A Few Notes on Safety:

1. ***Eye Protection is required in the Mass Spec Lab***. If you are handling pre-prepared closed sample vials, safety glasses or regular glasses are acceptable. If you are working with open sample vials or solvents, Safety Glasses are required.

2. ***If you are working with open solvents and chemicals***, lab coats should be worn. Lab coats are available in the Mass Spec lab for your temporary use.

3. ***In case of a Spill or broken sample***, please contact IMSERC staff. If this happens after working hours…….

4. ***In case of instrument error***, please contact IMSERC staff. *Do not attempt to fix errors, unless you have been instructed to do so by IMSERC staff.* If errors occur after working hours or staff is not available, fill out and put a **STOP SIGN** on the instrument and fill out a Bug report.

5. ***Please report ALL issues*** (instrument error, Instrument contaminations, Spills, area left in untidy manner, etc. to staff ***using BUG REPORT***.

Sample Preparation

- Samples for GCMS and LC-MS instruments should be prepared in appropriate ***Auto Sampler Vials.***

- ***Sample Concentrations*** should never exceed 500ug/ml for GCMS. For LC-MS/ESI instruments, concentrations should be 100ug/mL or less.

- ***Always use HPLC grade solvents***

- ***PLEASE SEE Sample prep guide for sample submission*** for more information on LC-MS-ESI and GC-MS sample prep, Autosampler vials, part numbers**:**
  http://imserc.facilities.northwestern.edu/mass-spectrometry/sample-submission/

- For Maldi: Sample Prep please consult with IMSERC Staff. Maldi sample plates can also be obtained from IMSERC staff.

To Get Trained…

- **To get trained you will need to apply for the appropriate instrument depending on:**

1. The type of samples you want to analyze- determined by the chemical structure, boiling point, solubility.

2. The type of data you require: Low resolution (reaction monitoring), High resolution (confirmation), Quantitation, MS/MS

Which Mass Spec is right for Me?

- You will also be given a quick tutorial of how to judge which instrument will work best for your samples based on ***Inlet (Sample intro) , Ionization source, and Mass Analyzer….***

Which Mass Spec is right for Me?

Tutorial:

The most important decision is which instrument to sit in front of, which instrument to choose; not just how you run it.

1) Safety- Unlike NMR or X-Ray (where your sample does not enter the instrument), you are always injecting/ entering your sample into the instrument- You can do damage!
2) Unlike NMR Mass Spectrometry has great variation in the instruments: LC-MS, GC-MS,MALDI-TOF, ESI, EI ionization, Ion Trap, TOF, Quadropole….etc. This can lead to confusion with the abbreviations and hyphenations…

3) Success- Do you get your Data?

3 Box Model of Mass Spec

You can break down any Mass Spectrometer into 3 Modules:

Inlet , Ionization source, and Mass Analyzer/detector

Inlet/sample intro > Ionization > Mass Analyzer (Spectrometer)

3 Box Model of Mass Spec

Any Mass Spec can be broken down into 3 modules. See examples below:

Sample Intro - Ionization - Mass Analyzer (Spectrometer)

Examples:

GC-MSD:

GC - EI (MS) - Quadrupole (MS)

LC/MS-TOF:

LC - ESI (MS) - TOF (MS)

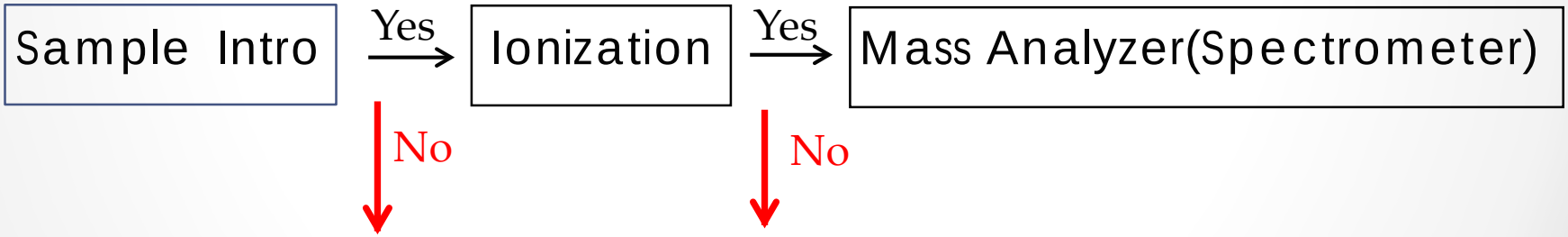
MALDI-TOF:

MALDI - TOF (MS)

3 Boxes

The main question however, is ***"Does your sample make it through each module?"***

Each box has ***limiting factors*** which allow any certain sample to pass or fail (pass or fail the inlet, pass or fail the ionization technique or analyzer) Let's look at the criteria for each....



For each box, if your sample meets the limiting factor then you consider next module; if not, then you are choosing the wrong instrument.

