SAND2015-7449PE

Biodetection using Mass Spectrometry

Sandia Biodetection Summit 2015

September 1-2, 2015

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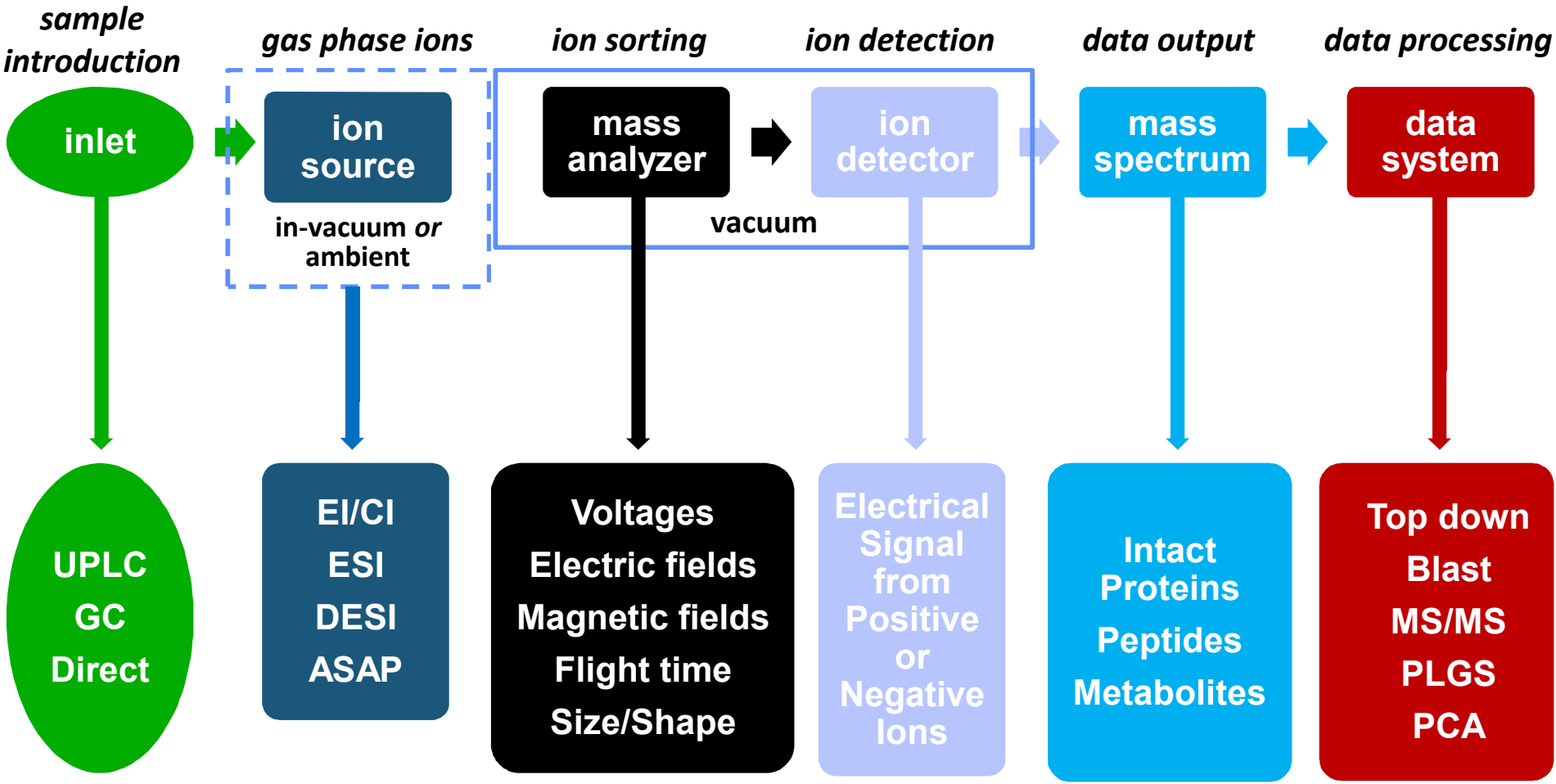
01852 – Materials Reliability

Sandia is a multi-program laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Overview

- Instruments
  - Waters Synapt G2 and Waters Xevo G2-xs
    - Waters Acquity Ultra Performance Liquid Chromatography (UPLC)
- Ionization Sources
  - Electrospray Ionization (ESI)
  - Desorption Electrospray Ionization (DESI)
  - Atmospheric Solids Analysis Probe (ASAP)
- Analytes
  - Proteins
  - Peptides
  - Biological Solids
  - Biological Fluids
- Software
  - ProteinLynx Global Server (PLGS) : Identification of unknown proteins
  - Progenesis: Metabolomics and Principle Component Analysis (PCA)

Introduction to Mass Spectrometry



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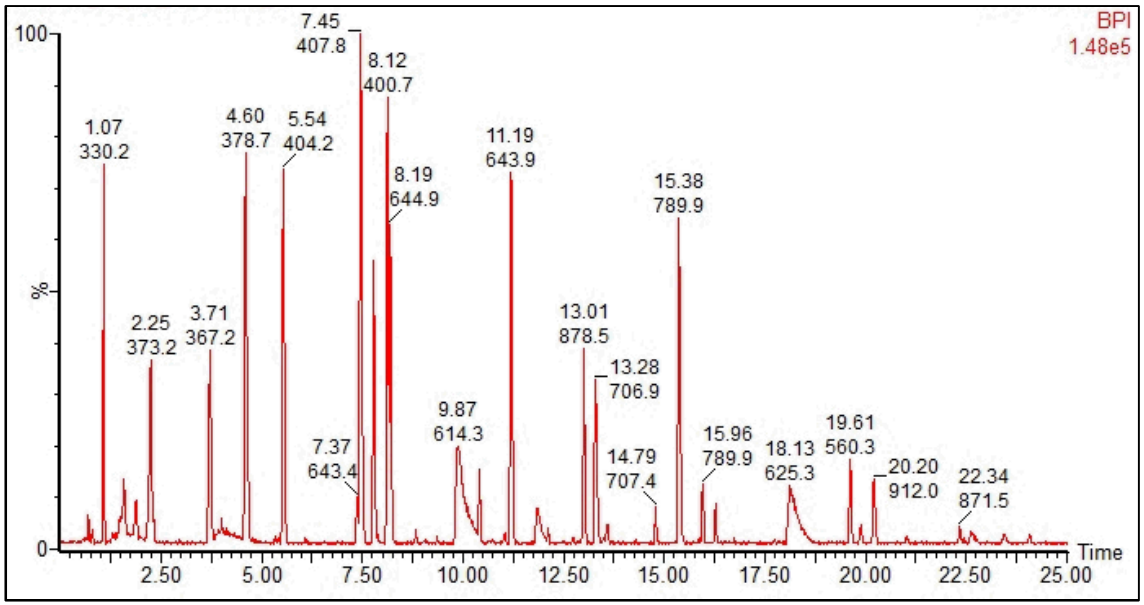
Waters Acquity UPLC

- Excellent separation of molecules (i.e. peptides) for analysis
  - Significant time and cost reduction from standard HPLC
- Can be easily connected on the front end of either MS system



