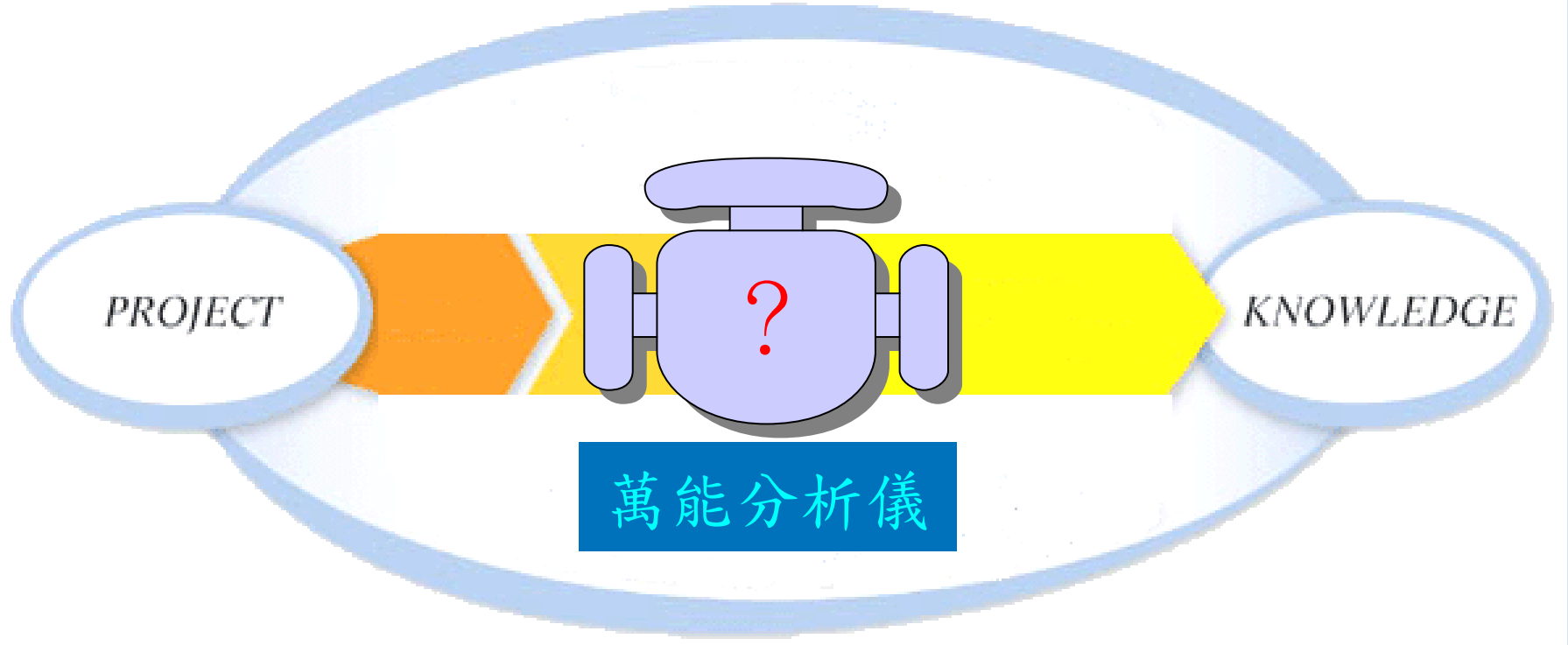
The skill of LC-ESI-Ion Trap

For Proteomics Research

James Yu, Application Specialist

科學家的共同夢想

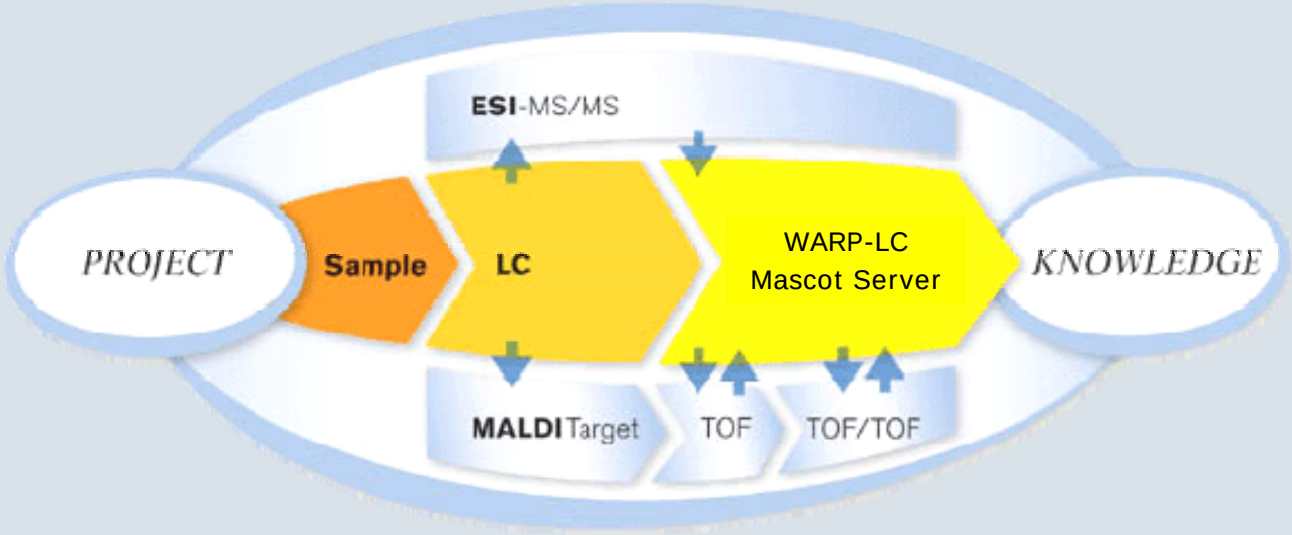


世事總難盡如人意

Proteineer LC ™ Workflow

Total Solution of Informative Driven Research

PTM Discovery

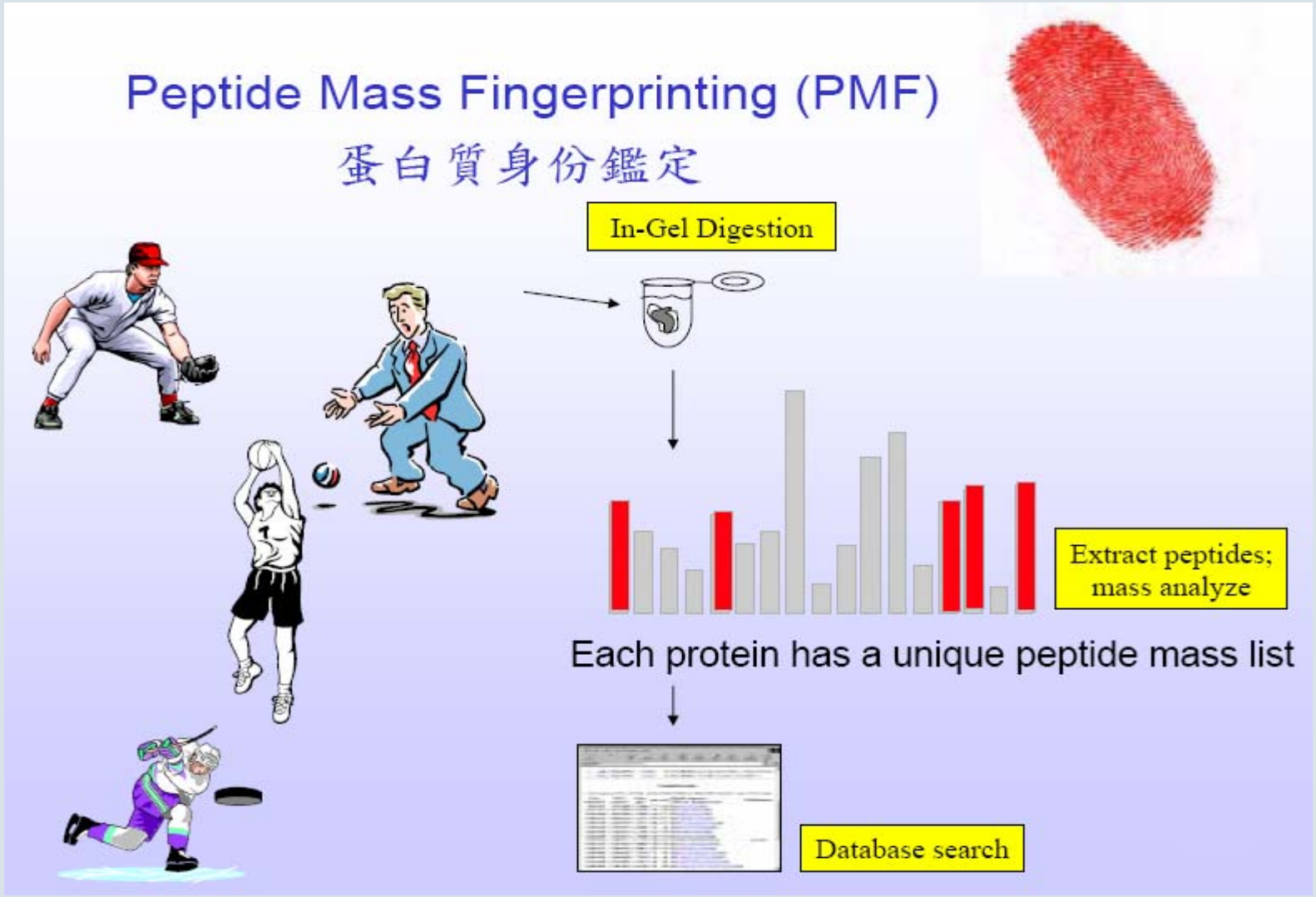


High Throughput Analysis

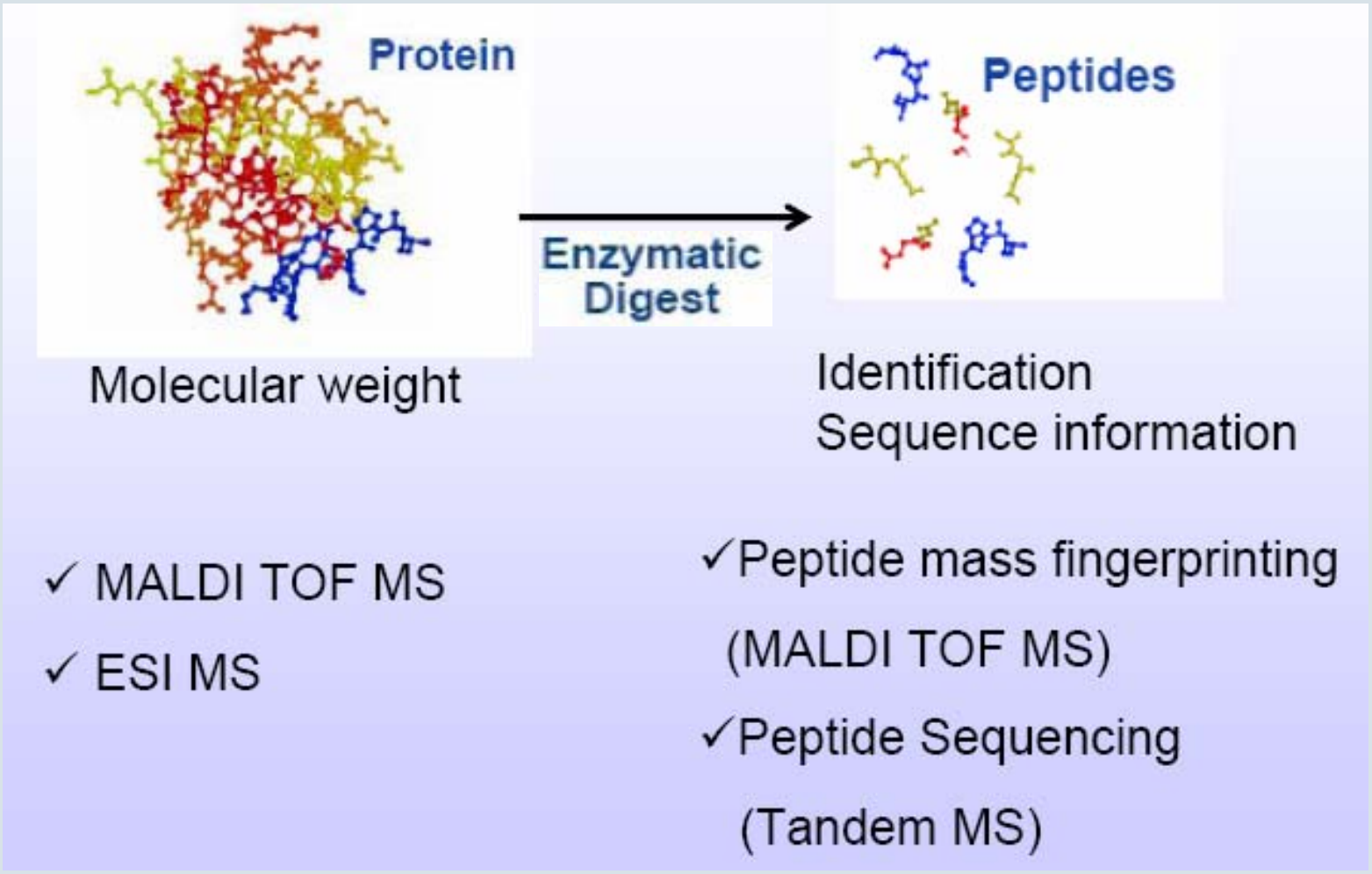
What Do We See?

Is  er?

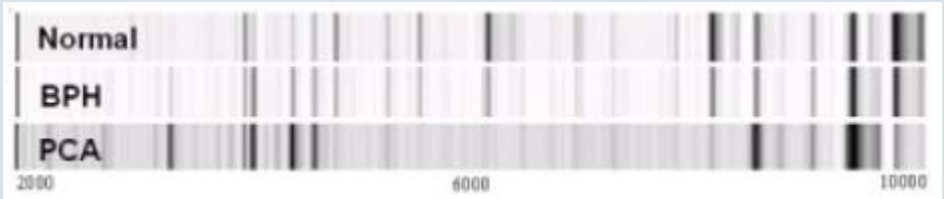
What‘s the different between these people?



Mass Spectrometry Methods

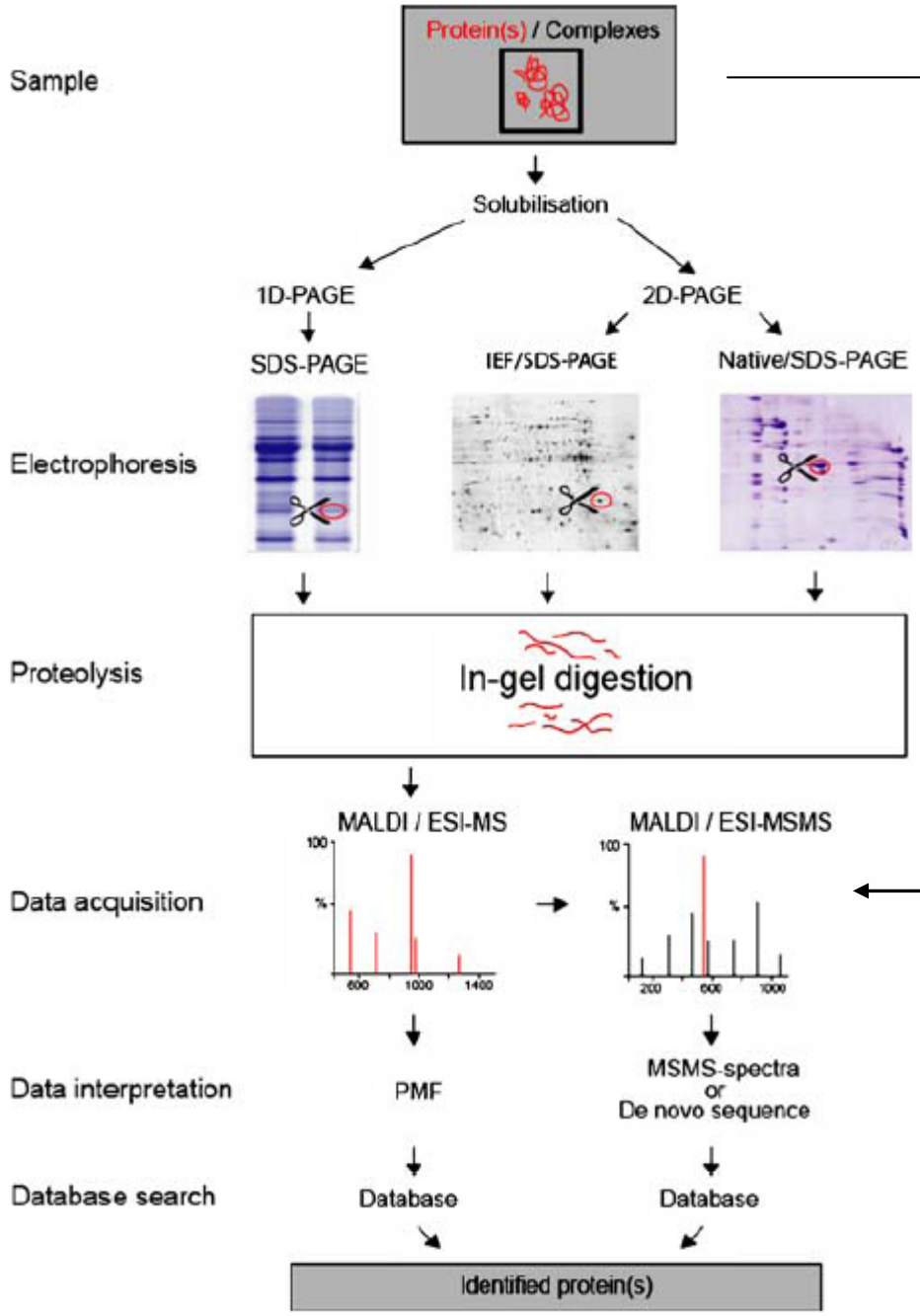


What‘s the different between these people?