Inlet

. **GC (Gas Chromatography):**

1. Gas(G)- Is your sample Volatile? Make it to Gas phase within 300^{o}C?
2. Chromatography(C) –Are you using the right column?

. **LC (Liquid Chromatography):**

1. Liquid (L)-Is your sample soluble in the Solvent used on the instrument?
2. Chromatography(C) –If you are not doing a direct injection, and doing chromatography- Are you using the right column?

. **MALDI:**

1. Are you using the right Matrix?
2. Are you using the correct Matrix/sample ratio?
3. Are you plating the sample correctly?

Ionization

- **Electron Impact (EI)-** relies on a 70eV electron beam impacting the Molecule, resulting in knocking an electron (+ ion) or a fragment off. (This produces Odd electron Ions). ***Sample in gaseous phase and usually works with smaller molecules.***

- **ElectroSpray Ionization (ESI)-** relies on ionization through charged droplets and formation of adduct ions (even electron) Therefore ***samples must have available functional groups to Protonate or Deprotonate or form other adduct ions.*** (Can make multiply charge ions and dimers, and may give fragments but does not rely on fragmentation for ionization).

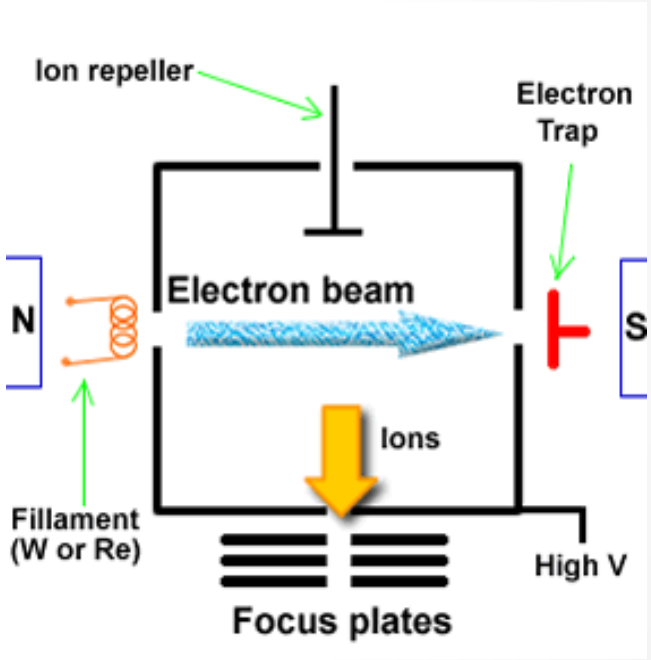
- **Matrix Assisted Laser Desorption Ionization (MALDI)-** relies on desorption and ionization of sample by laser enregy with the help of matrix. Can form adduct ions like ESI. Charging usually limited to single or double charges. ***Sample has to crystalizes or co-crystalize with Matrix on plate.***

Electron Impact Ionization (EI)

"Hard" Ionization Technique
- 70 eV electrons from W filament
  Small magnetic field to spin electrons
  Energy Transfer

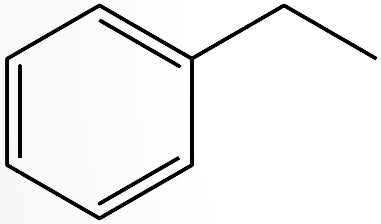
Fragmentation Data

- $OE^{+\bullet}$
- Predictable fragmentation pattern to piece molecules back together
- Stored in libraries -NIST
- Molecular Ion may be present



http://www.hull.ac.uk/chemistry/masspec3/images/electron%20ionisation.gif

Electron Impact Ionization (EI)

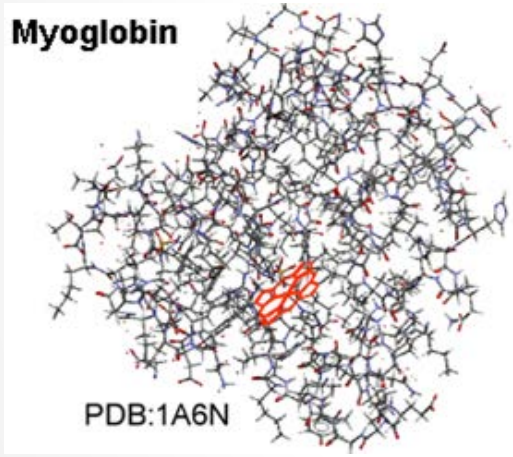


70eV

EI

OK- with fragmentation

Large Molecules (i.e.Proteins)