Chromatogram of Enolase trypsin digest – each peak represents a peptide

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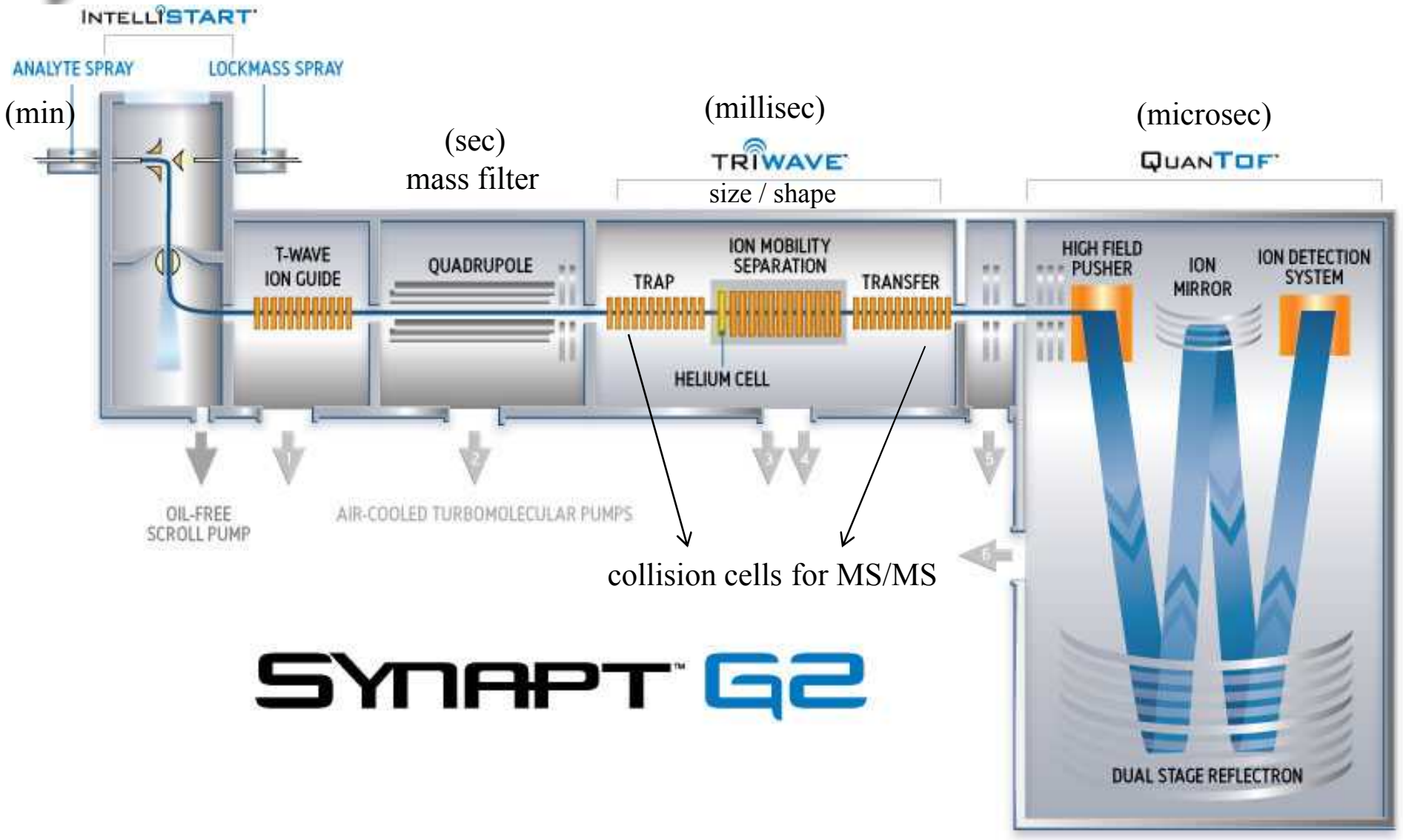
Waters Synapt G2 and Xevo G2-XS

- Cutting edge instrumentation
- High-resolution (10k to >50k @FWHM)
- Exact mass MS/MS
  - Identification of compounds in complex matrices
  - Unambiguous identification that prevents false positives
- Multiple ionization options and sources
  - Both positive and negative modes
  - Sources can be changed in minutes
- Wide range of samples detectable
  - Small biological molecules as well as proteins, polymers, *etc.*
  - Solution and gas phase, polar and less polar, surface deposits, *etc.*
- Powerful software package for visualizing data

Differentiation by size, shape, charge & mass



SYNAPT G2

Electrospray Ionization – Nobel Worthy

Dr. John Bennett Fenn receives the Nobel Prize for his development of Electrospray Ionization (2002)

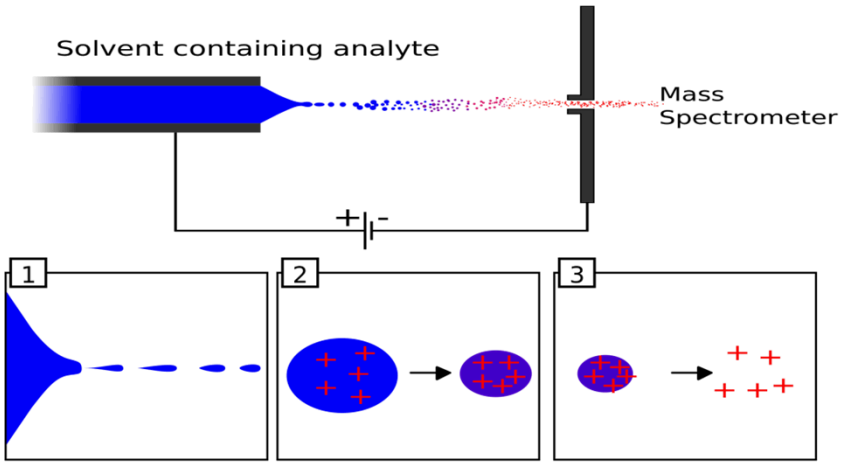
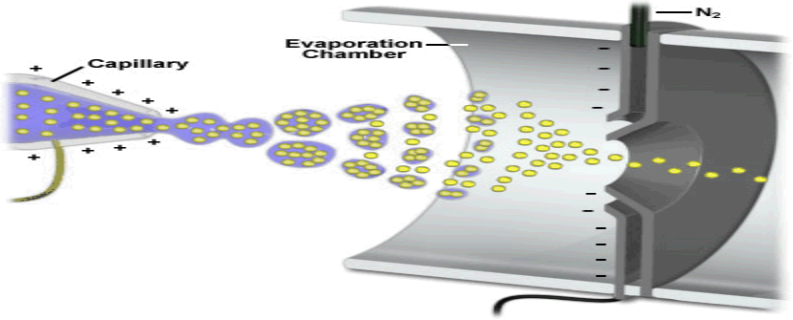


Diagram of Electrospray Ionization. (1) Under high voltage, the Taylor Cone emits a jet of liquid drops (2) The solute from the droplets progressively evaporates, leaving them more and more charged (3) When the charge exceeds the Rayleigh limit the droplet explosively dissociates, leaving a stream of charged ions