正常人
良性攝護腺腫瘤
攝護腺癌

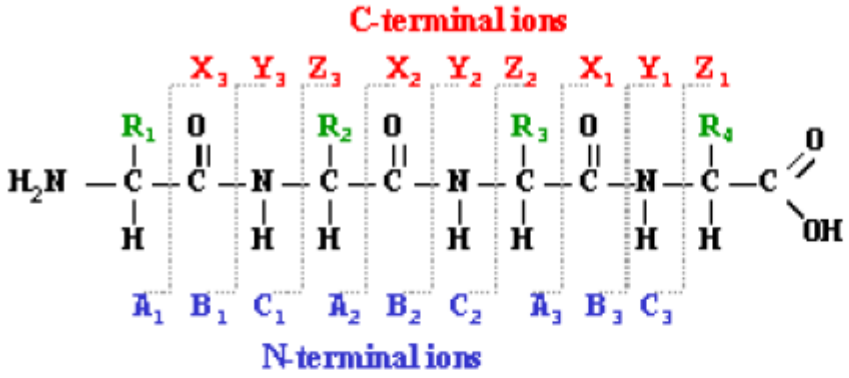
What is the protein we interesting?



In-solution digestion

2D-HPLC

Peptide Fragmentation



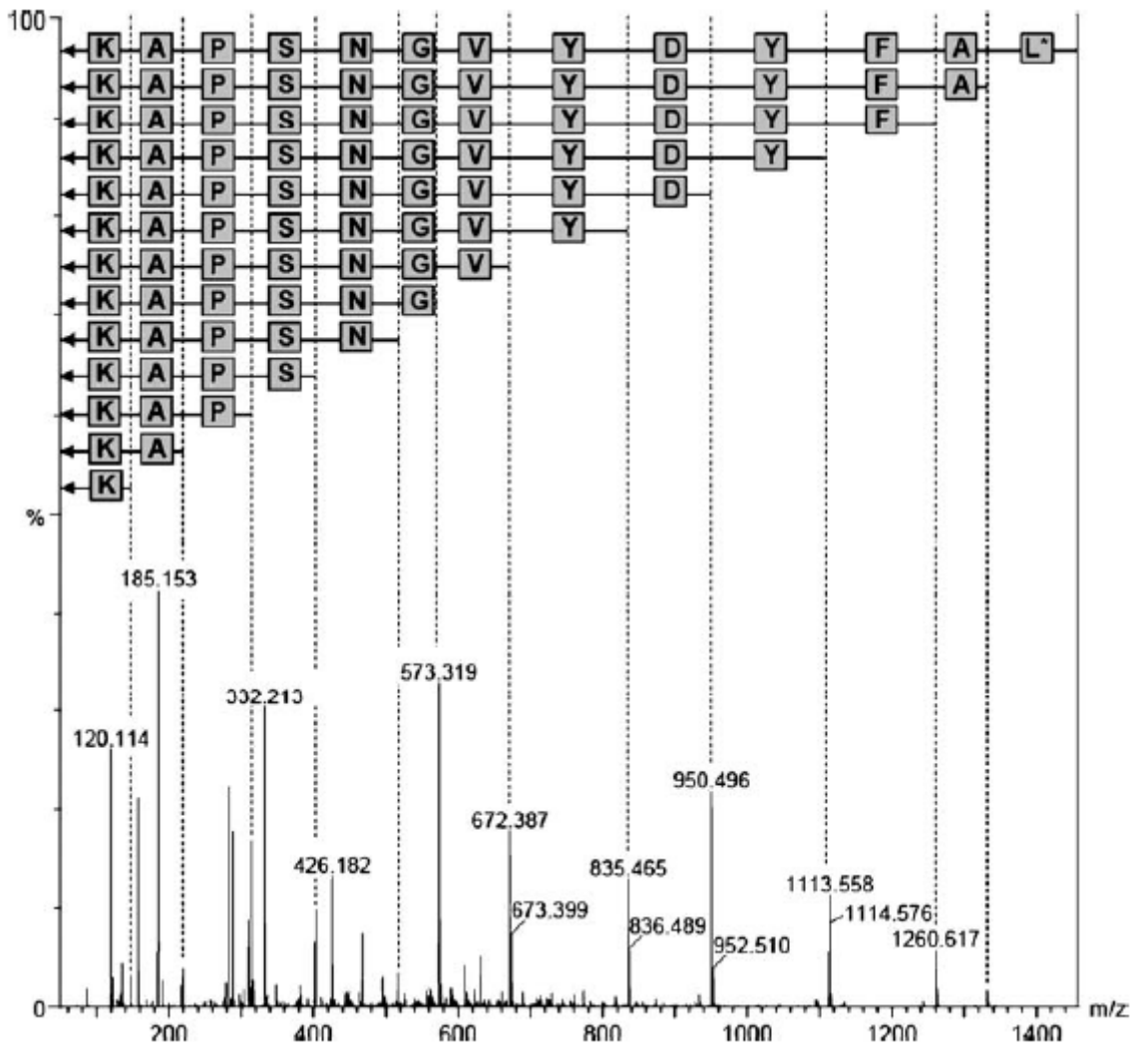
a,b,c – N-terminal side

x,y,z – C-terminal side

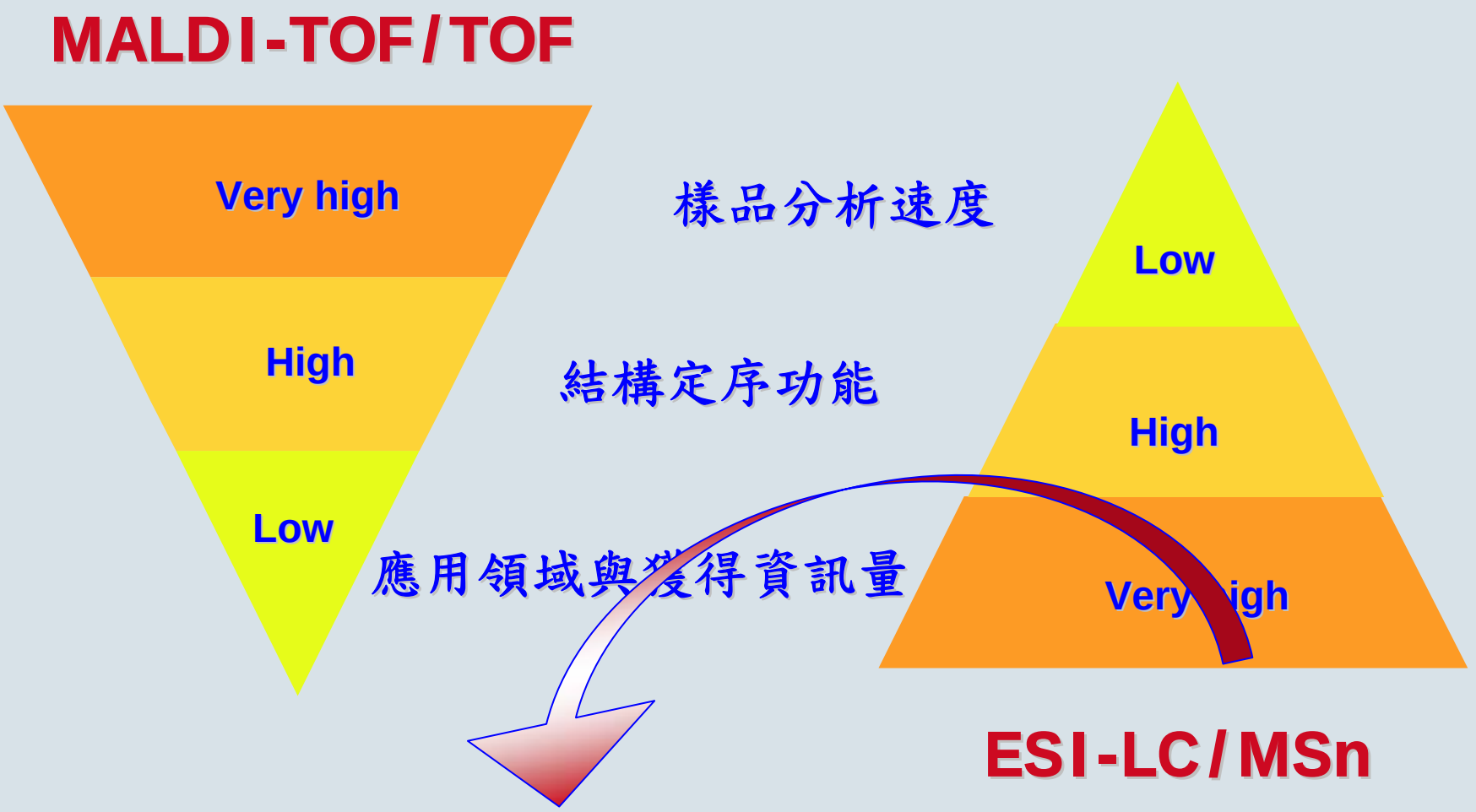
S-P-A-F-D-S-I-M-A-E-T-L-K

(protonated mass 1410.6)

mass+	b-ions	y-ions	mass+
88.1	S	PAFDSIMAETLK	1323.6
185.2	SP	AFDSIMAETLK	1226.4
256.3	SPA	FDSIMAETLK	1155.4
403.5	SPAF	DSIMAETLK	1008.2
518.5	SPAFD	SIMAETLK	893.1
605.6	SPAFDS	IMAETLK	806.0
718.8	SPAFDSI	MAETLK	692.3
850.0	SPAFDSIM	AETLK	561.7
921.1	SPAFDSIMA	ETLK	490.6
1050.2	SPAFDSIMAE	TLK	361.5
1151.3	SPAFDSIMAET	LK	260.4
1264.4	SPAFDSIMAETL	K	147.2


100
%
0
K A P S N G V Y D Y F A L*
K A P S N G V Y D Y F A
K A P S N G V Y D Y F
K A P S N G V Y D Y
K A P S N G V Y D
K A P S N G V Y
K A P S N G V
K A P S N G
K A P S N
K A P S
K A P
K A
K
185.153
573.319
120.114
672.387
426.182
835.465
950.496
673.399
836.489
952.510
1113.558
1114.576
1260.617
200
400
600
800
1000
1200
1400
m/z

LC/MSn *vs.* MALDI-TOF/TOF



Metabolomics, Drug and Small Molecules

ESI-, MALDI-MS/MS
Complementary

*MKWVTFISLL LLFSSAYSRG VFRR*DTHKSE IAHRFKDLGE
EHFKGLVLIA FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD
ESHAGCEKSL HTLFGDELCK VASLRETYGD MADCCEKQEP
ERNECFLSHK DDSPDLPKLK PDPNTLCDEF KADEKKFWGK
YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA
RLSQKFPKAE FVEVTKLVTD LTKVHKECCH GDLLECADDR
ADLAKYICDN QDTISSKLKE CCDKPLLEKS HCIAEVEKDA
IPENLPPLTA DFAEDKDVCK NYQEAKDAFL GSFLYEYSRR
HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ
VSTPTLVEVS RSLGKVGTRC CTKPESERMP CTEDYLSLIL
NRLCVLHEKT PVSEKVTKCC TESLVNRRPC FSALTPDETY
VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP
KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
STQTALA

BSA digest

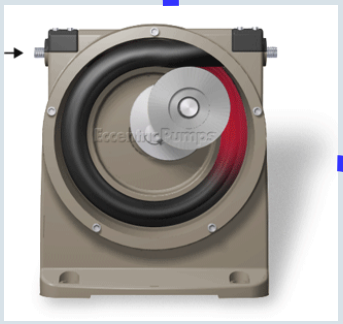
Pre-sequence
ESI
MALDI
both

What is “HPLC“?

High Performance Liquid Chromatography

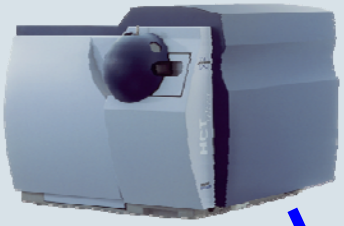
Individual Module of HPLC

Mobile Phase



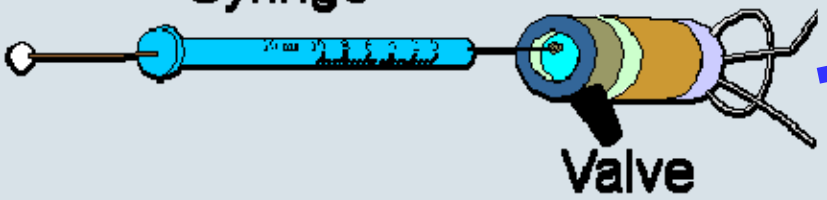
PUMP

Mass Spectrometer



UV Detector

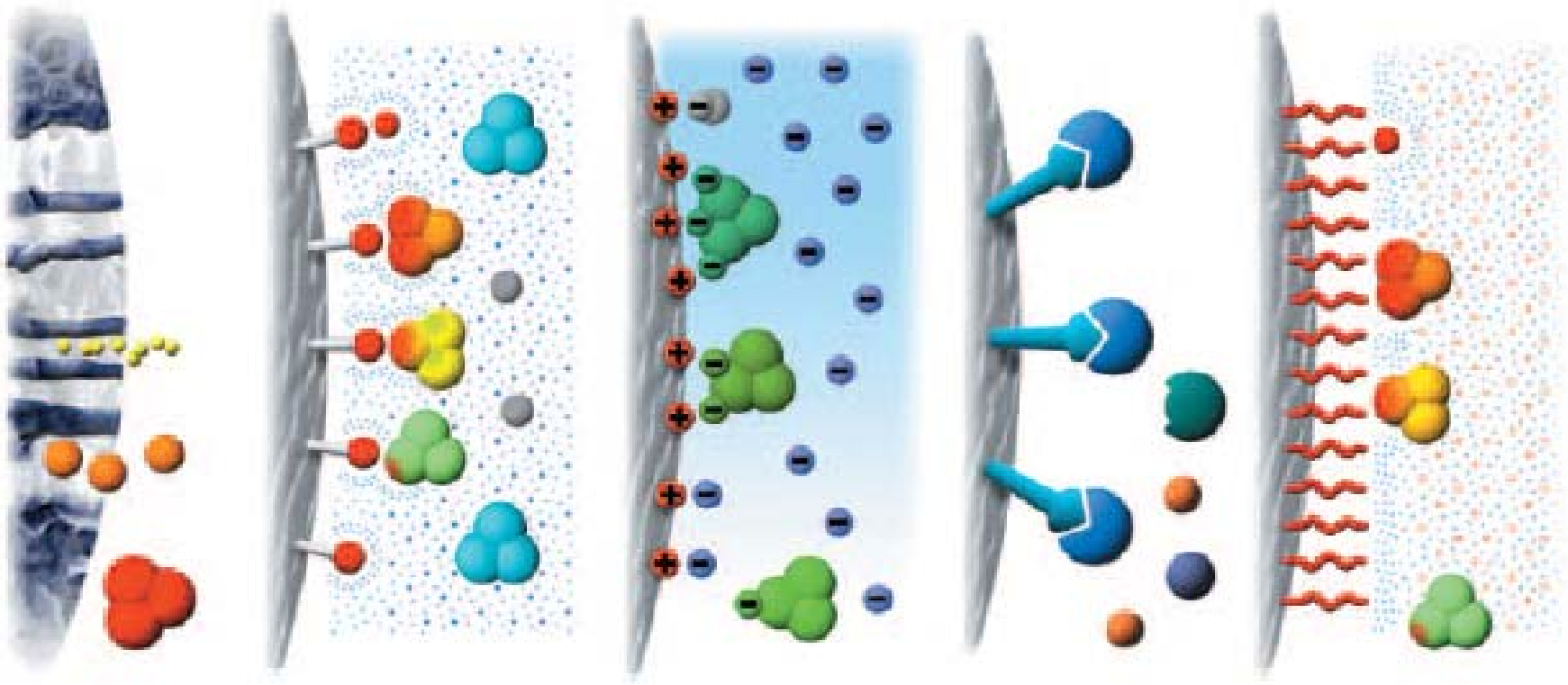
Injector



Column

Recorder

Category of Liquid Chromatography



Gel Filtration

Ion Exchange

Reverse Phase

Hydrophobic Interaction

Affinity

Column for Reverse Phase Analysis

What should I know for a column selection?