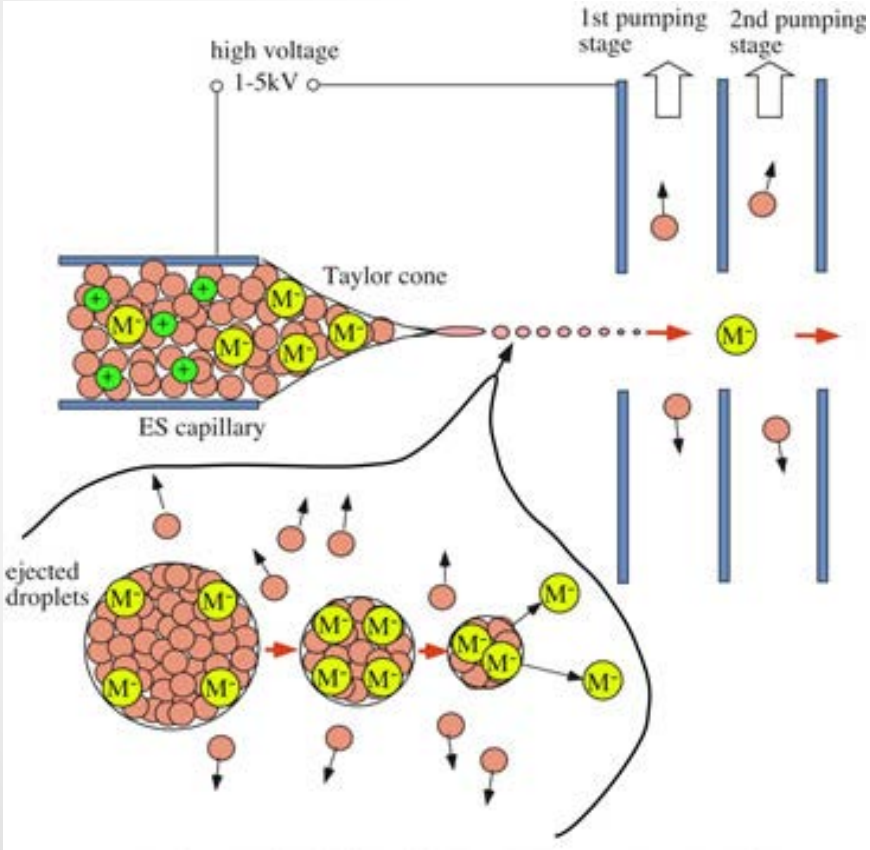
70eV

EI

X

Not Much happens- Not suitable
(Also Non-volatile)

Electrospray Ionization- ESI

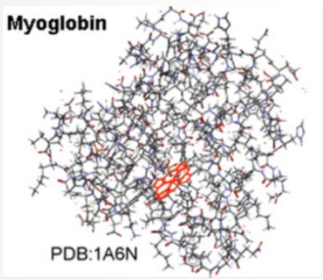


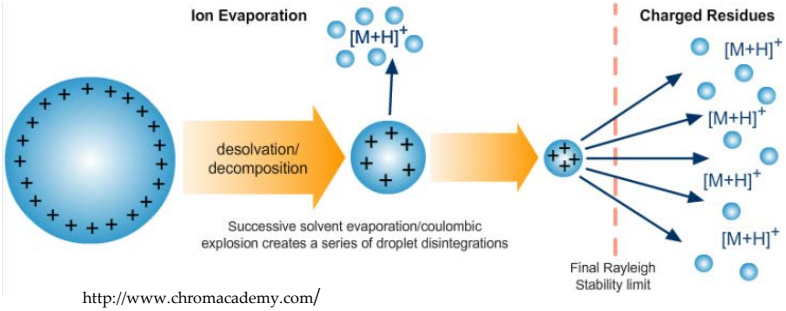
- Analyte is nebulized through charged needle, by sheath of gas
- Spray forms Taylor Cone
- Droplets desolvate becoming smaller and smaller , forcing charged molecules which are repelling each other, closer and closer
- Droplets approach Rayleigh Limit
- Charge Repulsion overcomes the surface tension of droplet
- Coulombic explosion to get desolvated ions in gas phase.
- Works with Polar Molecules, Proteins,peptides, nucleotides, oligosaccharides, polymers , etc.

http://rsl.eng.usf.edu/Images/Research/ElectrospraySchem.jpg

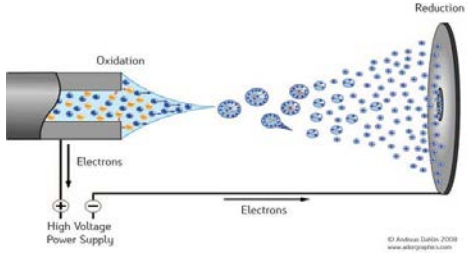
Large Molecules (i.e.Proteins)

ESI

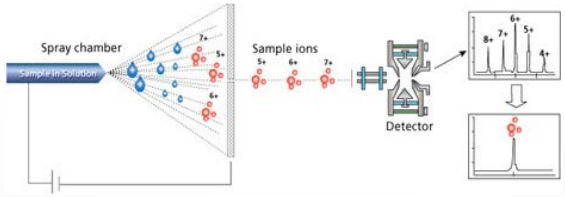




http://www.chromacademy.com/

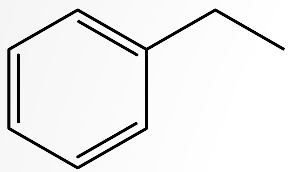


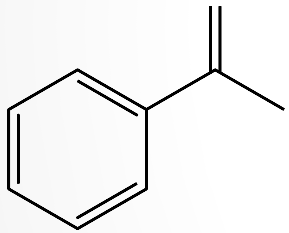
www.waters.com

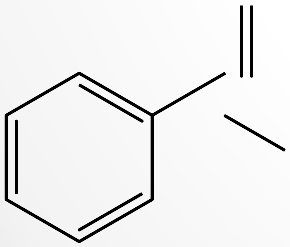


http://www.sigmaaldrich.com/technical-documents/articles/biology/custom-dna-oligos-qc-analysis-by-mass-spectrometry.html