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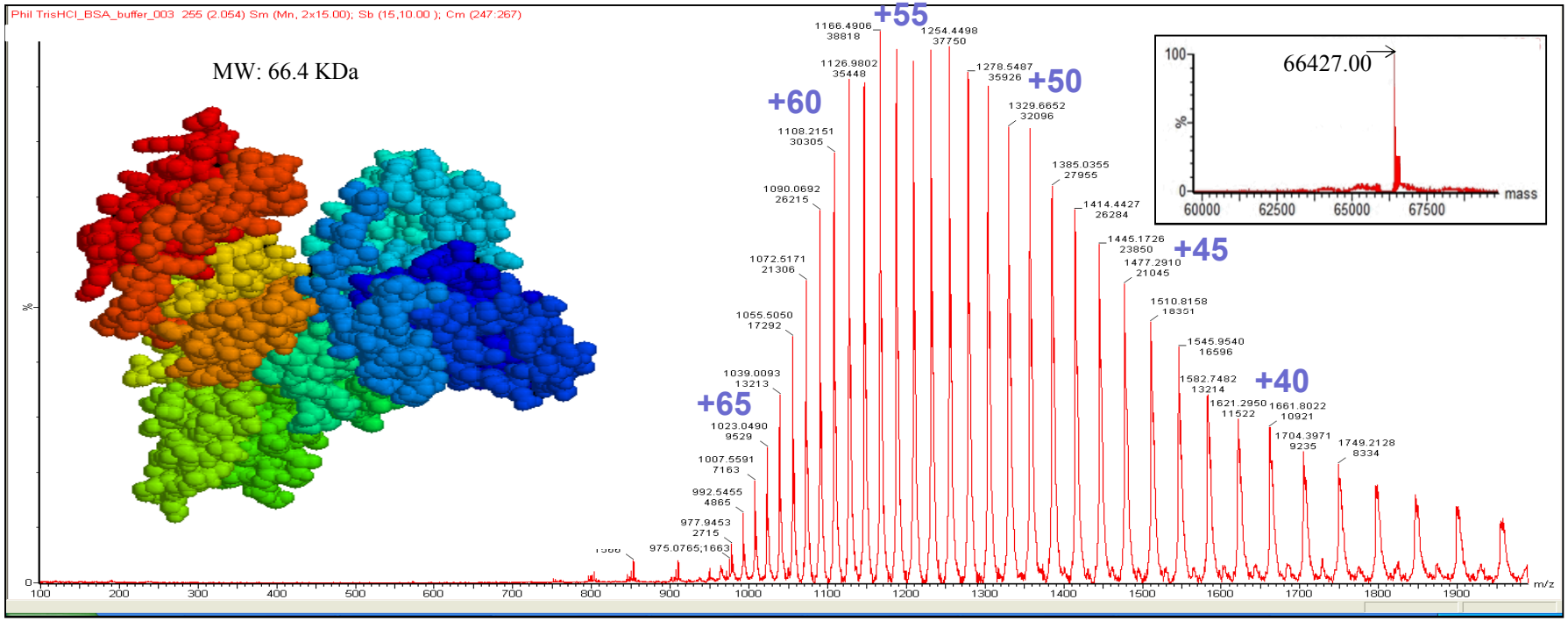
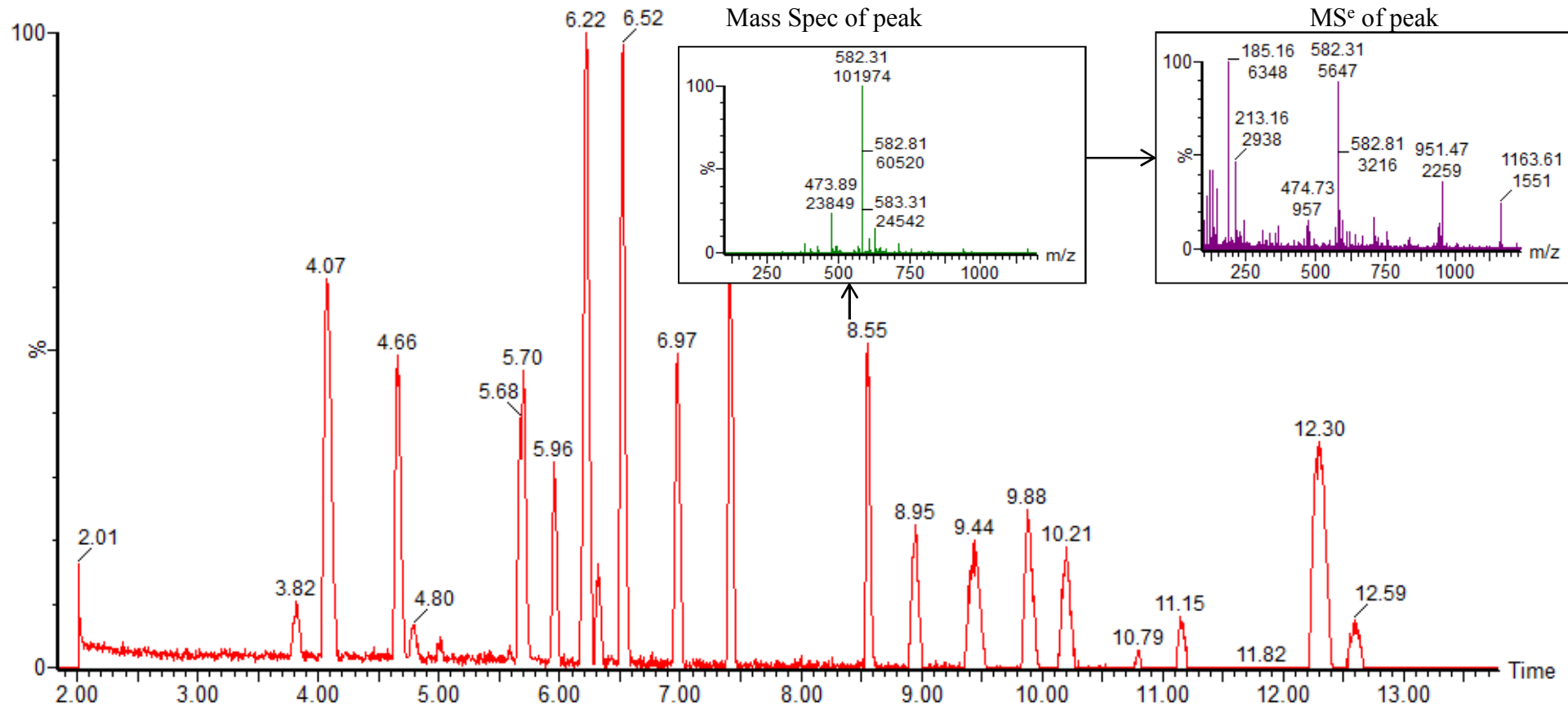
Electrospray Ionization (ESI)

- **Soft ionization technique allows for analysis of biological macromolecules (i.e. proteins)**
  - **Ability to produce multiply charged ions effectively extends mass range**
    - **Allows for Kilo-Dalton to Mega-Dalton sized molecules to be analyzed (mass to charge ratio)**
      - **Example – 66,427 amu protein with a charge state of +56 = analyzed mass is 1165 amu**



ESI Spectrum of Bovine Serum Albumin analyzed as an intact protein

ESI of Peptides



Trypsin digested BSA ran on the UPLC and Waters Synapt G2 showing total ion chromatogram with a mass spectrum of the peak at 8.55 minutes along with the MS^e fragmentation spectrum.