Chemistry of Reverse Phase Chromatography

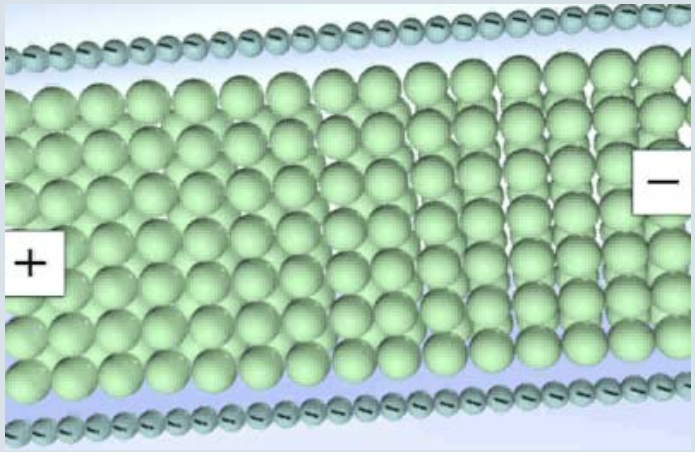
A. How much sample do you have?

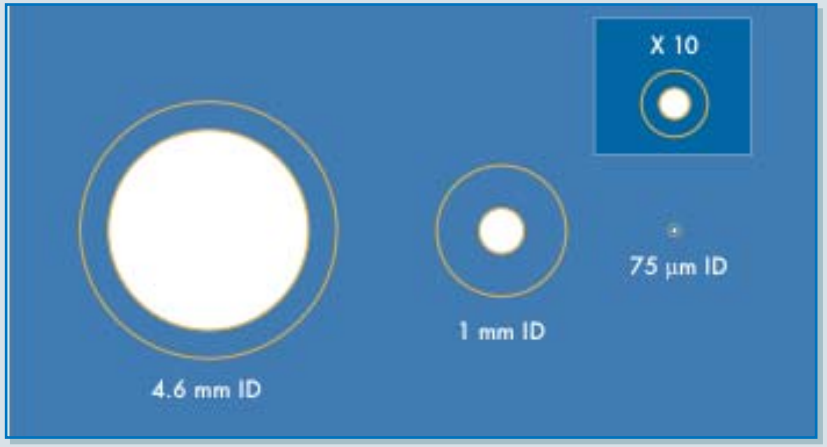
Column ID	Column Type	Flow Range (µL/min)	Optimum Flow per min	Analyte Capacity	Injector Volume
4.6 mm	Standard	500-3000	1000 ul	100ug~10ng	30 ul
2 mm	Microbore	100-1000	200 ul	10ug~1ng	5 ul
1 mm	Microbore	20-200	50 ul	1ug~100pg	1 ul
500 um	Microbore	5-50	12 ul	100ng~10pg	250 nl
300 um	Capillary	2-20	5 ul	10ng~1pg	128 nl
200 um	Capillary	1-10	2 ul	1ng~100fg	57 nl
100 um	Nanoscale	0.25-2.5	500 nl	100pg~10fg	14 nl
50 um	Nanoscale	0.05-0.5	100 nl	< 1pg	3 nl

Proteomics is here!!!

Why Use Nanobore LC?

平方倍數提高樣品分析靈敏度

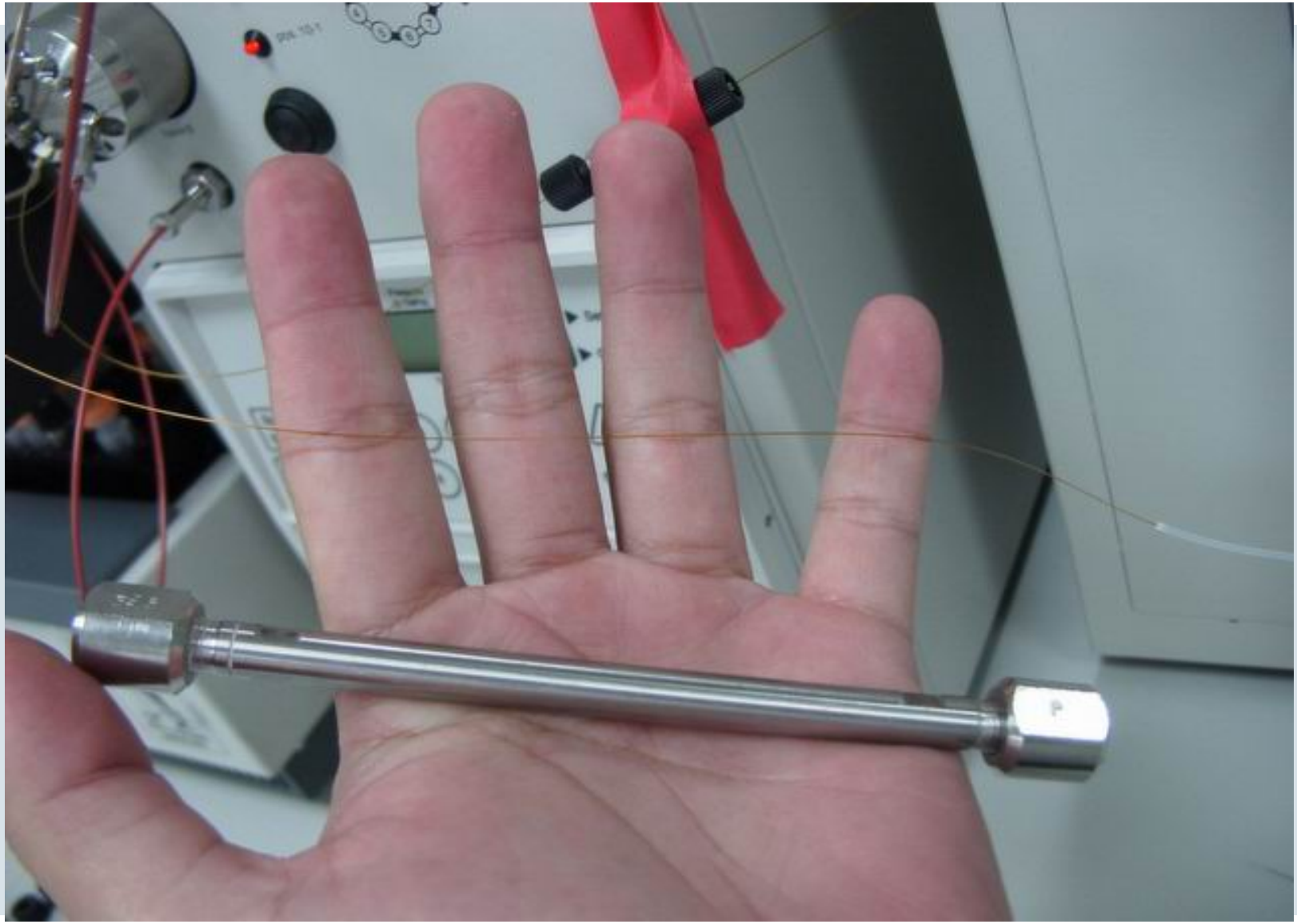




Column	ID	Flow Rate	Relative [C]
Standard	4.6 mm	1 mL/min	1
Microbore	1 mm	50 µL/min	21
Capillary	320 µm	5 µL/min	206
Nanobore	75 µm	250 nL/min	3,750
Nanobore	50 µm	150 nL/min	8,450

Adapted From Tomer & Moseley, *Mass. Spec. Rev.*, 1994, 13, 431

Chemistry of Reverse Phase Chromatography



Chemistry of Reverse Phase Chromatography

Mobile Phase Composition

Mobile Phase Composition

A. H_2O , 0.1% FA (Formic Acid), pH:~ 2

B. CH_3CN, 0.08% FA pH:~2

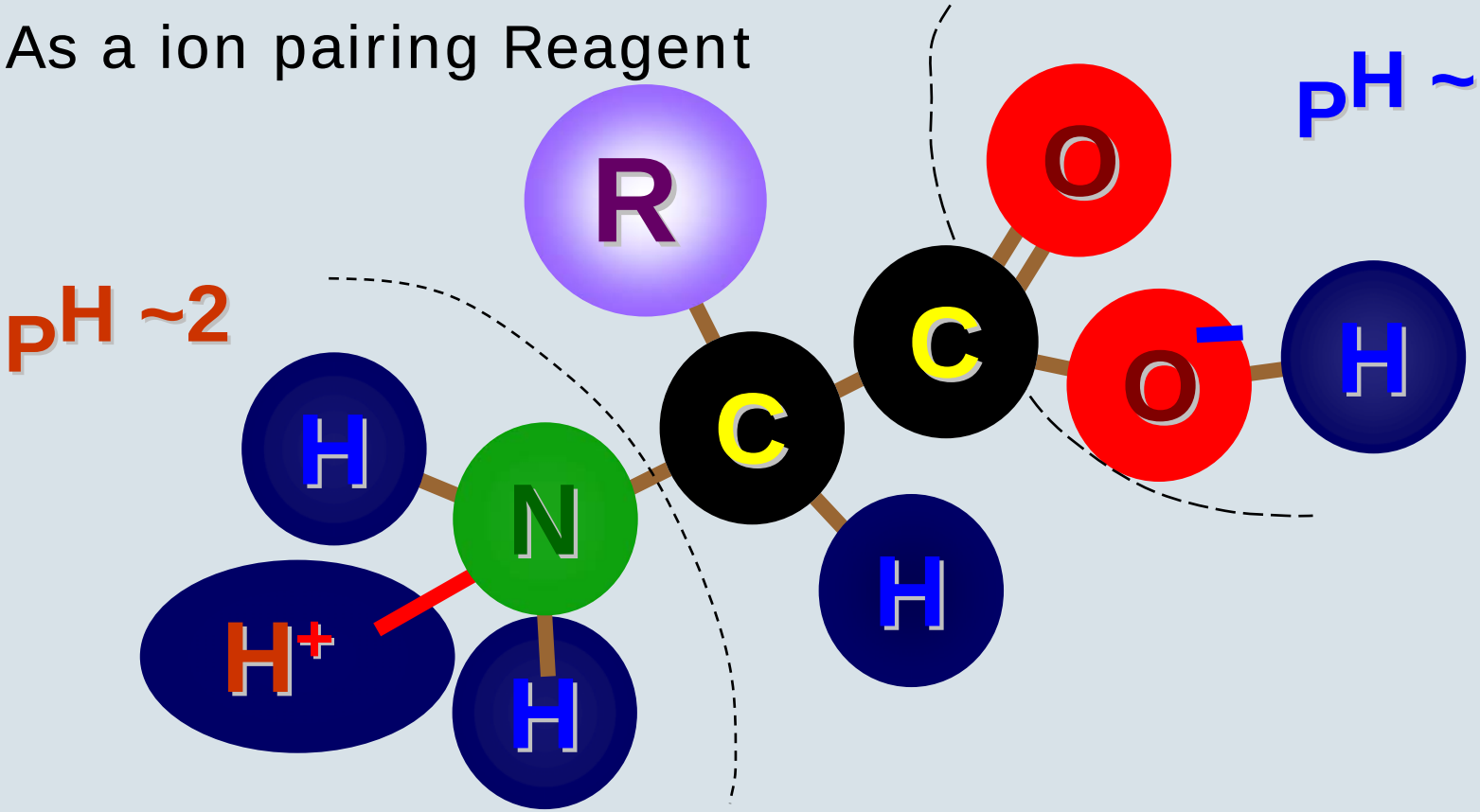
*. Filtered with 0.22um PVDF membrane

Mobile Phase For Cation Exchange

20~50% ACN, no acid

Why do we use FA?

As a ion pairing Reagent



Zwitterion Form

Why do we use these solvent?

TFA (Trifluoroacetic acid) :

A. Improve chromatographic retention – Block residual active silanols

B. TFA is very effective at ion suppressing

Solution : Change to use FA (Formic acid).

Mobile Phase :

MeOH : Strong nonpolar solvent,

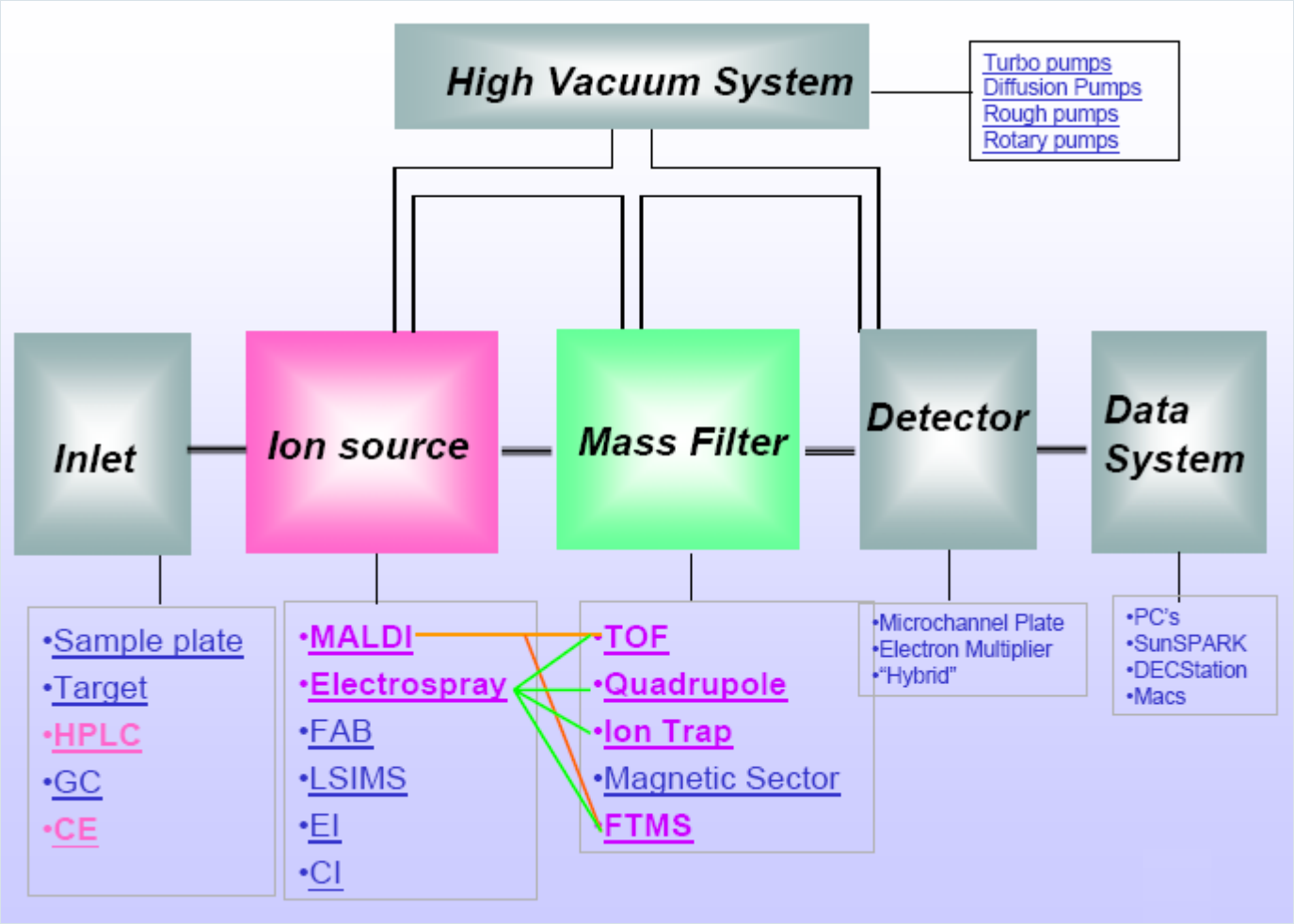
ACN : Less nonpolar effect, and good for peptide separation.