Electron Impact Ionization (EI) examples versus…







EI

70eV

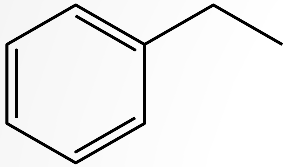
OK- with fragmentation

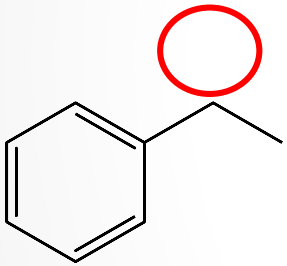
OK- with fragmentation

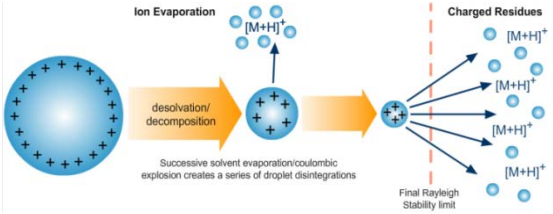
OK- with fragmentation

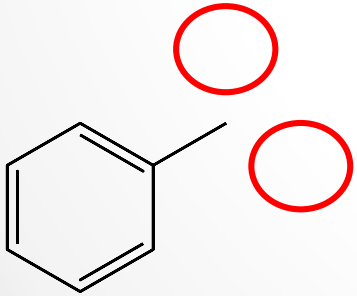
Electrospray Ionization (ESI)

ESI









X

No Signal

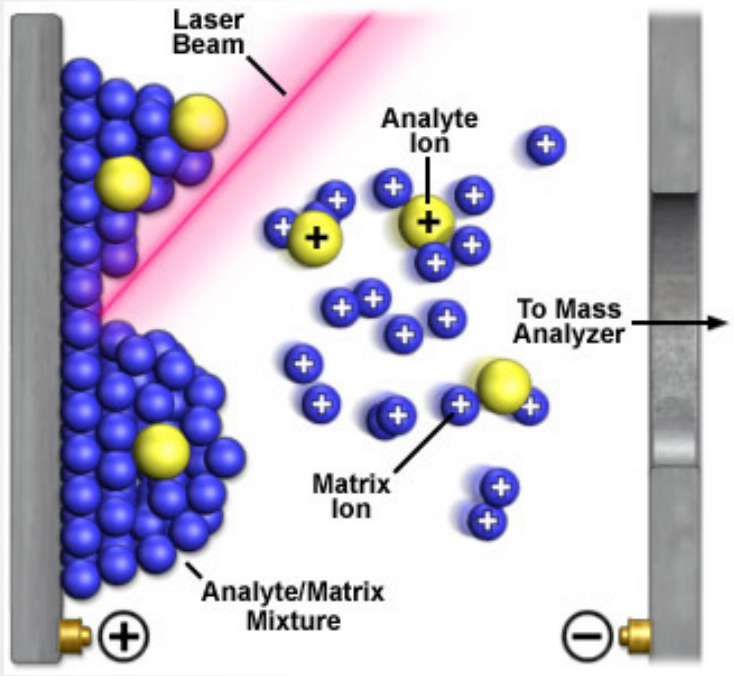
✓

OK - with $M+H^+$
$M+Na^+$, $M+NH4^+$,
etc.

✓

OK – Negative Ion with M-H,
(or potentially Pos Ion $M+Na^+$)

MALDI



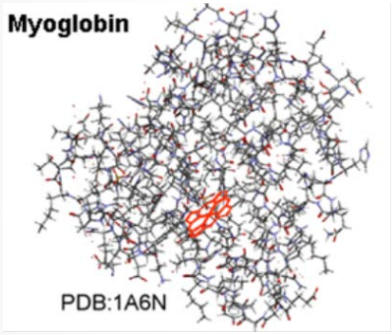
- Sample introduction coupled to ionization
- Analyte is co-crystallized with a matrix, a small molecule which can readily absorb the energy of laser and passes it to analyte.
- Laser ablates sample and provides energy to analyte via matrix to ionize it.
- Ionization dependent on laser power, matrix, and structure