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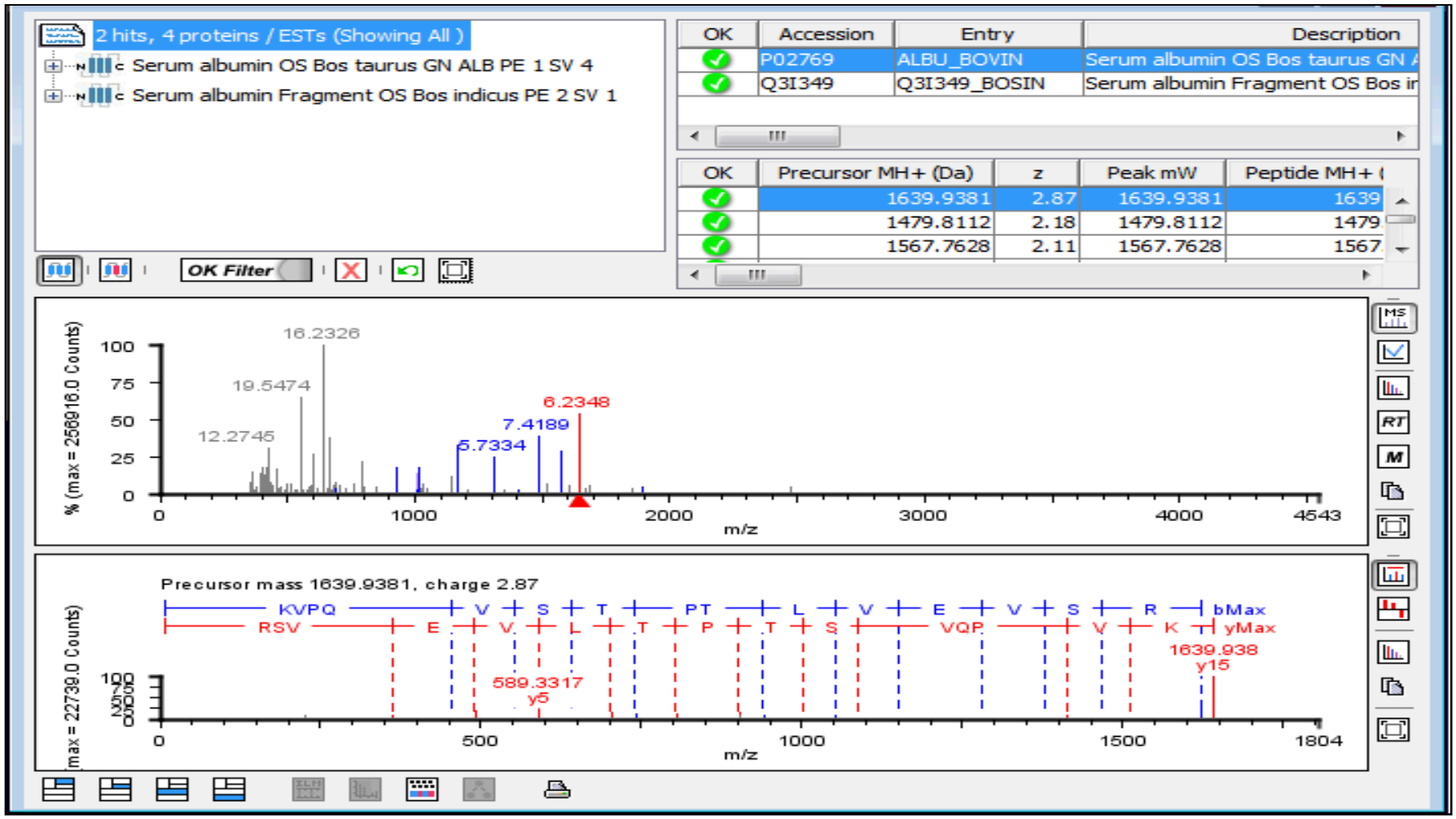
PLGS Match for Bovine Serum Albumin



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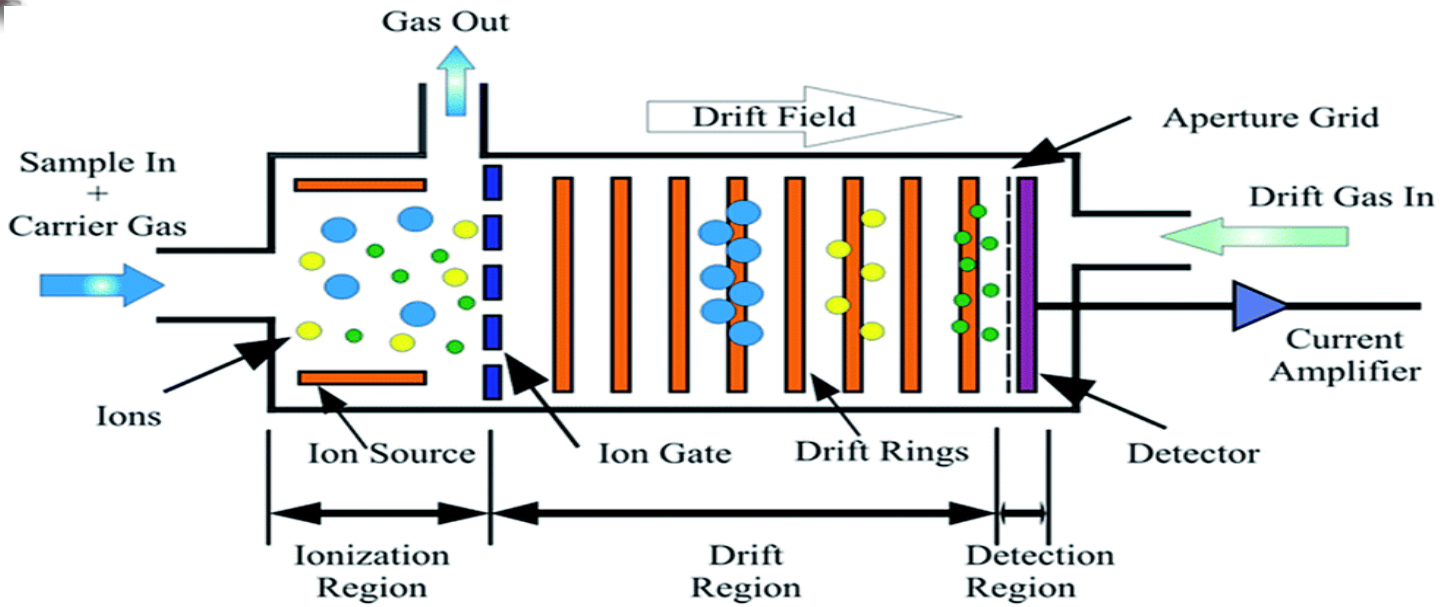
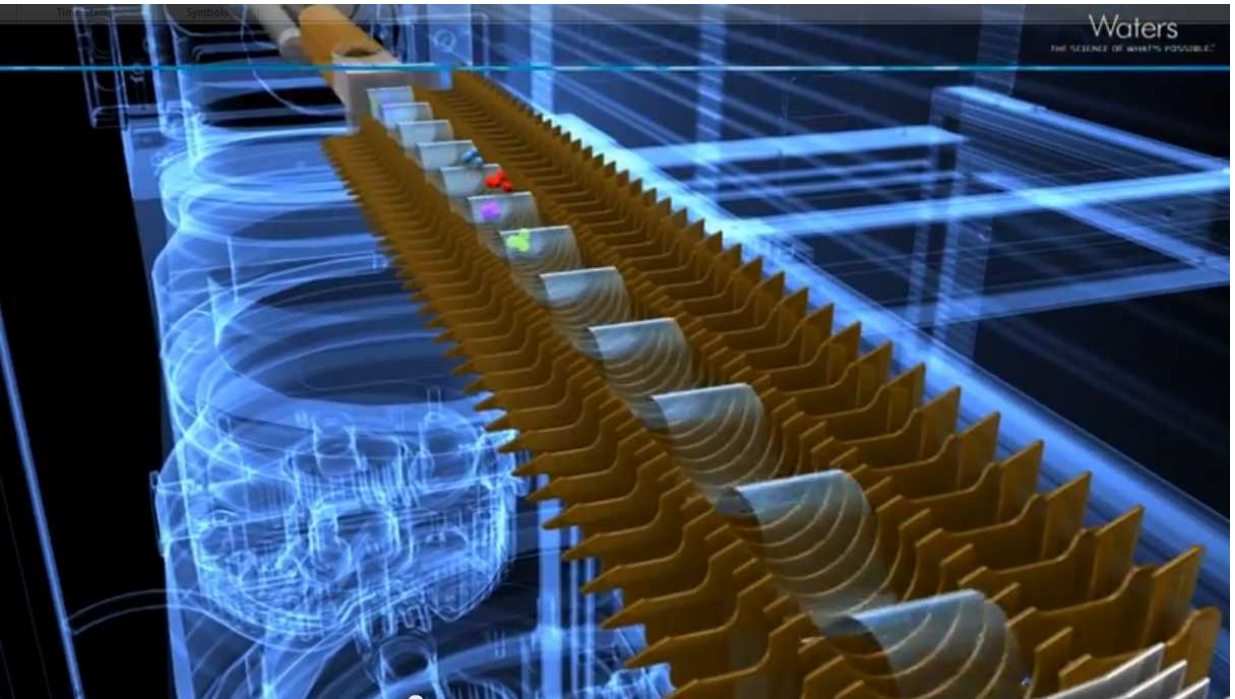
Ion Mobility