1D NanoLC Gradient Setup

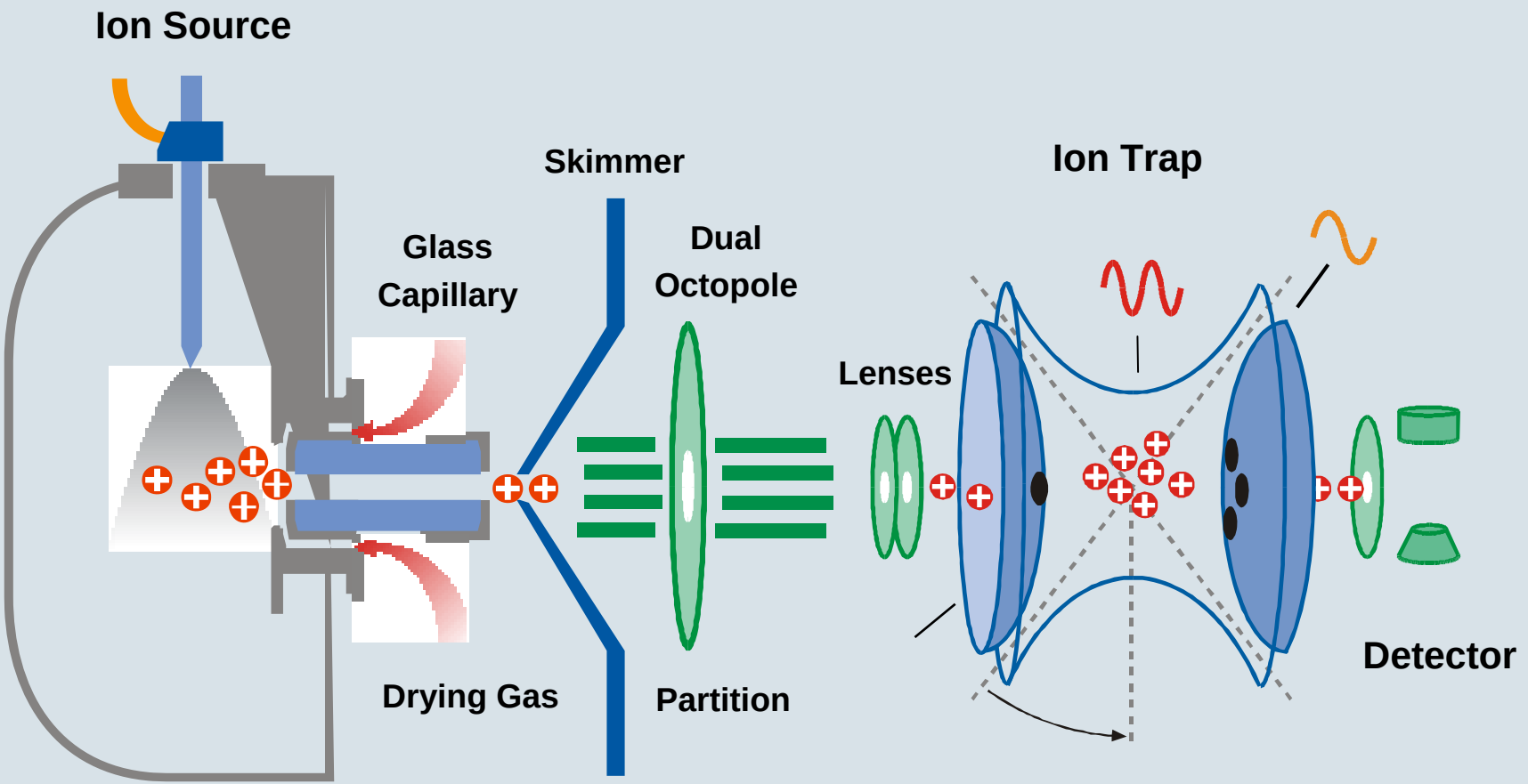
Common 1D LC Gradient

Time	A%	B%
0	97.0	3.0
2.0	97.0	3.0
2.1	92.0	8.0
26.1	76.0	24.0
31.1	61.0	39.0
33.0	5.0	95.0
38.0	5.0	95.0
38.5	97.0	3.0
52.0	97.0	3.0

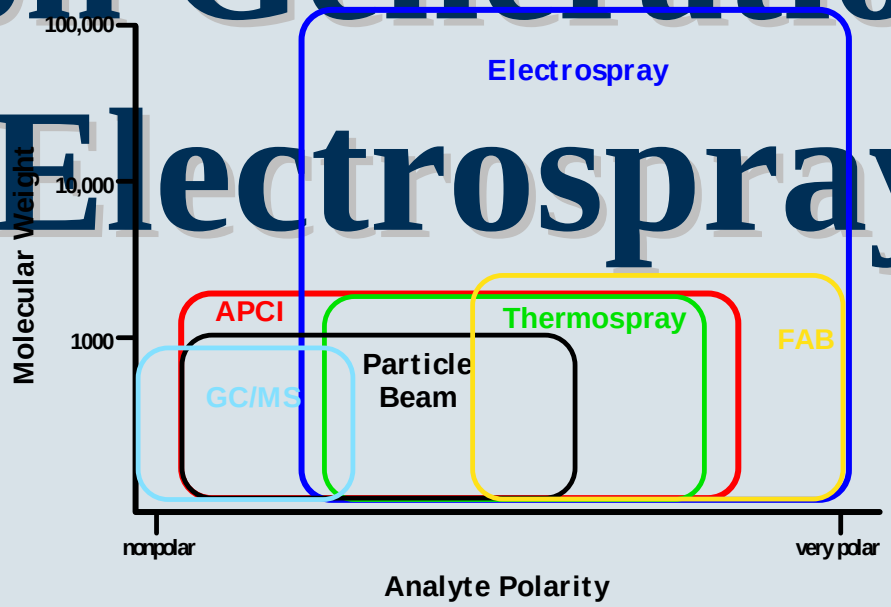
Elements of Mass Spectrometer



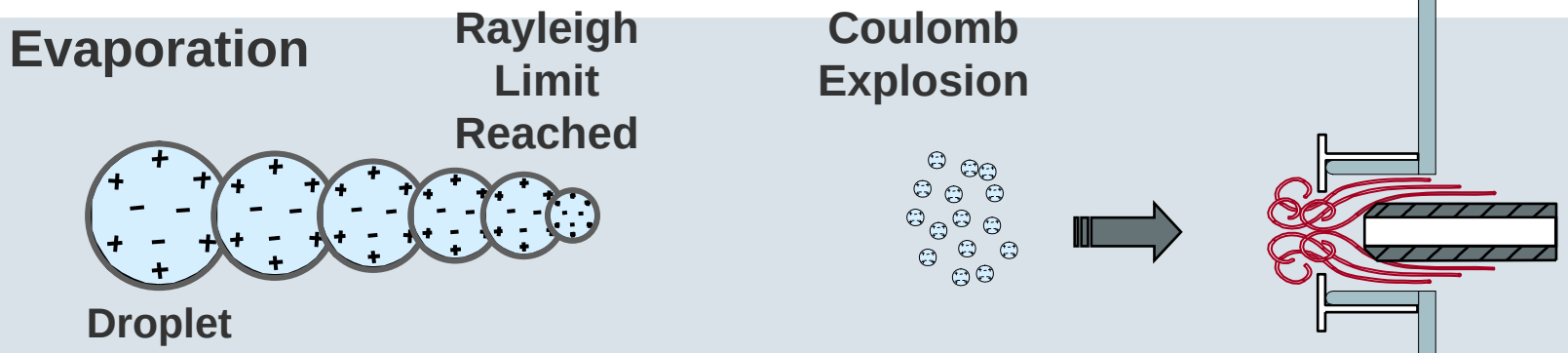
Experimental Setup of API - Ion Traps



Ion Generation: Electrospray



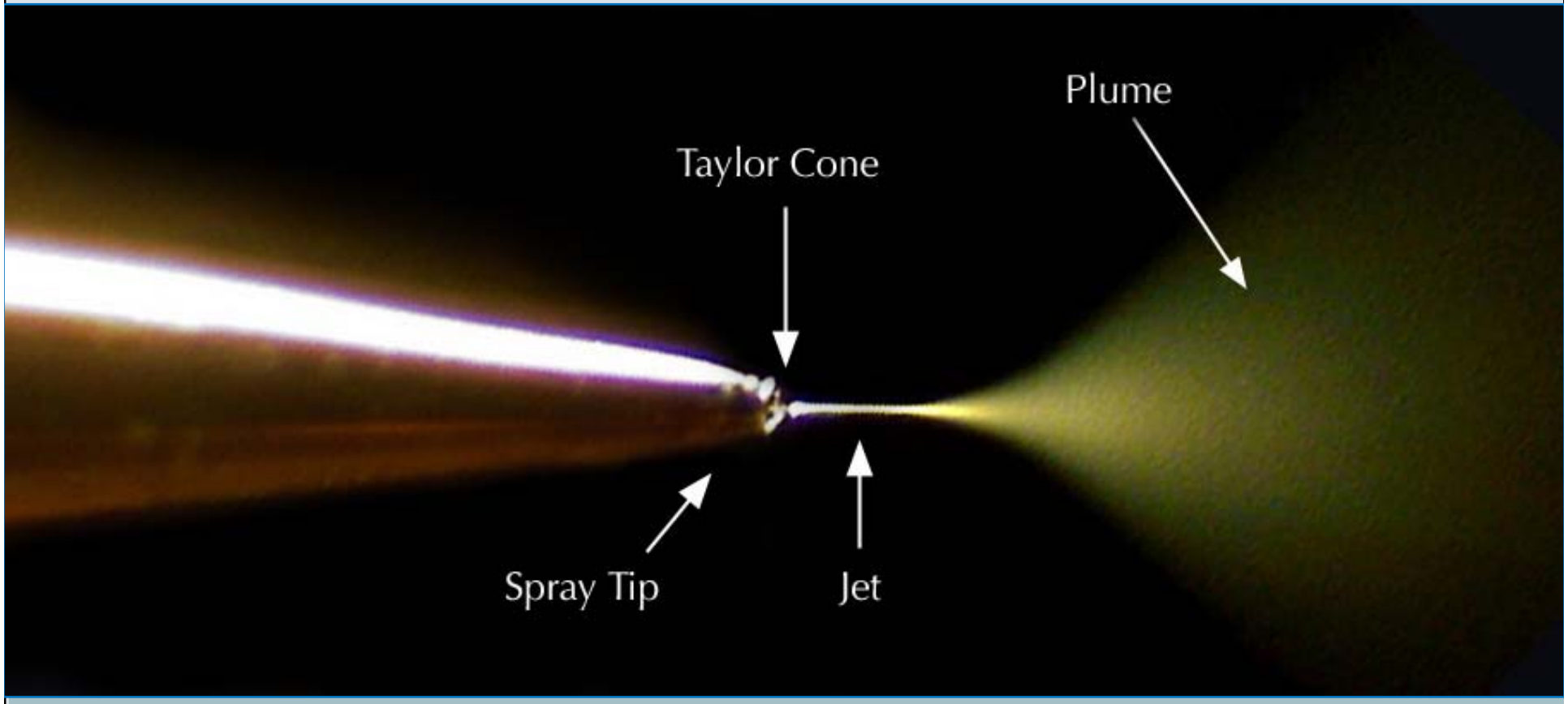
Generation of Ions (CRM)



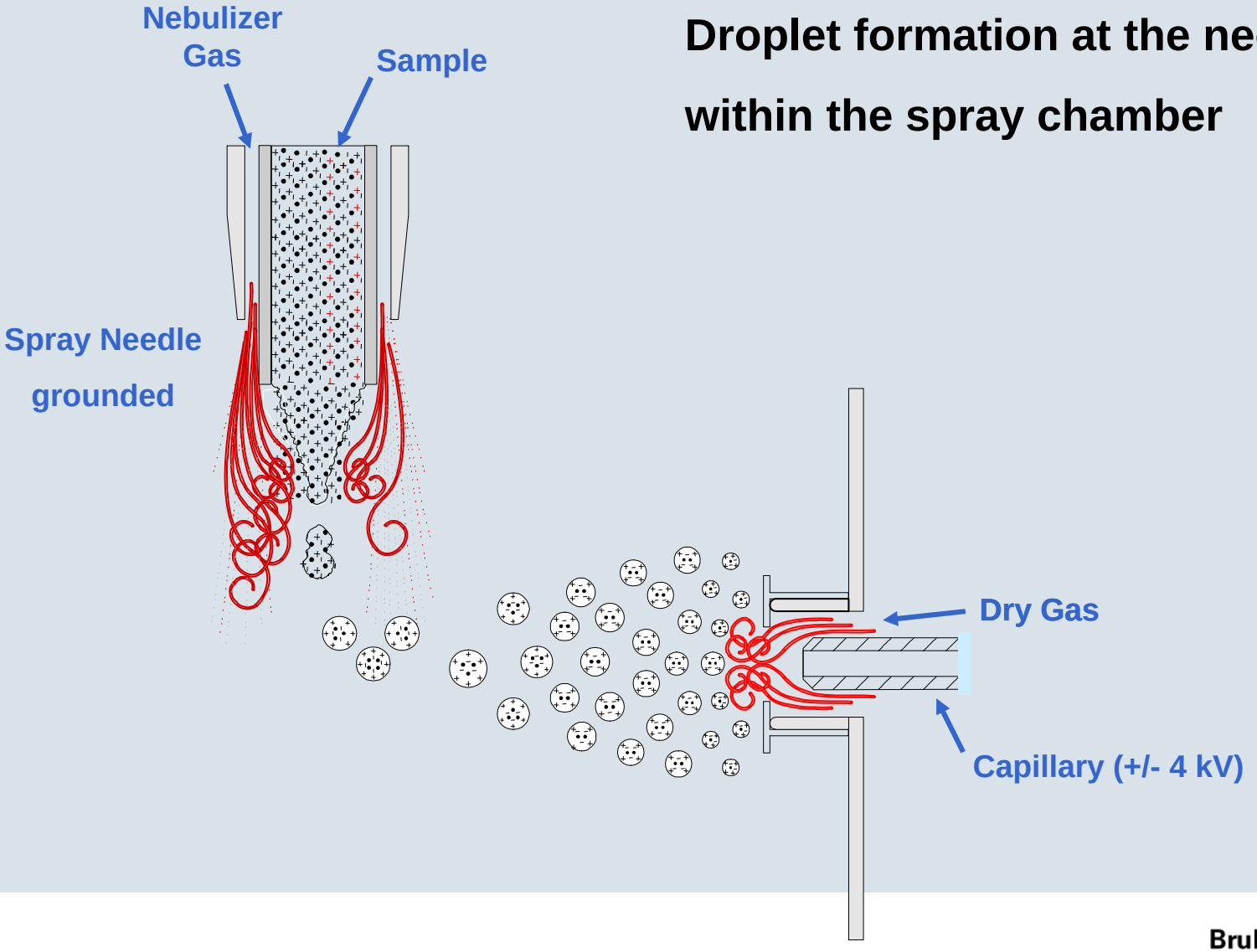
When the force of the Coulomb repulsion exceeds the surface tension of the droplet, the droplet explodes, producing charged daughter droplets that are subject to further evaporation.

Generation of Ions (IDM)

As solvent evaporates from the droplet, the surface becomes highly charged. When the field created by the ions at the surface of the droplets exceeds the surface tension, ions are emitted directly into the gas phase.


Plume
Taylor Cone
Spray Tip
Jet

Generation of Ions - Nebulization

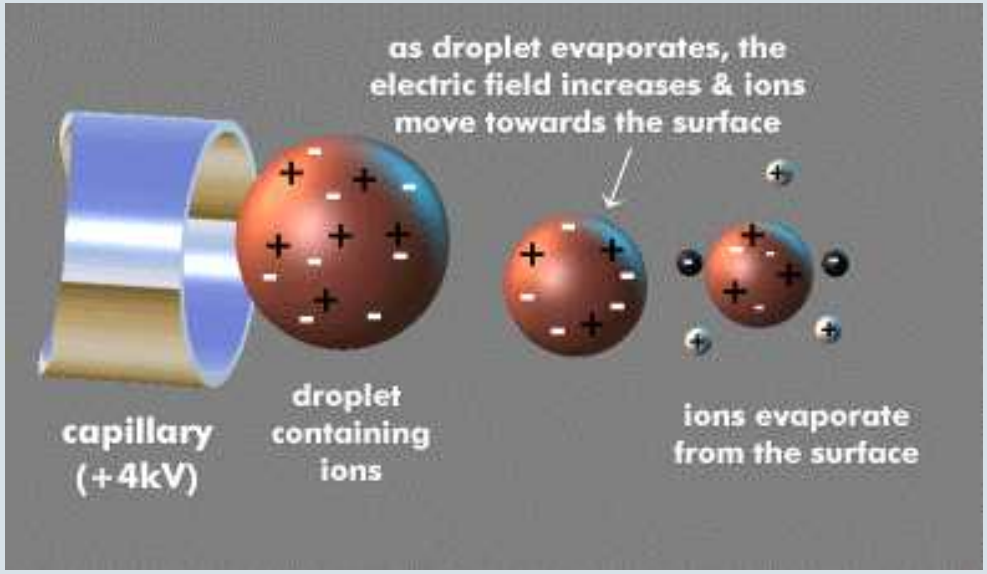


Droplet formation at the needle tip within the spray chamber

Atmospheric Pressure Ionization- Electrospray Ionization (API-ESI)

Ion generation for MS analysis

- Nebulization
- Desolvation
- Coulomb explosions
- Desorption



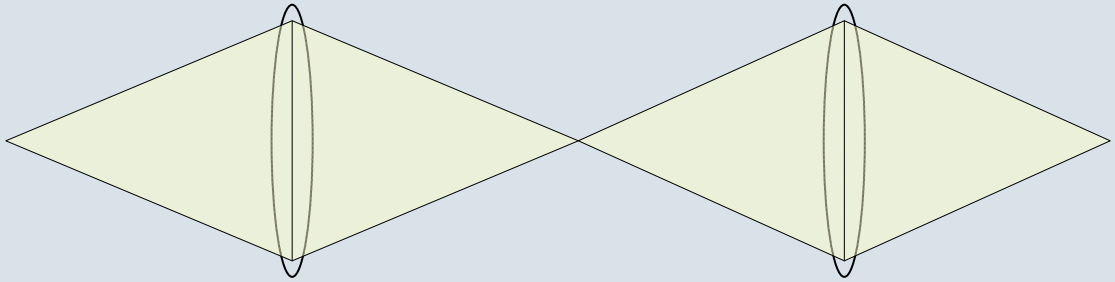
Under proper source conditions, individual ions only will enter the capillary. An unstable signal or capillary current may indicate a need for adjustment of gases, flow rate, or spray needle. See the User's Manual for Troubleshooting tips.