- Yields $[M+H]^+$ ions, soft ionization
- Preferable for large molecules, proteins, peptides, polymers as matrix forms clusters at low *m/z*
- Useful for imaging experiments

http://www.magnet.fsu.edu/education/tutorials/tools/images/ionization-maldi.jpg

Large Molecule (i.e.Protein) Maldi & ESI

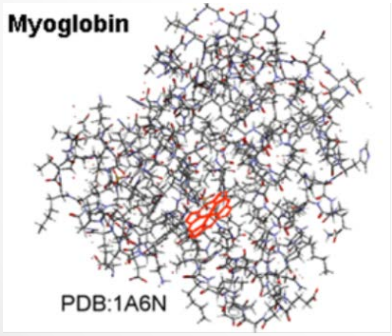
MALDI



Maldi usually produces singly or doubly charged ions:

z = 1 or 2 (rarely 3)

ESI

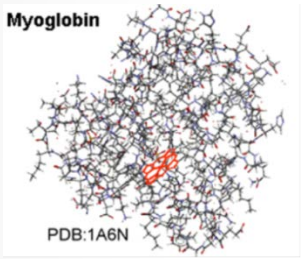


ESI can produce *Multiply* Charged ions:

z = 1,2,… 9, 10, 11, 12,…

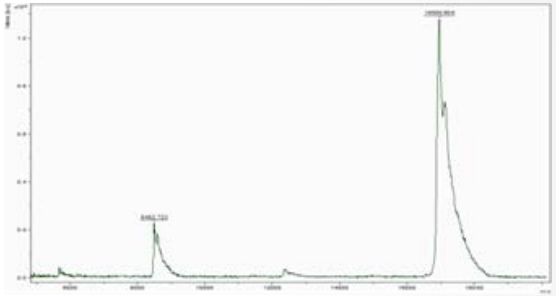
Large Molecule (i.e.Protein) Maldi & ESI

MALDI

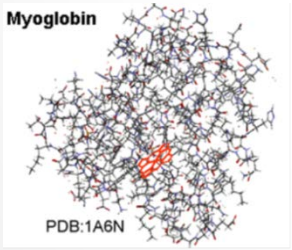


Maldi usually produces singly or doubly charged ions:

z = 1 or 2 (or rarely 3)

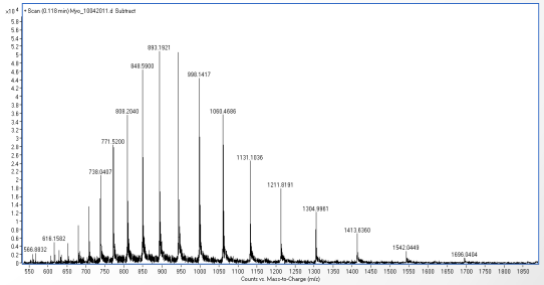


ESI



ESI can produce ***Multiple*** Charges:

Z = 9, 10, 11, 12, 13,….