- Ion mobility separates ions based on mobility through a buffer gas of helium
- Allows for better specificity of molecule detection by adding shape and size as factors for separation

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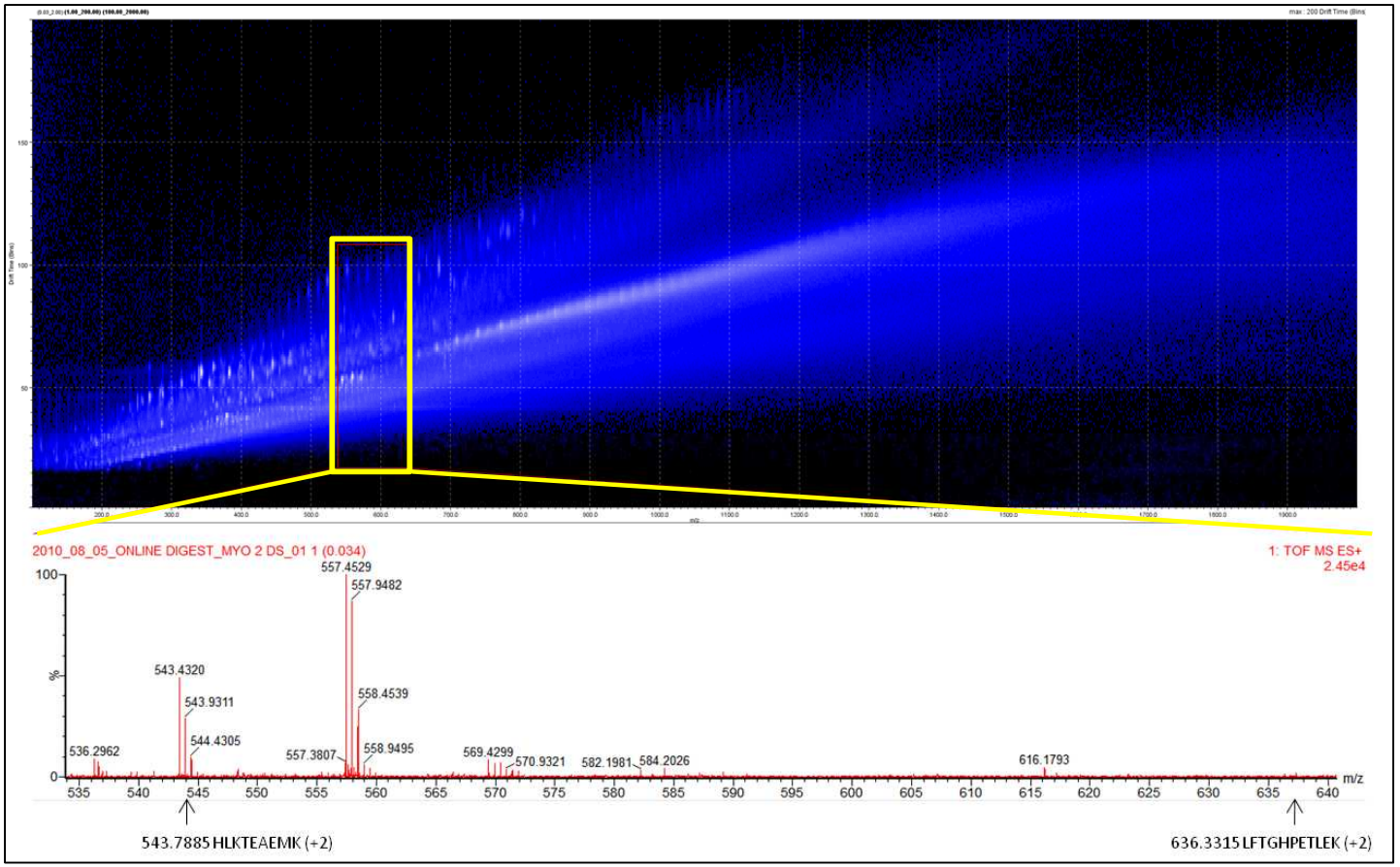
Ion Mobility – Drift Diagram

- Ion Mobility uses a helium cell to further separate molecules base on their shape



Ion Mobility drift diagram of Myoglobin trypsin digest – shows 2 molecules originating from the same mass but drifting apart in the helium cell due to isomeric/conformational differences.