Tune Parameters

Ion Optics

The ion beam is continuous. Formed from the nebulizer spray and electrostatic fields, the ion beam is drawn into the capillary by the voltage exerted on it and by the pressure gradient. As it exits the capillary there is a natural tendency for the beam to spread out—to radiate like a beam of light.

The ion optics focus the ions into the analyzer and propel them against the pressure gradient. At each stage the ion beam wants to expand, so it has to be focused back down with the applied voltages.



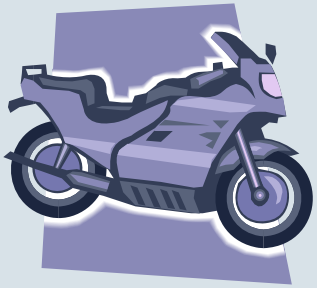
Tune Parameters

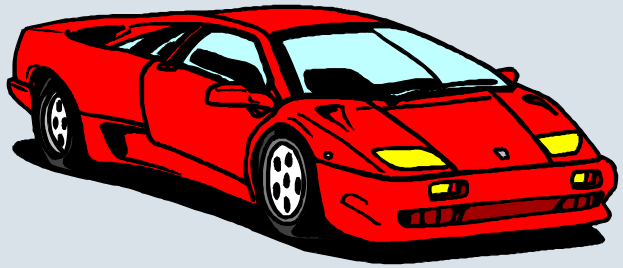
Ion Optics

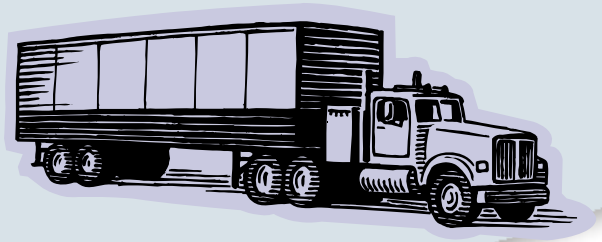
How the voltages affect the ions is largely mass dependent.

Smaller mass ions require less energy to control them.

and larger mass ions need more energy to direct them into the trap,



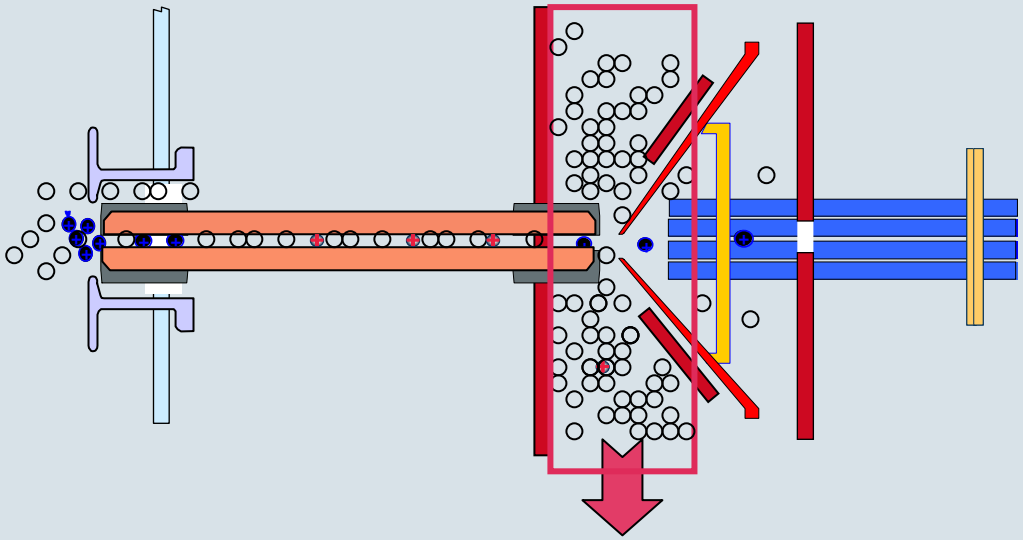




Thus we give the rule of thumb:
The larger the mass, the higher the value for the tuning parameters.

Tune Parameters

Capillary Exit Voltage

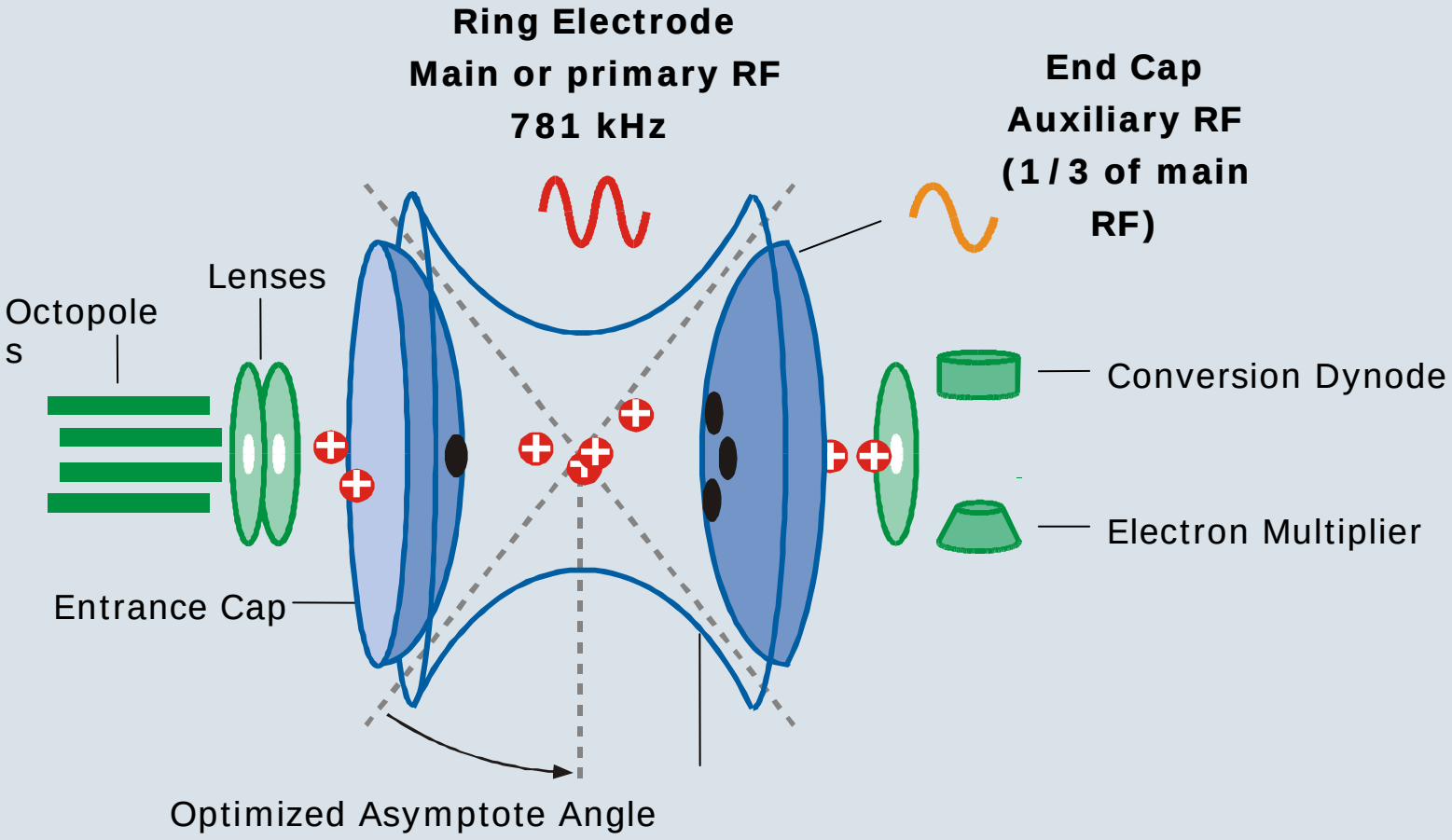


In-source fragmentation, or breaking the ions apart before they reach the ion trap, a balance must be struck between **Skimmer** and **Cap Exit** working in concert to set the voltage affecting the ions in this region. More unstable or fragile molecules need a lower Cap Exit voltage.

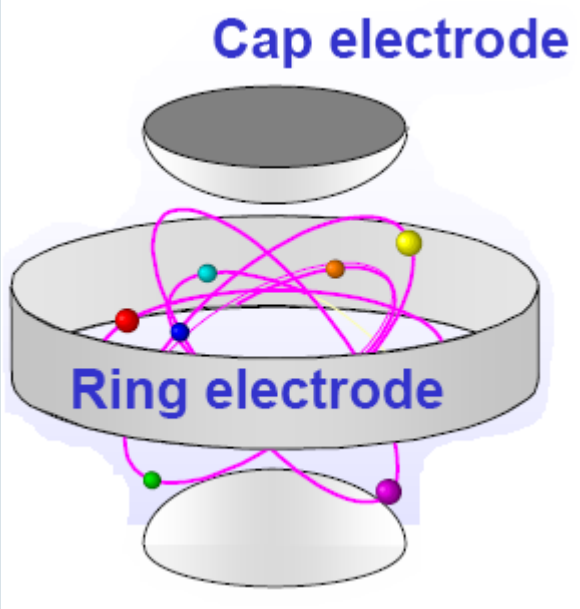
Ion Trap

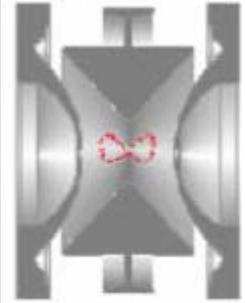
THEORY

Ion Trap and Detector System



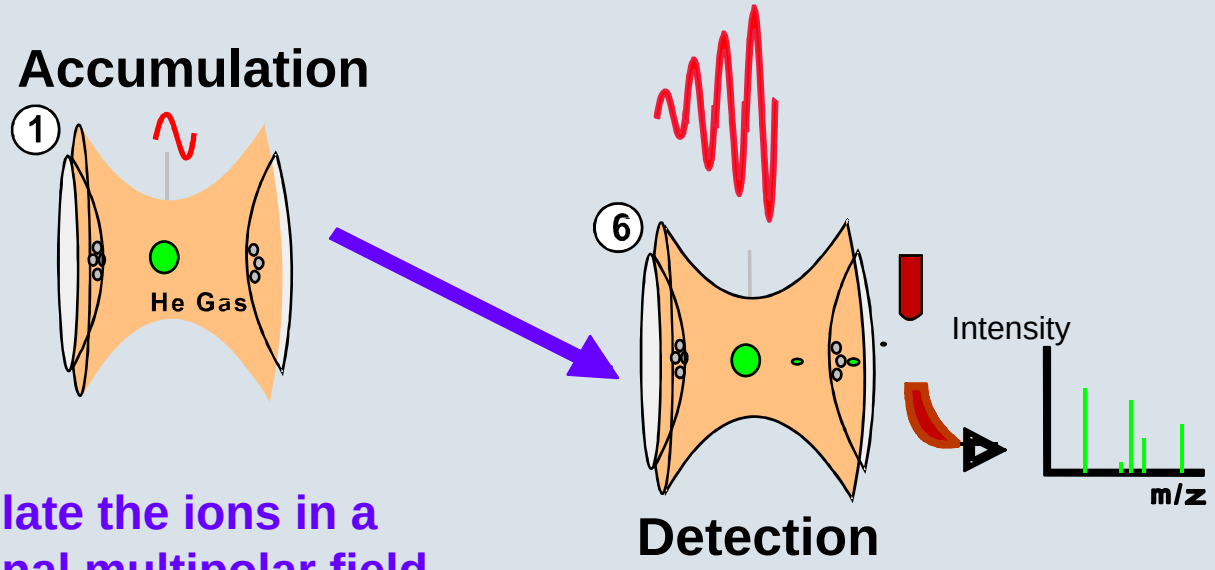
Ion Trap operations are performed by changing the voltages (amplitudes) of the primary and the auxiliary RF.





Ion path in a trap

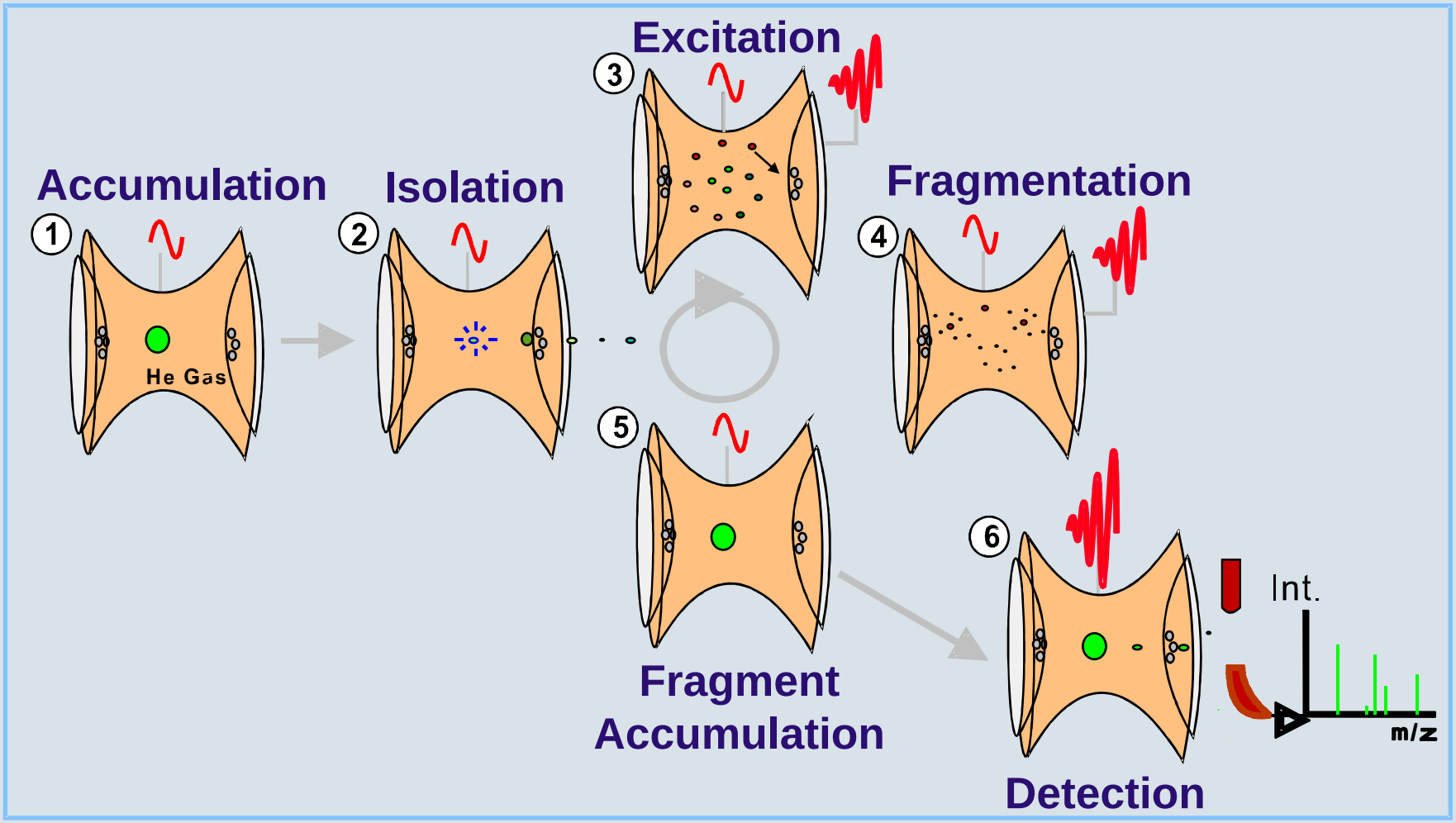
Generating a Mass Spectrum- overview



Accumulate the ions in a 3-dimensional multipolar field.

Scan the ions out of the trap in increasing mass by increasing the RF amplitude and measure the response at the detector.

