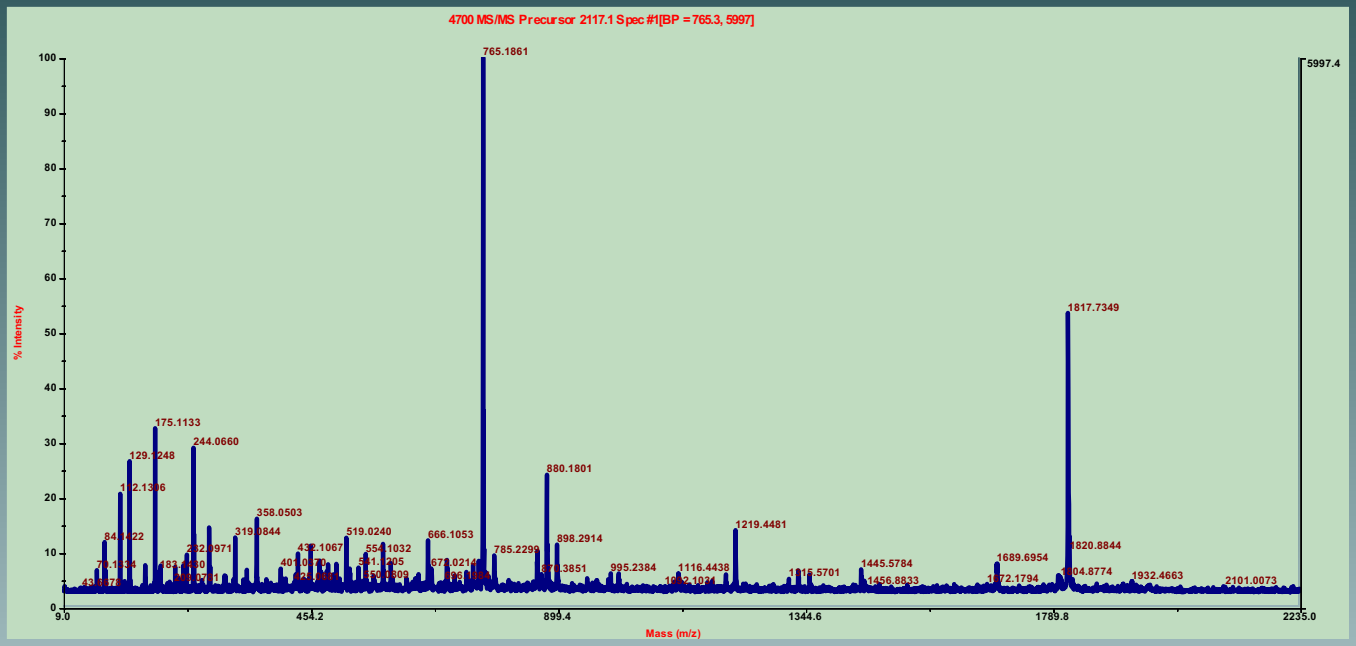


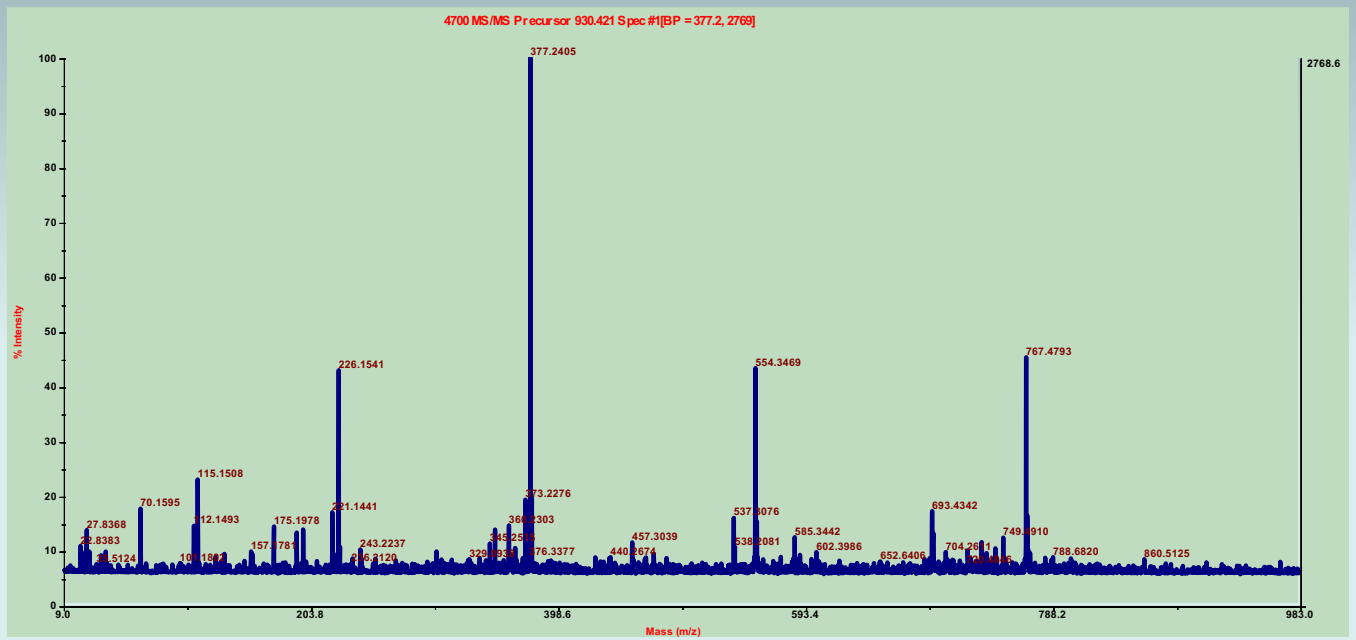
•MS/MS (Tof/Tof) Profiles of fragmented peptides precursor

Precursor, 2117.1 Da



Precursor, 930.4 Da





How does the data work?