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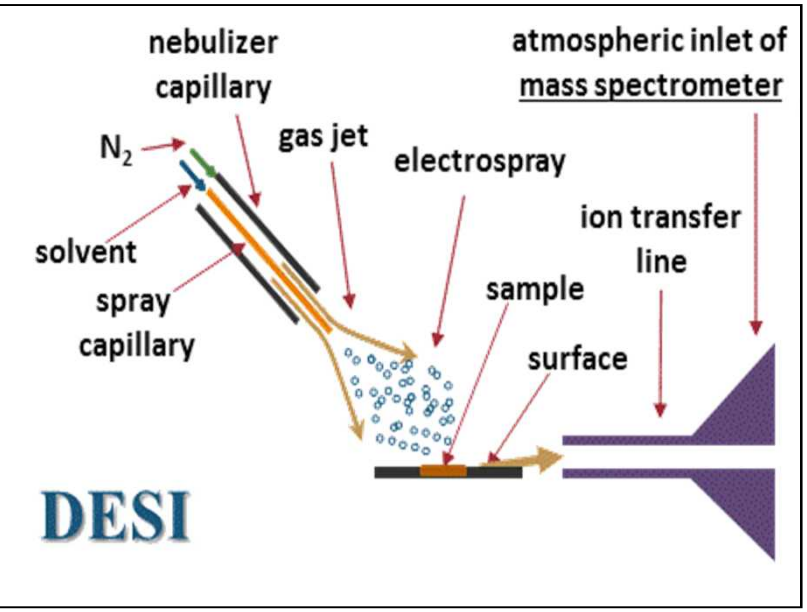
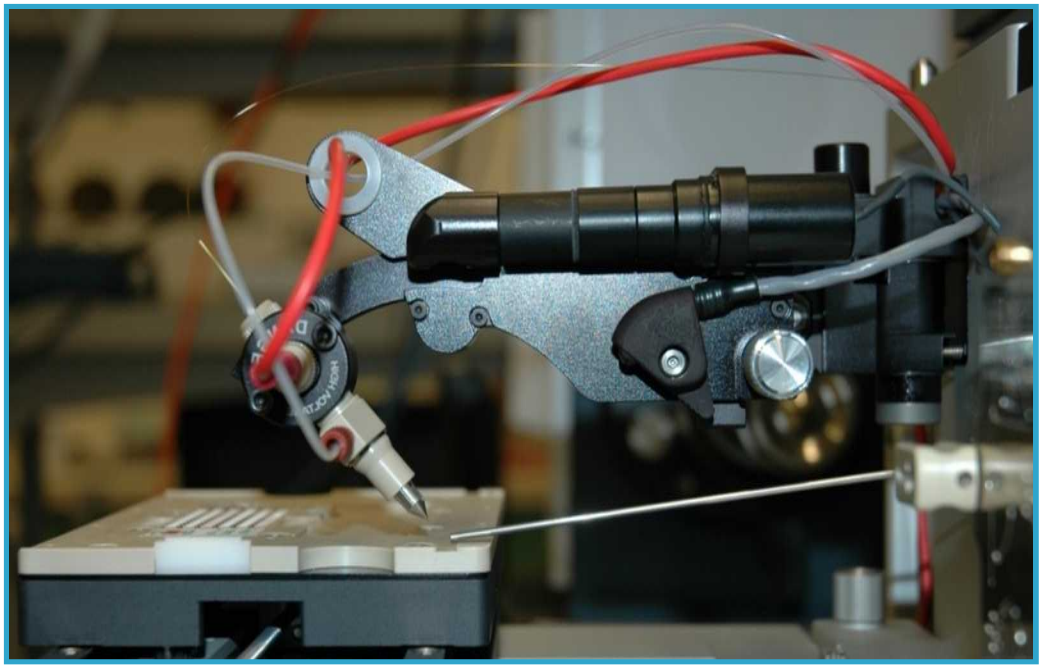
Desorption Electrospray Ionization (DESI) Source

Dependent on a ***CRITICAL*** combination: **(1)** solvent, **(2)** analyte, & **(3)** surface



Advantages of Ambient Ionization Surface Analysis

- Little to no sample preparation
- Rapid, direct analysis of a wide range of compounds
- **ALLOWS for IMAGING using MASS SPECTROMETRY!**



Our DESI source showing inlet tube, spray nozzle and sample stage.

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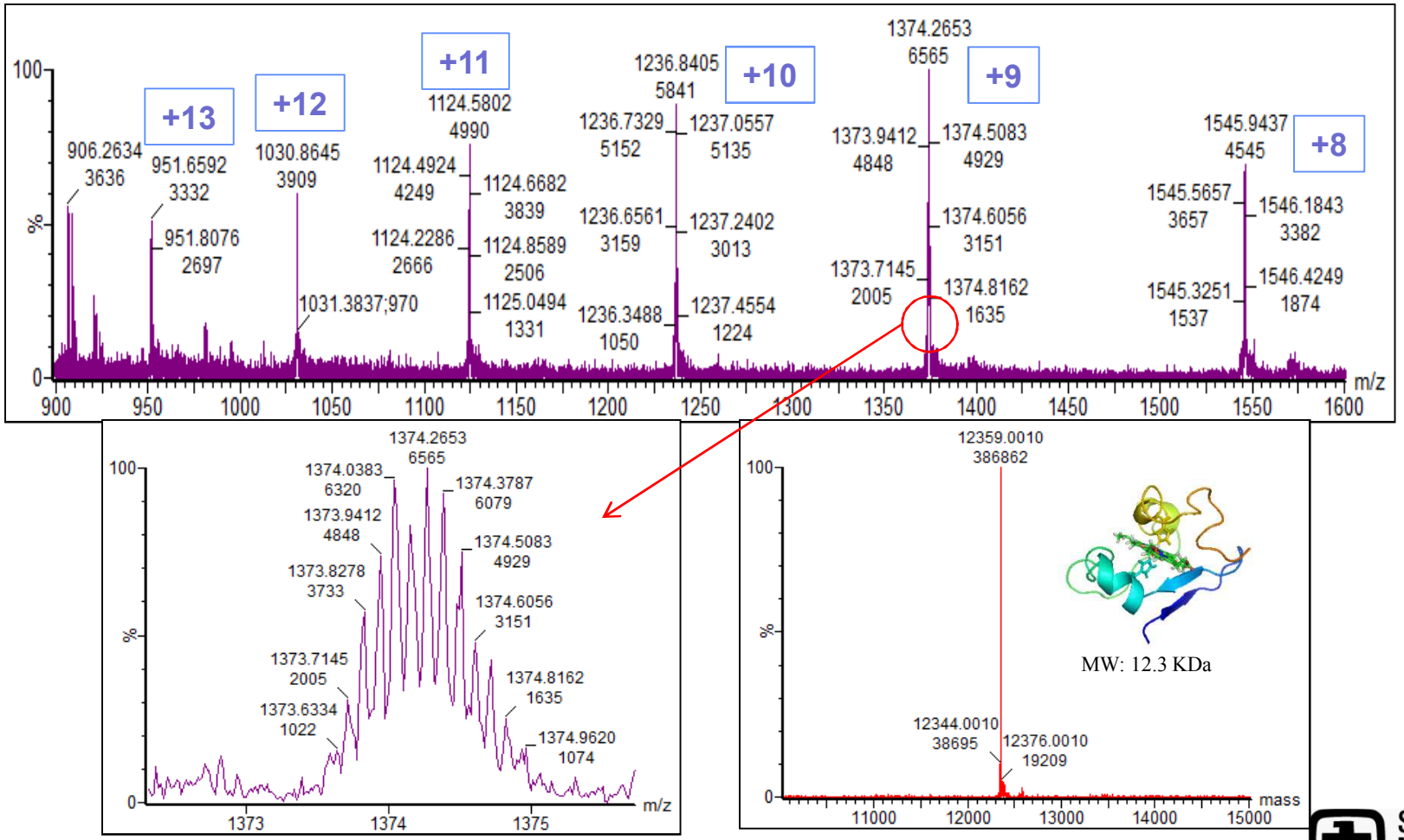
DESI Bio Data