Principles of Fragmentation in an Ion Trap

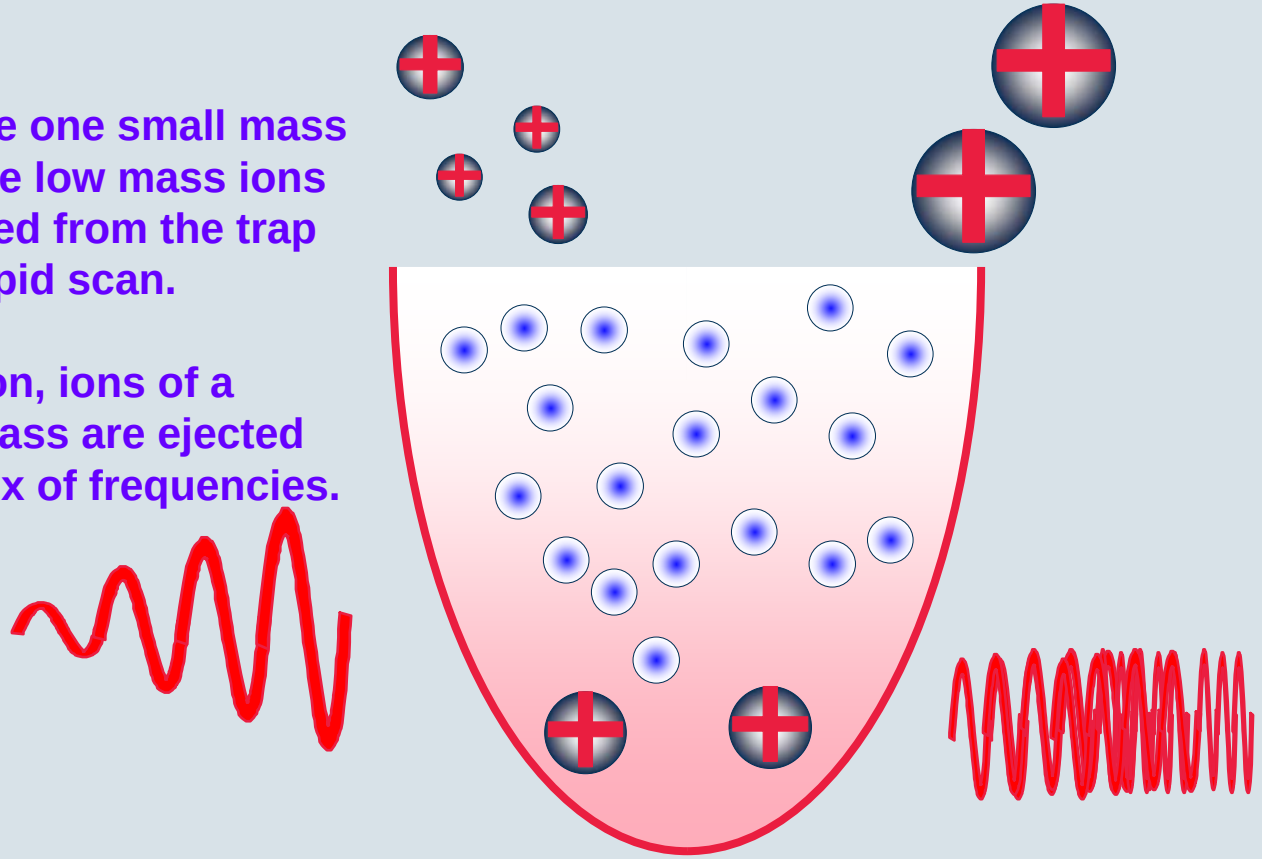


Generating MS^n spectra

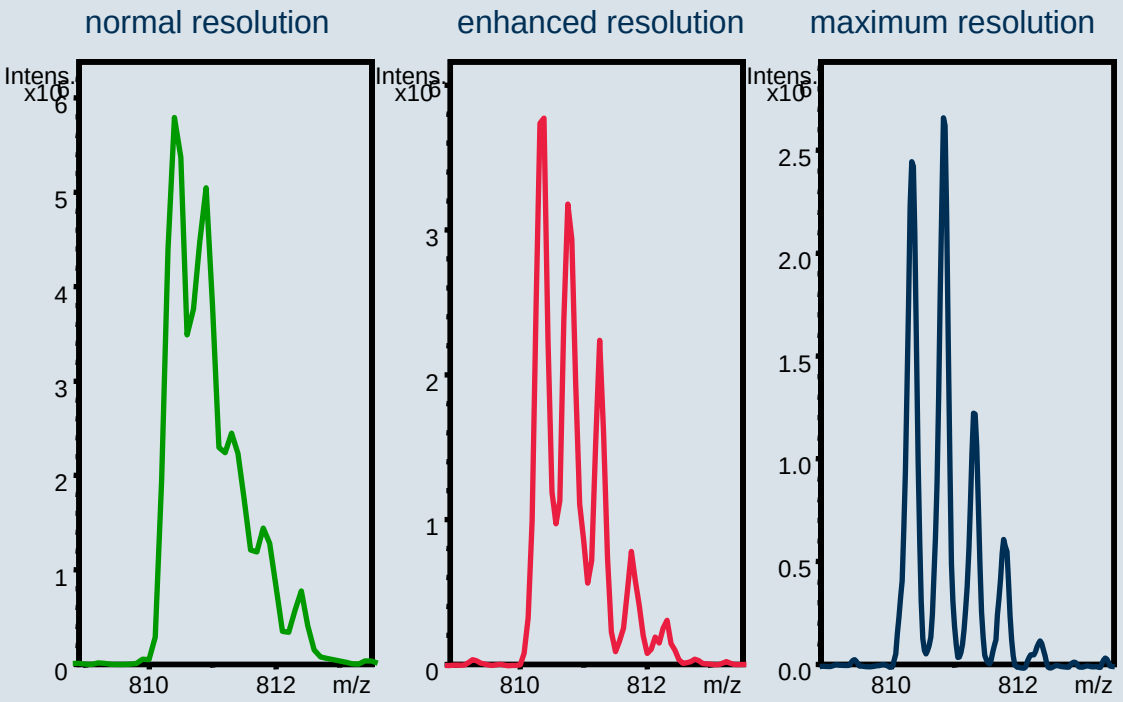
Inside the trap - isolation

To isolate one small mass range, the low mass ions are ejected from the trap with a rapid scan.

In addition, ions of a higher mass are ejected with a mix of frequencies.



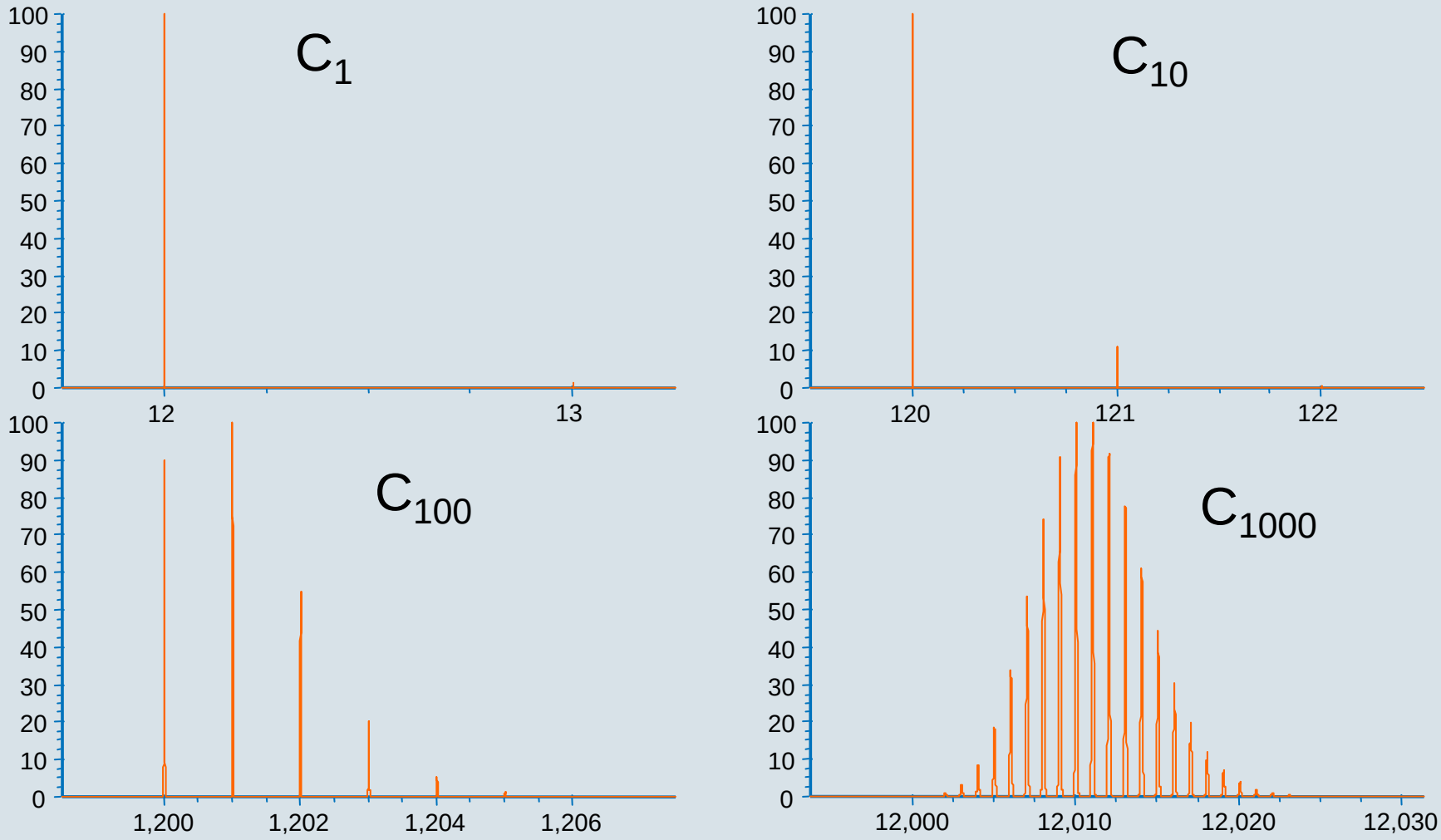
Mass Resolution depends on the scan rate



2+ ions

Here is an example of the resolution that results for a 2+ ion using different scan rates. All the scan rates are available over the full mass range of the trap. The trade off is that for more resolution there is a slower scan rate, so there are fewer data points across an LC peak. With infusion of a sample this time factor is not important.

Isotopic Distribution Patterns



Average & Monoisotopic Masses

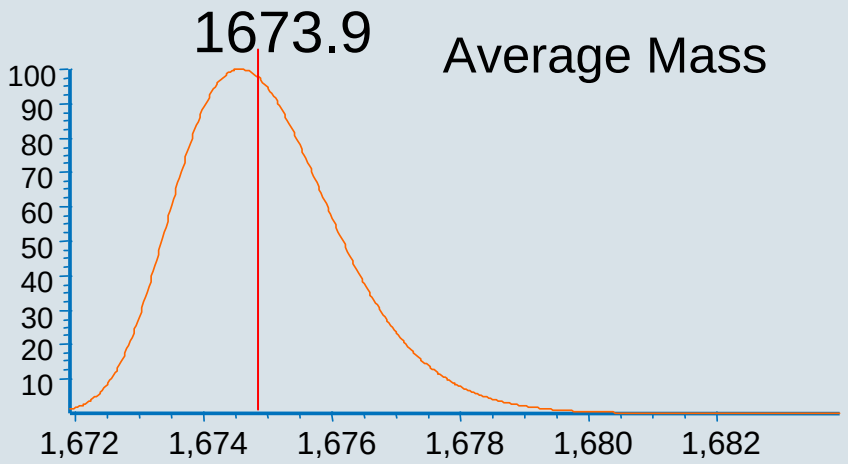
Neurotensin

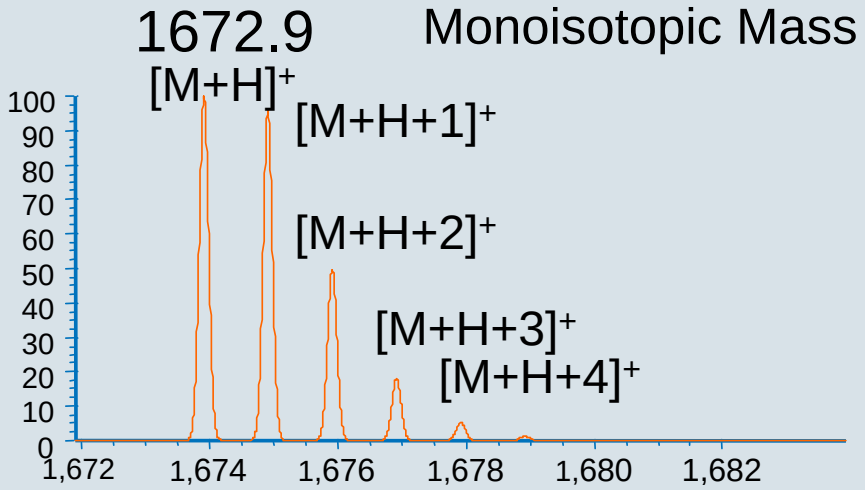
Res. 1’000

pQLYENKPRRRPYIL
MW 1672.9

Res. 10’000

Resolution = (m/z) / FWHM

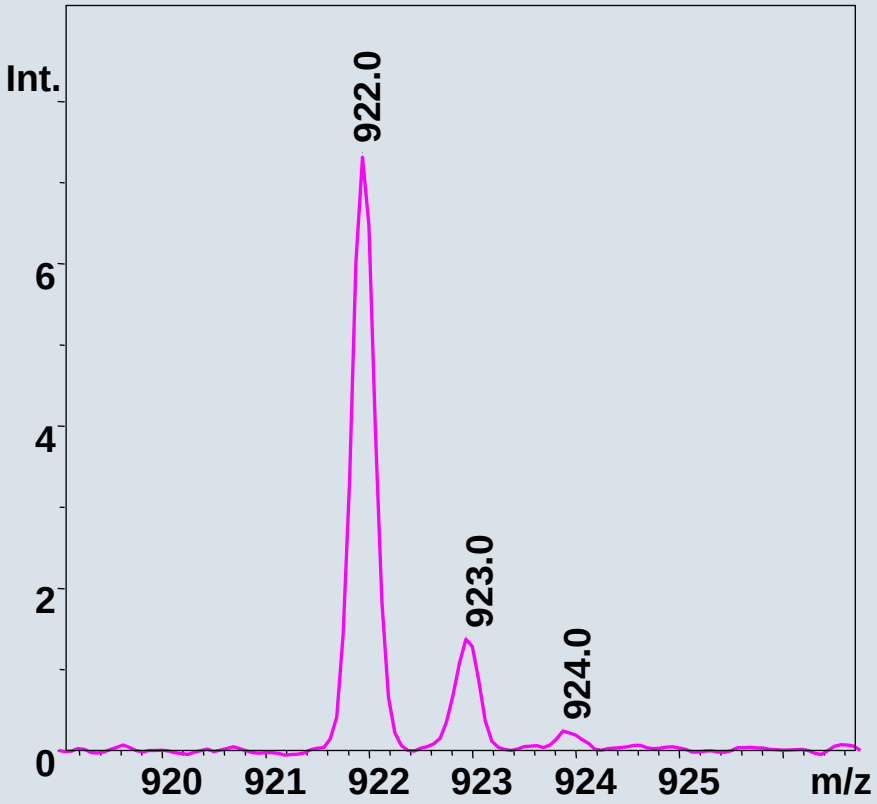




What is the peak width at 10,000 resolution?_____

Gating Ions :

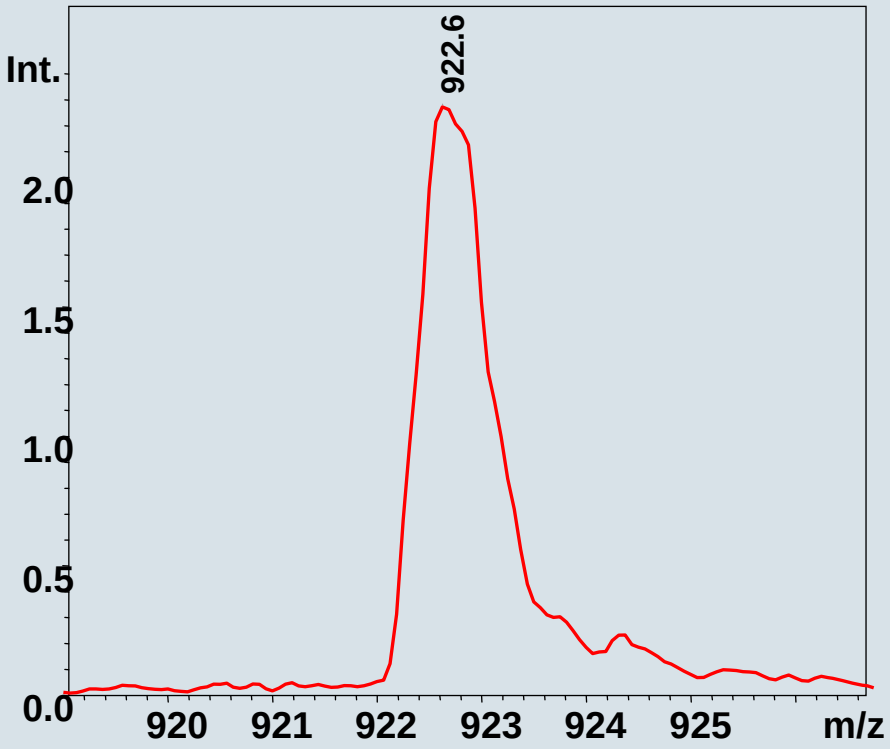
Controlling the number of ions in the trap