ESI Adduct Ions

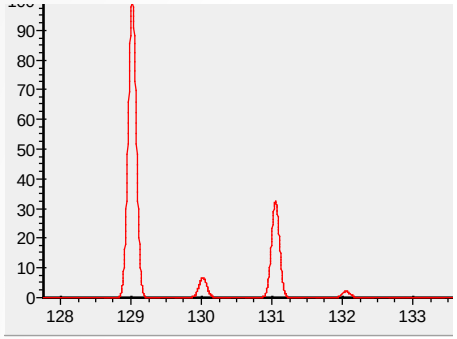
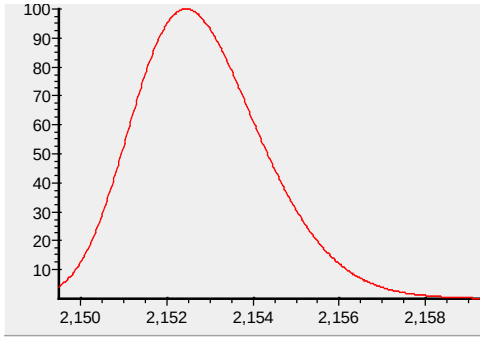
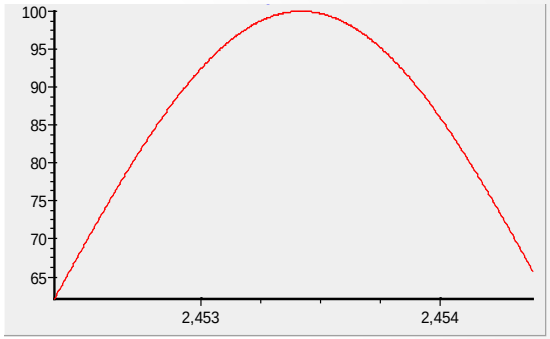
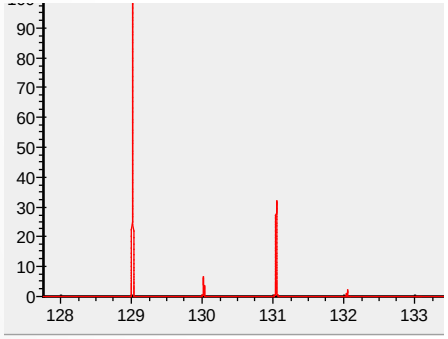
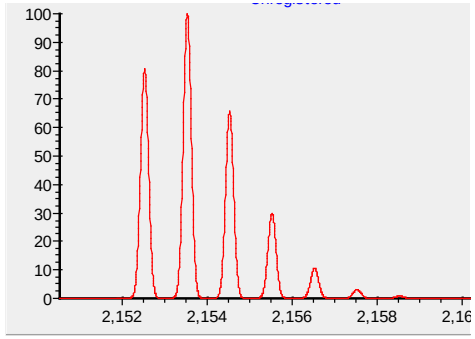
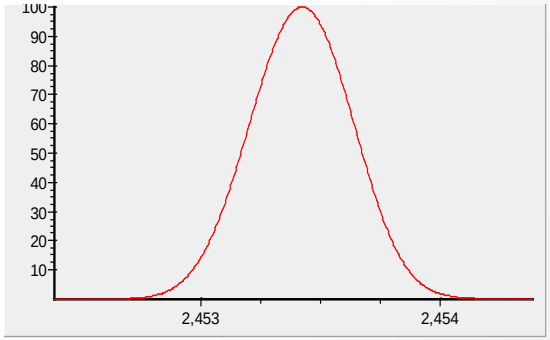
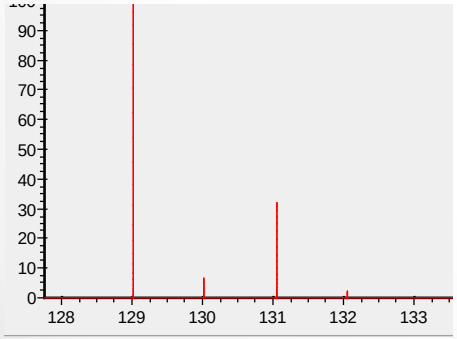
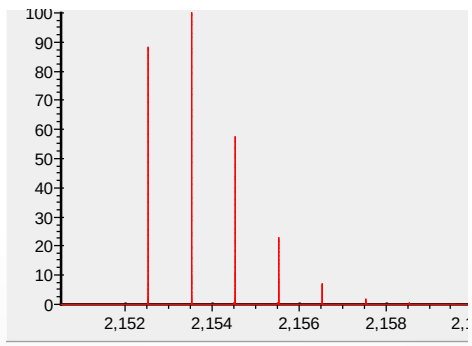
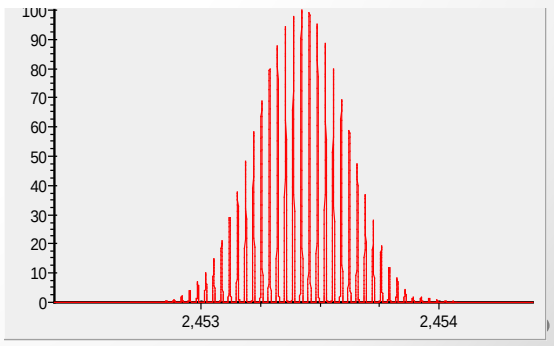
NEG IONS		POS IONS
$M\text{-}CH_3COO^-$ (M+49)		
$M+Cl^-$		
$M\text{-}H^+$		
(Cl , Br, organometallics)	M*	(Organometalics, M-Cl)
		$M+H^+$
		M+NH4 (M+18)
		M+Na (M+23)
		M+K (M+39)
		$[M]^{+x}$ x= # $[PF6]^-$ (M-145)
		$[M]^{+x}$ x= # $[TFA]^-$

Mass Analyzers

In IMSERC:

Time of Flight (TOF)	- High Resolution: resolution 10,000. Can resolve peaks <0.1amu apart. Can show distinct isotopes peaks for species with 10 charges, z=10)
Ion Trap	- Mid Resolution: resolution 3000+. Can resolve peaks 0.4amu apart can show distinct isotope peaks for species with 4 charges, z=4)
Quadropole	- Low Resolution(resolution1000-2000. Can resolve peaks 0.5-1amu apart. Can distinct isotope peaks for species with 1-2 charges, z=1 or 2)

Impact of Resolution

	Small Molecule	Peptide	Protein 73K at 30+
1000 Quad			
10000 TOF			
500,000 FTMS			

Sample Prep "Do's and Don'ts"

In order to prevent contamination and consistently obtain high quality data, adhere to the following ***Do's*** and ***Don'ts***:

Do:

Use only HPLC grade solvents.
Transfer solvents from large containers to smaller containers (<100 mL) to limit impacts of contamination events.
Use only new, clean pipette tips when transferring solvents. Use separate pipettes to transfer concentrated samples.
Immediately cap solvents when not in use.
Store organics in glass containers and water in plastic containers.
Rinse / Rinse / Rinse:

- Syringes before storing
- Glassware before transferring solvents

Don't:

Ever put anything but a clean pipette in HPLC grade solvent container.
Return solvents to an HPLC grade solvent container
Use a sample container with a lid liner which is glued in place. Solvents leach glues and cause contamination of the instruments
Take samples from deuterated solvents, especially if samples contain exchangeable protons.
Submit samples in DMF or DMSO. These solvents dissolve the HPLC tubing which then must be replaced.
Run samples with high Salt and non-volatile Buffers.
Run sample if they are not fully dissolved

Sample Preparation for Mass Spec

DILUTE your SAMPLES!