Cytochrome C was spotted on a glass slide and successfully identified



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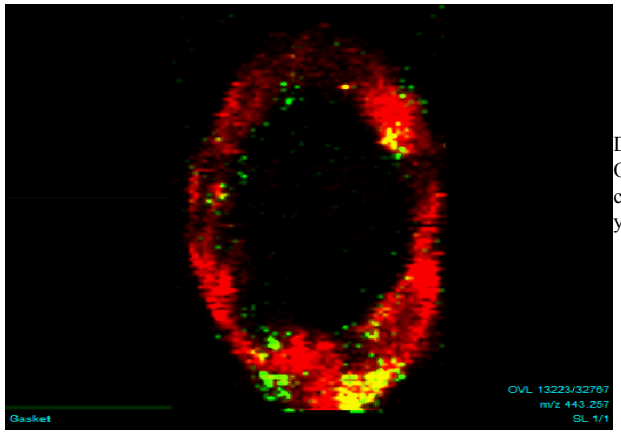
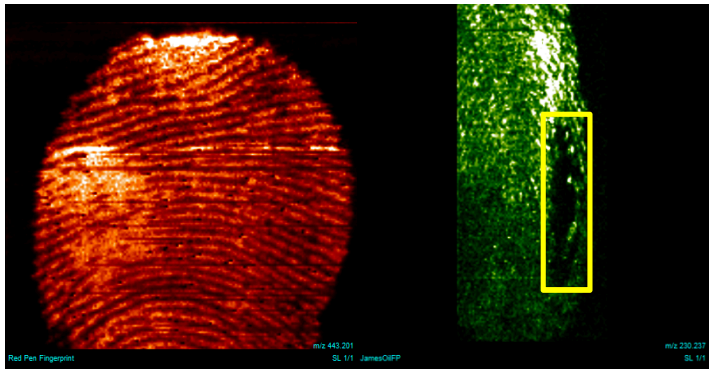
DESI Imaging

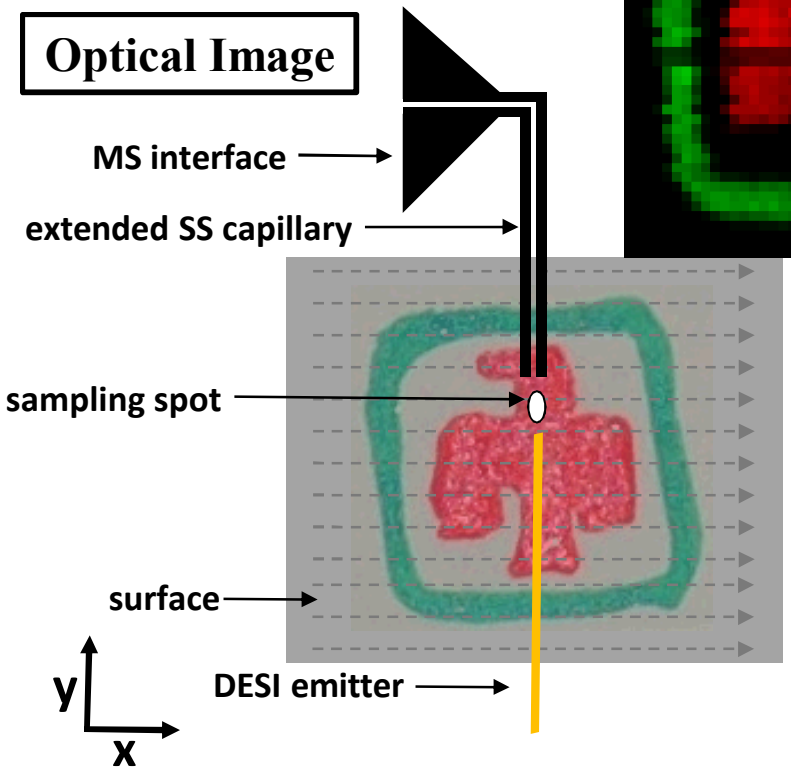
- Can move sample stage in 100 micron resolution across a surface in a horizontal line.
- Perform next scan at 100 microns down from the last scan.
- Identify chemical species of interest and include only data from that mass.
- Use the location and mass intensity data to plot an image from mass spec data.

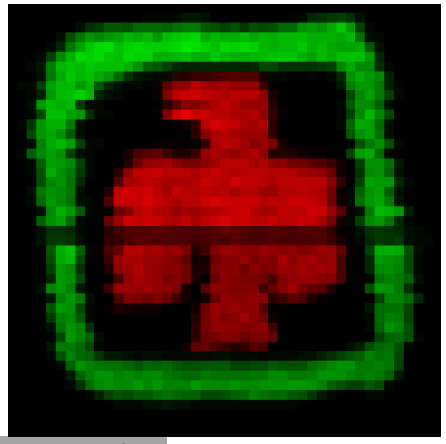


DESI image of an O-ring showing contamination in yellow and green



Example of two fingerprint images generated, within our lab, from the presence of red ink on a finger (red image on left) and from nose oil (green image). Both images show the resolution possible using DESI and key features of the fingerprint such as scar tissue from the oil image (highlighted in yellow).





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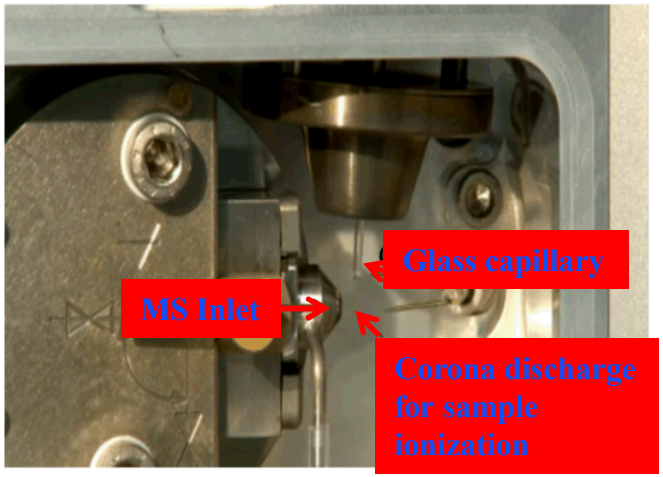
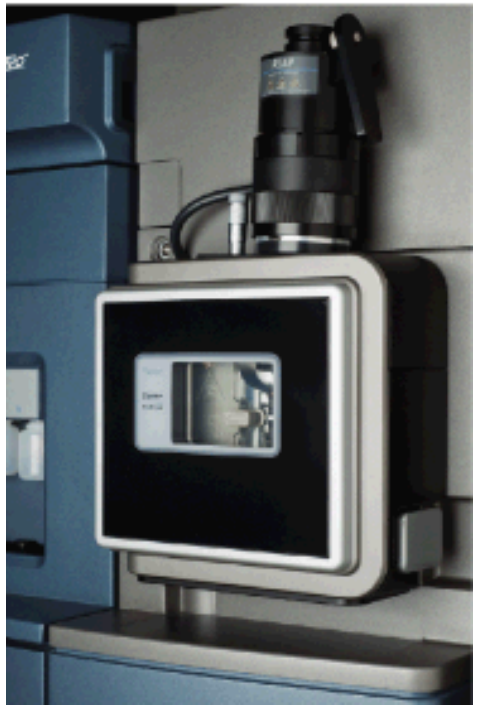
Atmospheric Solids Analysis Probe (ASAP)