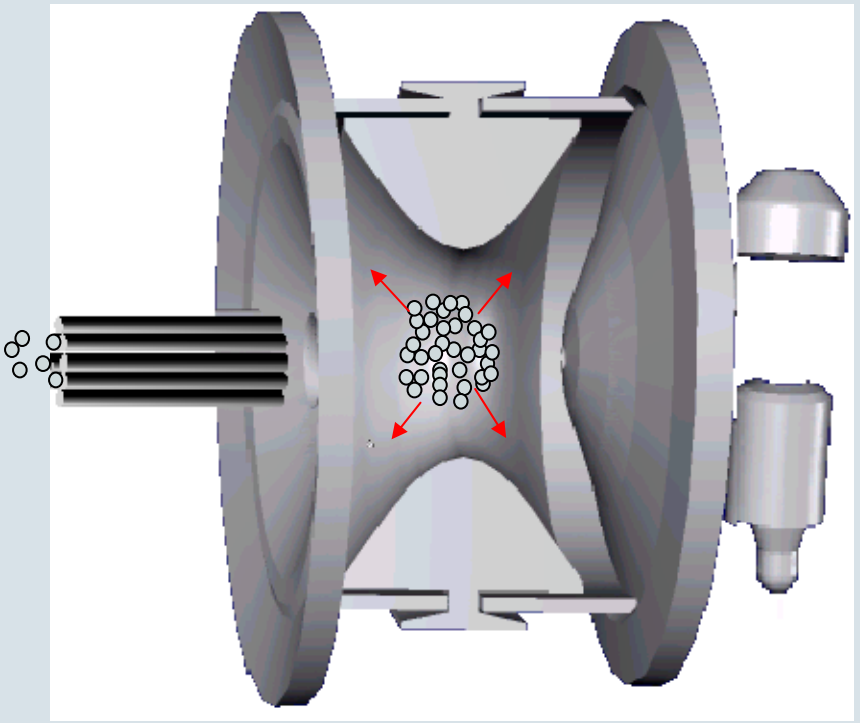
Appropriate load gives good resolution

Gating Ions :

Space Charging Effects



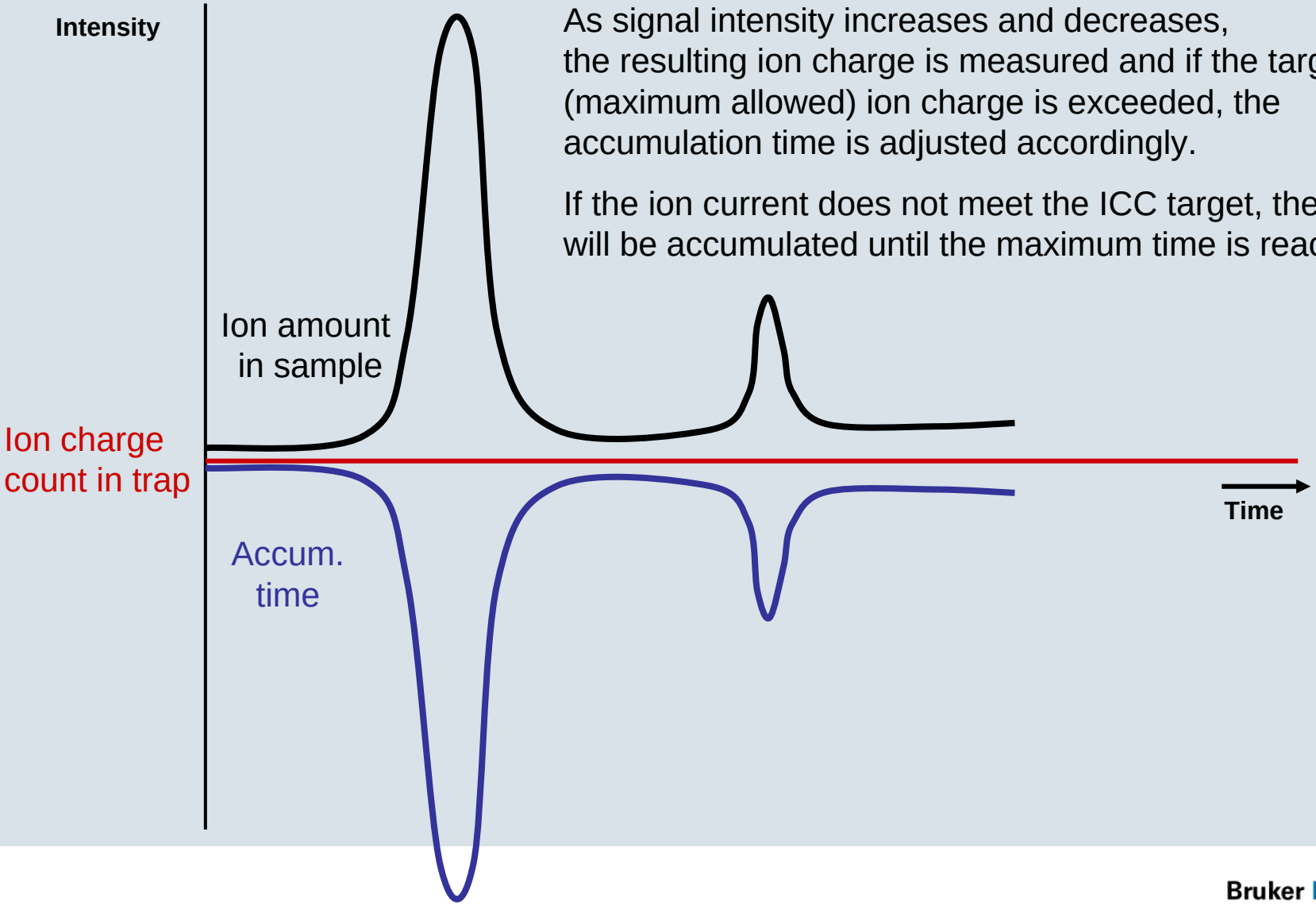
Ions “see” each other and not just the ion trap field.



Too many ions: **OVERLOAD** !

- poor mass resolution
- poor mass accuracy
- poor peak area determination

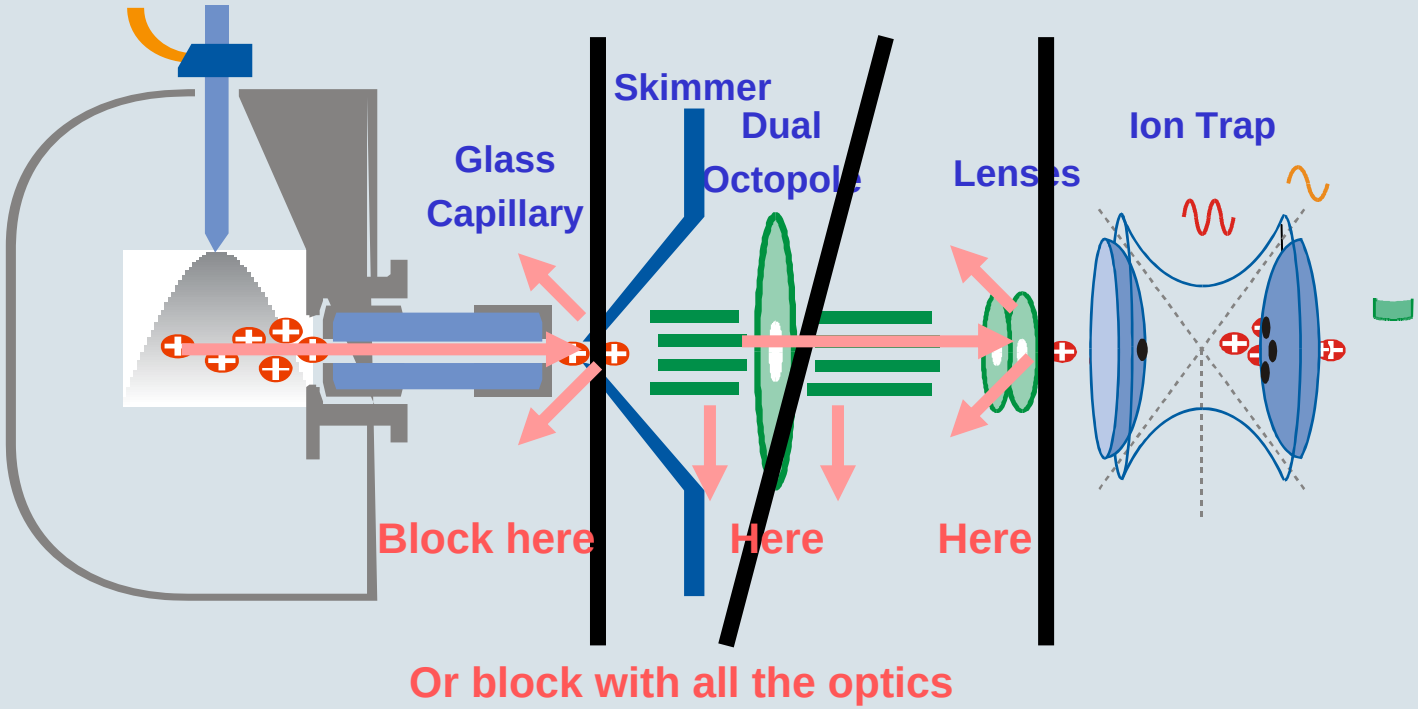
Gating ions: Ion Charge Control (ICC)



As signal intensity increases and decreases, the resulting ion charge is measured and if the target (maximum allowed) ion charge is exceeded, the accumulation time is adjusted accordingly.

If the ion current does not meet the ICC target, then ions will be accumulated until the maximum time is reached.

Blocking Voltages

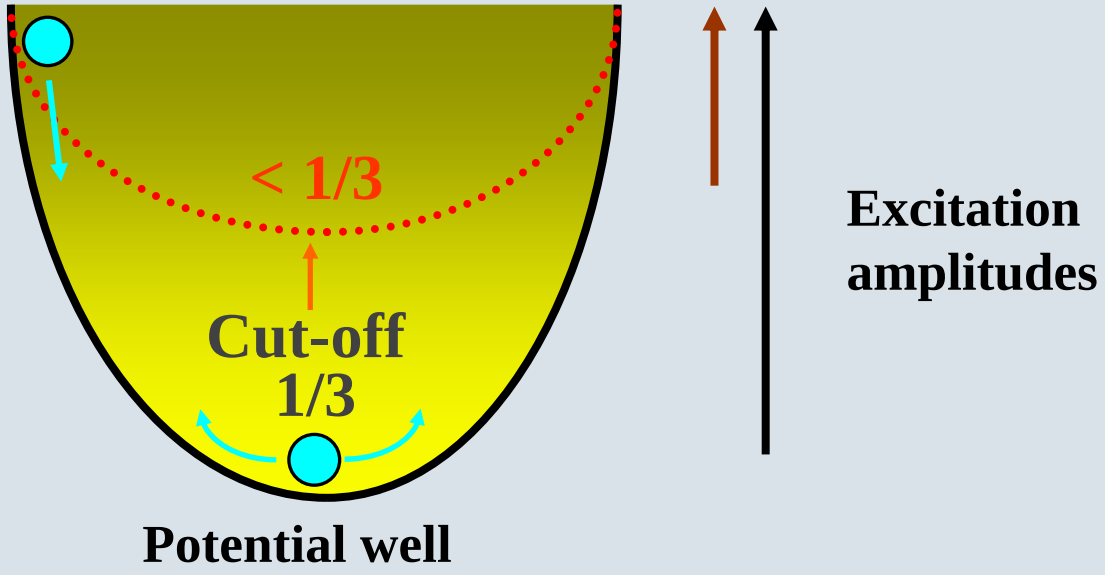


MS/MS below the 33% Limit

Changing the low m/z Cut-off for MS/MS Fragments

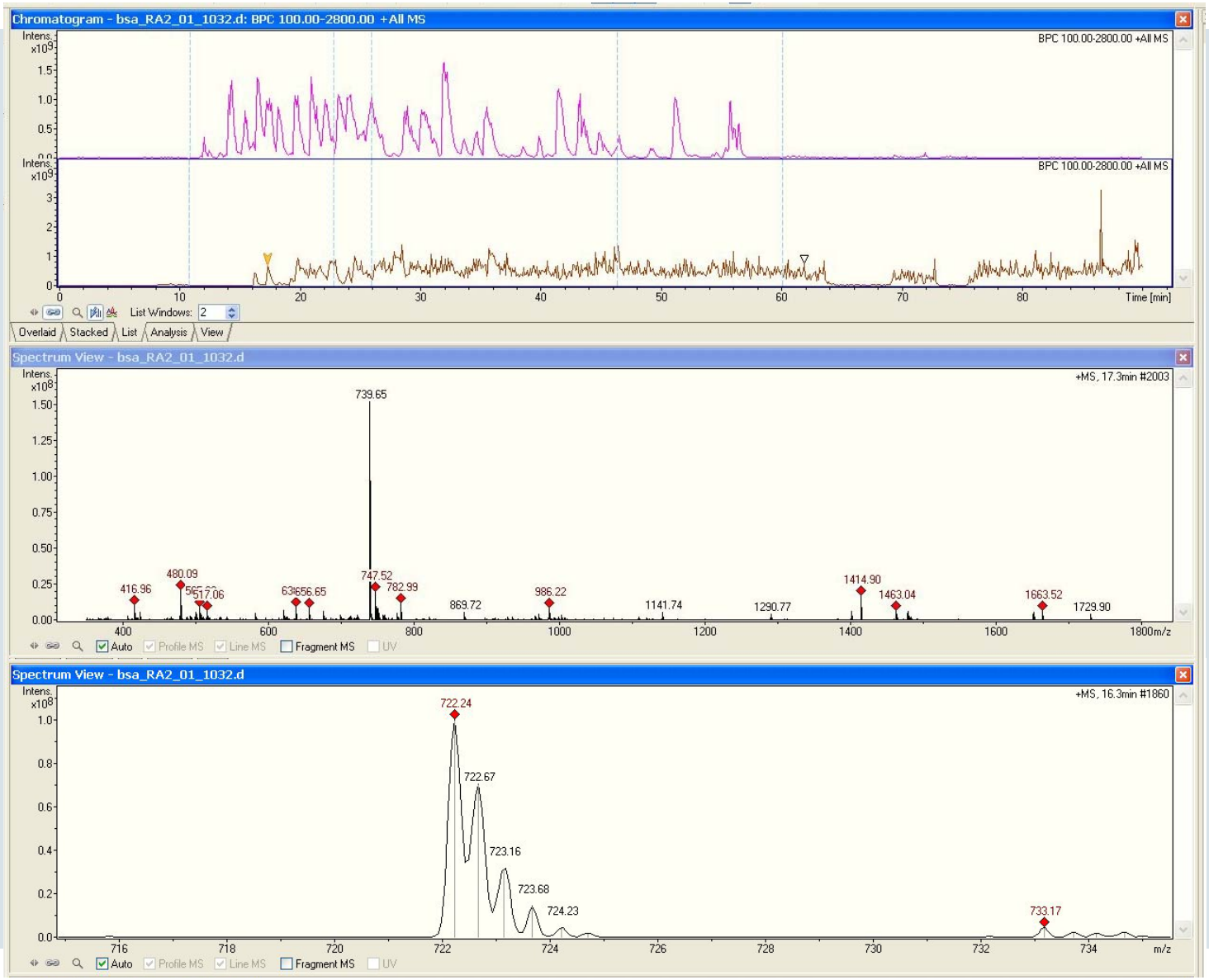
With MS/MS fragmentation, we use a preset cut-off of slightly under 1/3 of the mass that is isolated. This parameter is chosen to ensure that the isolated ion is excited most efficiently, to give the best MS/MS spectrum - the trade-off is that fragment ions of a mass below that 1/3 cut-off are not retained in the trap.

Other values can be set, to override the default cut-off. This is particularly useful if there is a fragment ion expected a little below the 1/4 value.