- Mass Specs are >1000x more sensitive than an NMR
- Always best to run a LOD test to determine how low you can go!!!
- Series of different concentrations
- Sample prep varies for different instruments

ESI:

AmaZon X

- Non-volatile compounds
- Dissolved in polar solvents (MeOH, ACN, H_2O, aqueous buffers (50 mM or less))
- Concentration should be between 1 and 100 µg/mL
- Remember salt concentration should be low! – ion suppression

AmaZon SL

- Non-volatile compounds
- Dissolved in non-polar solvents (DCM, hexane, benzene, MeOH)
- Concentration should be between 1 and 100 µg/mL
- Remember salt concentration should be low! – ion suppression
- Samples must be run with a small amount of MeOH

For ESI instruments DO NOT use DMF, THF, DMSO unless they are under 10% of the total volume of solvent!

Sample Preparation for Mass Spec

MALDI-ToF

- Sensitive instruments! Dilute!
- Non-volatile Compounds
- Dissolved in a volatile solvent(MeOH, ACN, DCM, hexane, etc.)
- Concentration should be between 0.05 and 1mg/mL of the stock analyte solution
- Concentration of matrix should be ~10 mg/mL
- Mix together 1 µL of stock analyte solution with 9 µL of Matrix solution (solvents should be miscible)
- Spot 1 to 2 µL on your plate (nanograms of material on the plate!!!)
- **VERY IMPORTANT** to do a Concentration range with first sample
- Other cations can be added to increase ionization, Ag+, Na+, K+

MALDI is LESS sensitive at HIGHER concentrations
More sensitive within the femtomole to picomole range of compound

GC/MS

- A relatively low boiling point (<250^{o}C)
- Dissolved in a volatile solvent(DCM, hexane, etc.)
- Concentration should be between 0.05 and 1mg/mL

QUIZ

- Take a few minutes to go through these questions below to see what you retained, and how this applies to *YOUR* sample

What if an error occurs?

If an instrument error occurs…
(Check All That Apply):

A. Reboot computer
B. Contact IMSERC staff
C. If Autosampler error power cycle Autosampler
D. Fill out and put “Stop Sign” on instrument, if Staff not available
E. Fill Bug report
F. Remove sample leave instrument just as is.

Sample Prep

Which ones are “DO’s” and which ones and “DON’Ts”

• Use concentrated samples (since it gives more intensity)	Do?	Don’t?
• Use HPLC Grade solvents even if you are not doing HPLC	Do?	Don’t?
• Use Screw cap vials (Screw cap with glued cap liner)	Do?	Don’t?
• Use Screw cap vials (Agilent Type Auto sampler)	Do?	Don’t?
• Use DMSO as solvent for submission of samples with difficult solubility	Do?	Don’t?
• Run samples if they have salts as long as the salts are dissolved	Do?	Don’t?
• Run samples partially dissolved- as long as some is dissolved	Do?	Don’t?

What is the ***main*** limiting factors in each case?

GC:

A) Molecular weight
B) Solubility
C)Polarity
D) Boiling Point/ Volatility
E) Structure/ Functional Group

LC:

A) Molecular weight
B) Solubility
C)Polarity
D) Boiling Point/ Volatility
E) Structure/ Functional group

ESI:

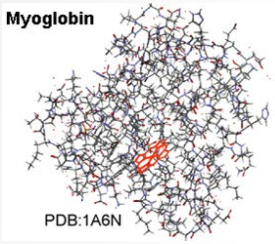
A) Molecular weight
B) Solubility
C)Polarity
D) Boiling Point/ Volatility
E) Structure/ Functional Group

EI:

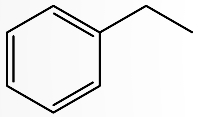
A) Molecular weight
B) Solubility
C) Polarity
D) Boiling Point/ Volatility
E) Structure/ Functional Group

Ionization

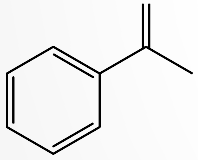
Which ionization technique is suitable for each analyte?
(Choose All that apply to each)



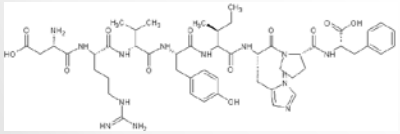
A) EI B) ESI C) Maldi



A) EI B) ESI C) Maldi



A) EI B) ESI C) Maldi

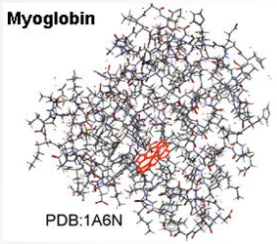


Peptide (Angiotensin II)