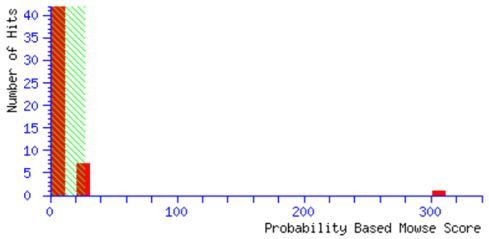
- **Database match and search result; MS Profile**

{MATRIX SCIENCE} Mascot Search Results

```
User           :
Email          :
Search title   : SampleSetID: 237, AnalysisID: 387, Path=\Biology Core-2008\Kyu-082008\Lu-cong-082008-Ecoli
Database       : NCBInr  (2367365 sequences; 802797248 residues)
Taxonomy       : Escherichia coli (28171 sequences)
Timestamp      : 20 Aug 2008 at 21:25:46 GMT
Significant hits: gi|1942723  Chain C, Elongation Factor Complex Ef-TuEF-Ts From Escherichia Coli
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 27 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

Format As: Peptide Summary — Help

Significance threshold p< 0.05 — Max. number of hits 10

Standard scoring ◉ MudPIT scoring ○ — Ions score cut-off 0 — Show sub-sets ☐

Show pop-ups ◉ Suppress pop-ups ○ — Sort unassigned Decreasing Score — Require bold red ☐

Select All | Select None | Search Selected | ☐ Error tolerant | Archive Report

1. gi|1942723 Mass: 42150 Score: 307 Queries matched: 8
   Chain C, Elongation Factor Complex Ef-TuEF-Ts From Escherichia Coli
   ☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
☑ 1	837.4638	836.4565	836.4868	-0.0303	0	50	0.00029	1	R.EHILLGR.Q
☑ 3	1027.5627	1026.5554	1026.5821	-0.0267	0	43	0.0014	1	R.AGENVGVLLR.G
5	1233.5809	1232.5736	1232.6090	-0.0354	0	11	2.5	3	K.GYRPQFYFR.T
☑ 6	1376.5917	1375.5844	1375.6255	-0.0411	0	44	0.0011	1	R.AFDQIDNAPEEK.A
☑ 9	1728.7891	1727.7818	1727.8365	-0.0547	1	26	0.053	1	K.FESEVYILSKDEGGR.H
☑ 10	1803.8333	1802.8260	1802.8799	-0.0538	0	38	0.0037	1	R.GITINTSHVEYDTPTR.H
☑ 11	1964.9021	1963.8948	1963.9527	-0.0579	0	61	1.4e-005	1	R.ELLSQYDFPGDDTPIVR.G
☑ 12	2117.0898	2116.0825	2116.1568	-0.0742	0	32	0.0089	1	R.AIDKPFLLPIEDVFSISGR.G

Database Search and Match Result

Gel Idx/Pos Plate [#] Name	274/L2 [1] 7659-061208-Kyu	Instr./Gel Origin Instrument Sample Name	ak167/7659-061208	Process Status Spectra	Analysis Succeeded 14

Rank	Protein Name	Accession No.	Total Ion Score	Total Ion C. I. %
1	Chain A, E. Coli Elongation Factor Ef-Tu Complexed With The Antibiotic Kirromycin, A Gtp Analog, An	gi|49258331	435	100

Protein Group

Chain A, Ef-Tu.Kirromycin Coordinates Fitted Into The Cryo-Em Map Of Ef-Tu Ternary Complex (Gdp.Kir	gi|38492965
Chain B, Crystal Structure Of Elongation Factor, Tu (Ef-Tu-Mggdp) Complexed With Ge2270a, A Thiazol	gi|11514298
Chain C, Elongation Factor Complex Ef-TuEF-Ts From Escherichia Coli	gi|1942723
Elongation factor Tu [Escherichia coli CFT073]	gi|26249935
protein chain elongation factor EF-Tu (duplicate of tufB) [Shigella flexneri 2a str. 301]	gi|24115265
protein chain elongation factor EF-Tu [Shigella flexneri 2a str. 2457T]	gi|30064737

Peptide Information

Calc. Mass	Observ. Mass	± da	± ppm	Start Seq.	End Seq.	Sequence	Ion Score	C. I. %	Modification	Rank	Result Type
837.4941	837.457	-0.0371	-44	117	123	EHILLGR	28	96.486		1	Mascot
1027.5895	1027.5609	-0.0286	-28	270	279	AGENVGVLLR	60	99.998		1	Mascot
1233.6163	1233.5822	-0.0341	-28	325	333	GYRPQFYFR	47	99.951		1	Mascot
1376.6328	1376.5886	-0.0442	-32	45	56	AFDQIDNAPEEK	31	97.954		1	Mascot
1728.8439	1728.796	-0.0479	-28	304	318	FESEVYILSKDEGGR	64	99.999		1	Mascot
1780.9448	1780.8859	-0.0589	-33	358	373	MVVTLIHPIAMDDGLR	26	93.765		1	Mascot
1803.8872	1803.8304	-0.0568	-31	59	74	GITINTSHVEYDTPTR	48	99.964		1	Mascot
1964.96	1964.903	-0.057	-29	155	171	ELLSQYDFPGDDTPIVR	62	99.999		1	Mascot
2117.1641	2117.0957	-0.0684	-32	205	223	AIDKPFLLPIEDVFSISGR	67	99.999		1	Mascot