掃描取點越多越好！

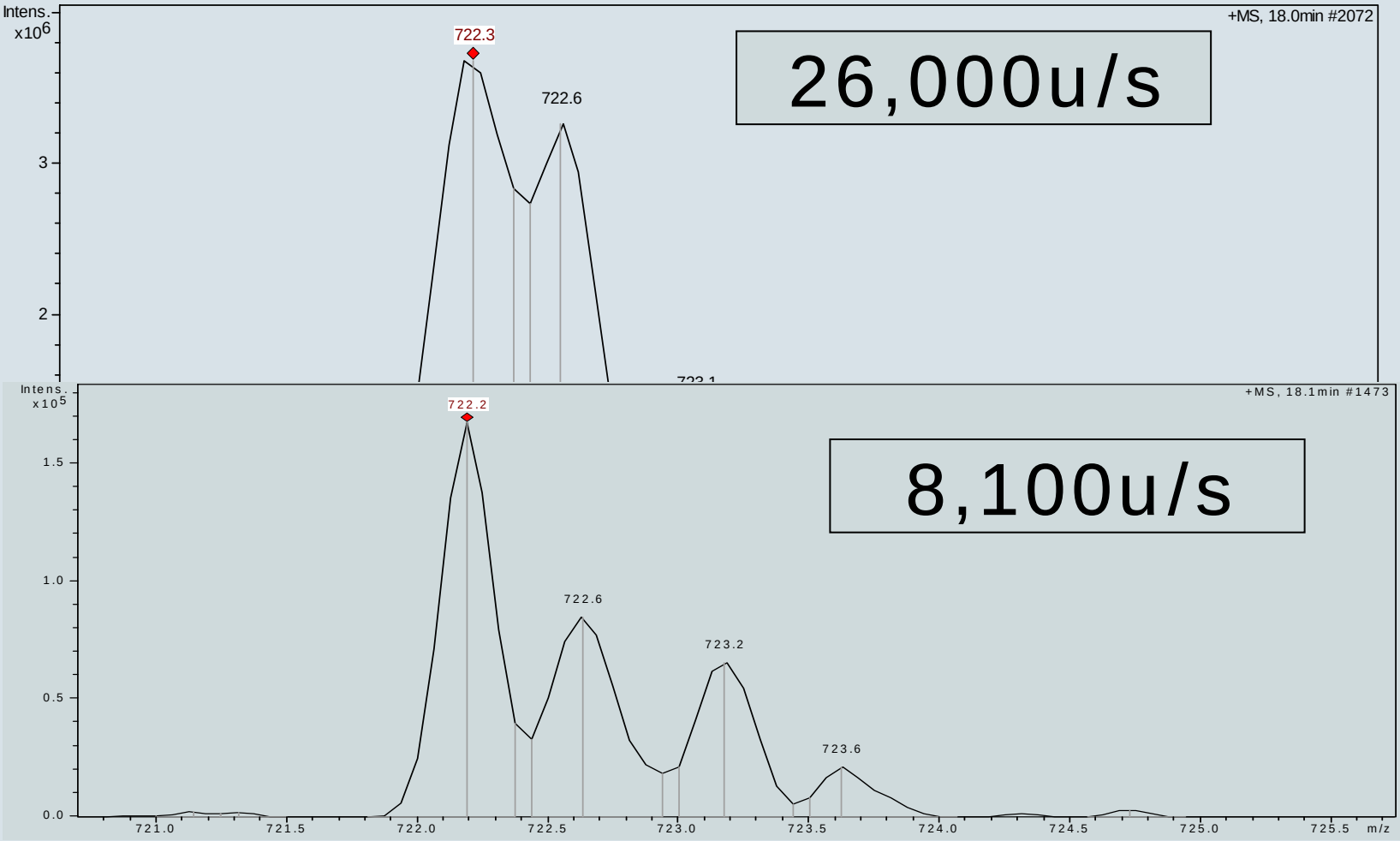
1. How complex your sample is? →LC separation
2. Pick up valuable signals! →2+/3+ ions


Chromatogram - bsa_RA2_01_1032.d: BPC 100.00-2800.00 +All MS
BPC 100.00-2800.00 +All MS
BPC 100.00-2800.00 +All MS
Time [min]
List Windows: 2
Overlaid
Stacked
List
Analysis
View
Spectrum View - bsa_RA2_01_1032.d
+MS, 17.3min #2003
739.65
480.09
416.96
517.06
656.65
747.52
782.99
869.72
986.22
1141.74
1290.77
1414.90
1463.04
1663.52
1729.90
m/z
Auto
Profile MS
Line MS
Fragment MS
UV
Spectrum View - bsa_RA2_01_1032.d
+MS, 16.3min #1860
722.24
722.67
723.16
723.68
724.23
733.17
m/z
Auto
Profile MS
Line MS
Fragment MS
UV

掃描速度越快越好？

速度越快，解析度越差

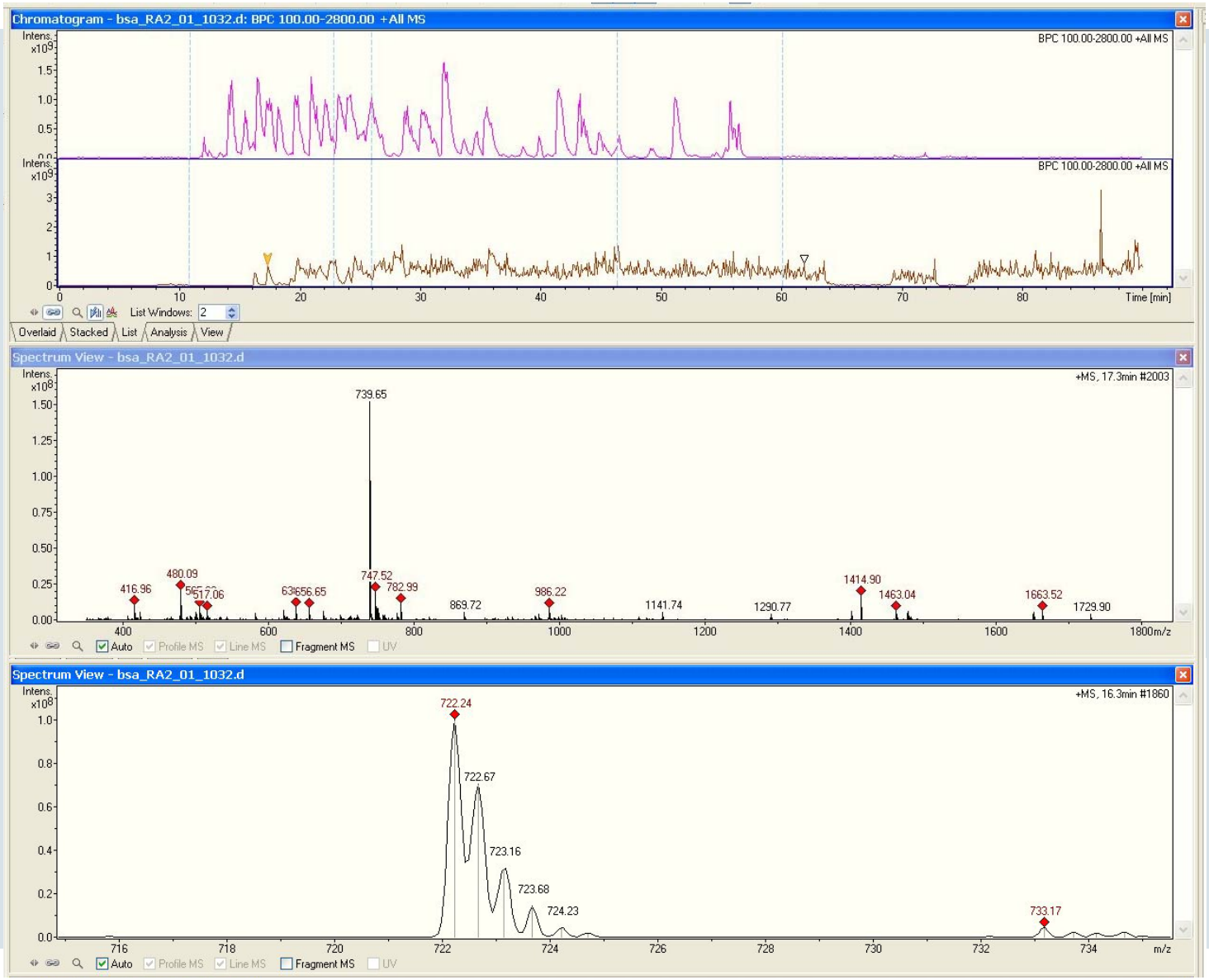
速度越慢，漏失訊號越多

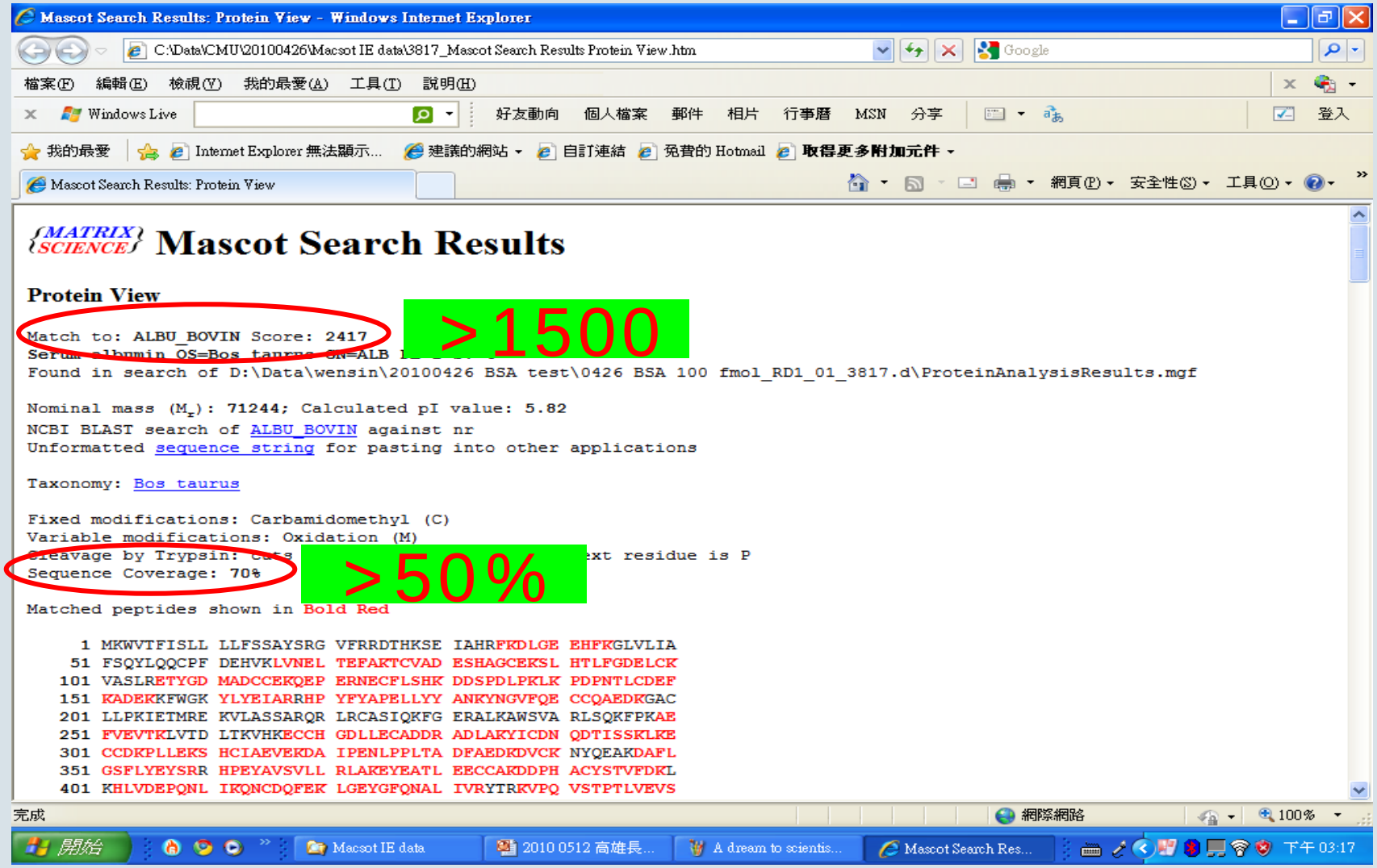

Intens.
x10^6
722.3
722.6
+MS, 18.0min #2072
26,000u/s
3
2
Intens.
x10^5
722.2
+MS, 18.1min #1473
8,100u/s
1.5
1.0
722.6
723.2
0.5
723.6
0.0
721.0
721.5
722.0
722.5
723.0
723.5
724.0
724.5
725.0
725.5
m/z

掃描範圍越大越好？

1. Ions charge state → 2+/3+
2. MS ←→ MS/MS setting
3. Duty cycle

Is the system good enough for sample test?


Chromatogram - bsa_RA2_01_1032.d: BPC 100.00-2800.00 +All MS
BPC 100.00-2800.00 +All MS
BPC 100.00-2800.00 +All MS
Time [min]
List Windows: 2
Overlaid
Stacked
List
Analysis
View
Spectrum View - bsa_RA2_01_1032.d
+MS, 17.3min #2003
739.65
480.09
416.96
517.06
656.65
747.52
782.99
869.72
986.22
1141.74
1290.77
1414.90
1463.04
1663.52
1729.90
m/z
Auto
Profile MS
Line MS
Fragment MS
UV
Spectrum View - bsa_RA2_01_1032.d
+MS, 16.3min #1860
722.24
722.67
723.16
723.68
724.23
733.17
m/z
Auto
Profile MS
Line MS
Fragment MS
UV


Mascot Search Results: Protein View - Windows Internet Explorer
C:\Data\CMU\20100426\Macsot IE data\3817_Mascot Search Results Protein View.htm
Mascot Search Results: Protein View
MATRIX SCIENCE Mascot Search Results
Protein View
Match to: ALBU_BOVIN Score: 2417
>1500
Found in search of D:\Data\wensin\20100426 BSA test\0426 BSA 100 fmol_RD1_01_3817.d\ProteinAnalysisResults.mgf
Nominal mass (Mr): 71244; Calculated pI value: 5.82
NCBI BLAST search of ALBU_BOVIN against nr
Unformatted sequence string for pasting into other applications
Taxonomy: Bos taurus
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Sequence Coverage: 70%
>50%
Matched peptides shown in Bold Red
1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
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A dream to scientis...
Mascot Search Res...
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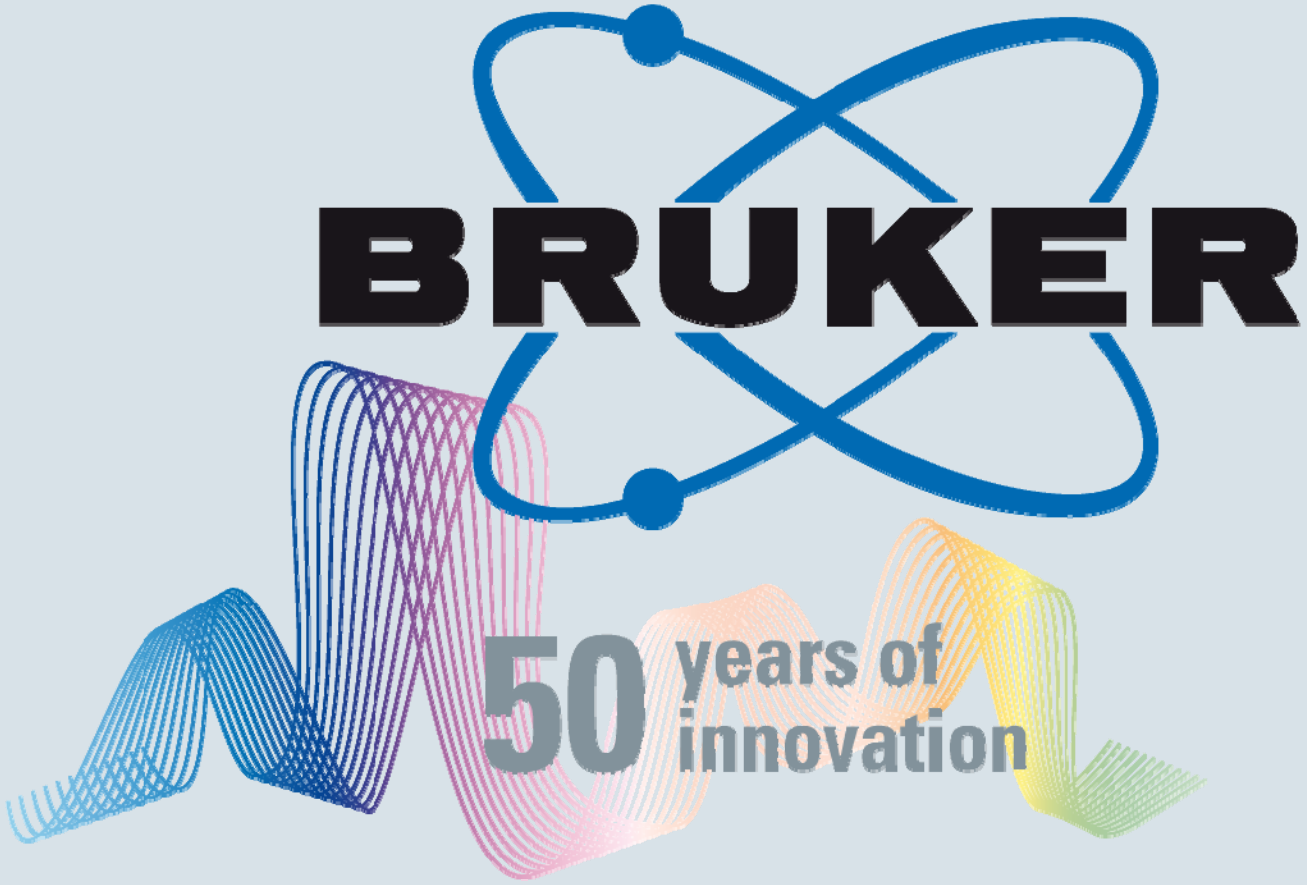

Bruker BioScience, Taiwan