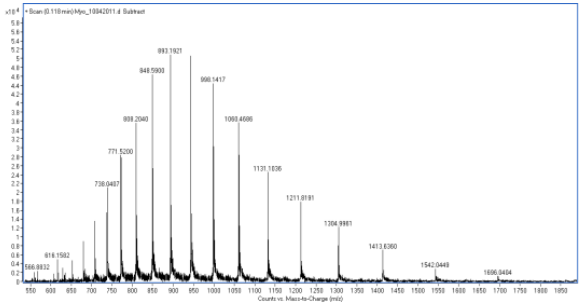
A) EI B) ESI C) Maldi

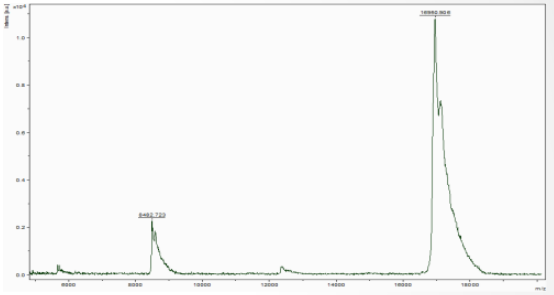
Data

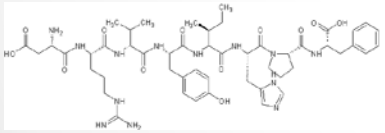
Match the data to Ionization Technique



A) ESI
B) Maldi

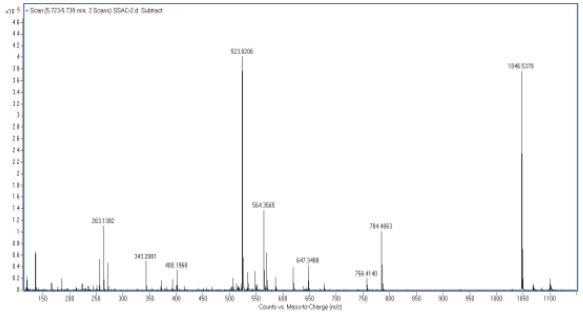


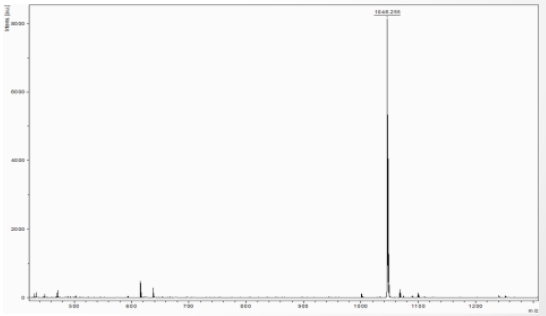




Peptide (Angiotensin II)

A) ESI
B) Maldi





My Sample

- What is the structure of your sample?
- What does is dissolve in?
- What kind of data do you need?
- Put you sample through the 3 “boxes” and choose the appropriate instruments you need training on:

1)
2)
3)

Mass Spectrometers Available in IMSERC for Use

	Instrument	Injection Mode	Solvents	Typical samples	Capabilites
ESI	Amazon SL	Direct	Methanol, Acetonitrile Water Dichloromethane	Flexible up to 2500 mass range: Synthesized compounds, peptide, organometallics, polymers, etc. Can see higher masses if multi-charged	Identification
	Amazon X	HPLC	Methanol, Acetonitrile, Water	Flexible up to 2500 mass range: Synthesized compounds, peptide, organometallics, polymers, etc. Can see higher masses if multi-charged	Identification, PRIMARILY USED for Quantitation, MS/MS capability
	LC-TOF	Direct/ HPLC	Methanol, Acetonitrile, Water, Dichloromethane	Flexible up to 300-4000 mass range: Synthesized compounds, peptide, organometallics, etc., Proteins, Oligonucleotides	High resolution Accurate Mass determination, Protein and Oligo exact mass determination using multi-charginging mass deconvolution
	Impact II	Direct/ HPLC	Methanol, Acetonitrile, Water	Flexible up to 300-4000 mass range: Synthesized compounds, peptide, organometallics, etc., Proteins, Oligonucleotides	Very High resolution Accurate Mass determination, Protein and Oligo exact mass determination using multi-chrginging mass deconvolution; MS/MS capability, Species Identification using fragmentation and MS/MS data, quantitation
MALDI	Autoflex II			Flexible: Synthesized compounds, peptide, organometallics,polymers, etc. crude MW of Preteins and Oligonucleotides	MW determination, High mass range
	Rapiflex			Surface and tissue analysis, Flexible: Synthesized compounds, peptide, organometallics,polymers, etc. crude MW of Preteins and Oligonucleotides	Imaging, High resolution MALDI data, High mass range
EI	GCMSD-DB5	Liquid injection-GC seperation	volatile solvents	Volatile small molecules (up to 300^{o}C)	Identification using library, quantitation
	GCMSD-HeadSpace	Headspace Gas -GC seperation		Volatiles components evaporated from solids, reaction mixtures, water, enviromental analysis	Identification using library, quantitation