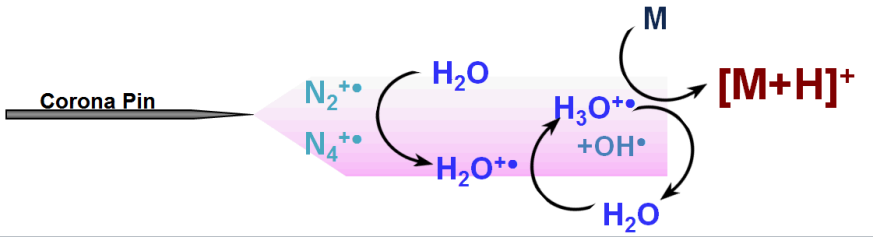
- Uses glass rod probe in place of ESI sprayer
  - Rod is dipped/swiped on solid or liquid to pickup sample
  - Probe is inserted into source
  - Desolvation gas is used to thermally desorb analytes off of glass rod surface
  - Analytes are ionized through Corona ionization
- Capabilities
  - Fingerprinting complex systems, such as:
    - bacteria, pollen, other biological solids and fluids
  - Thermo-chemical mechanisms
  - Real-time reaction product characterizations

A reaction chamber





ASAP-MS source setup



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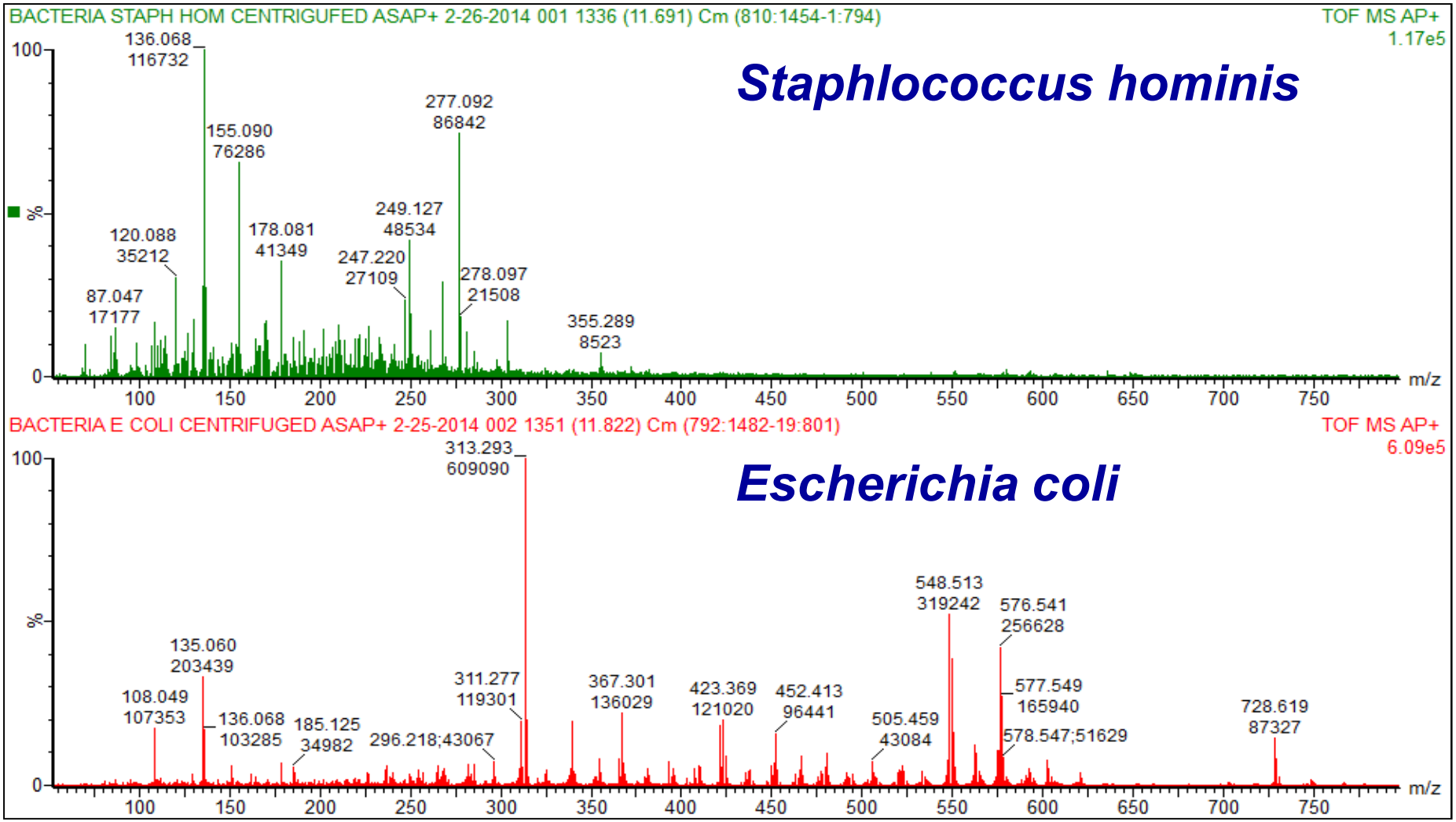
ASAP of Intact Bacteria



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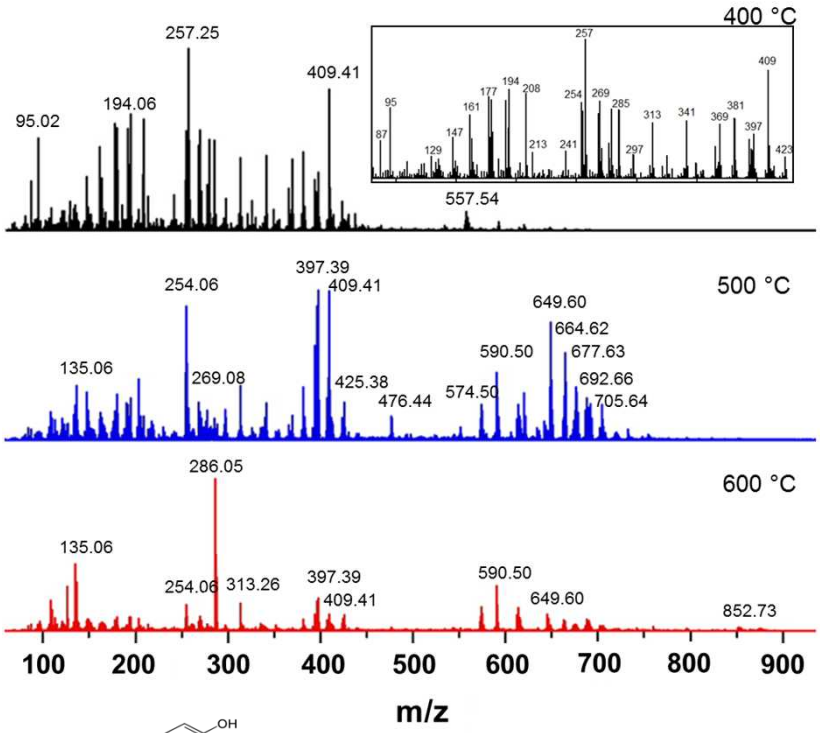
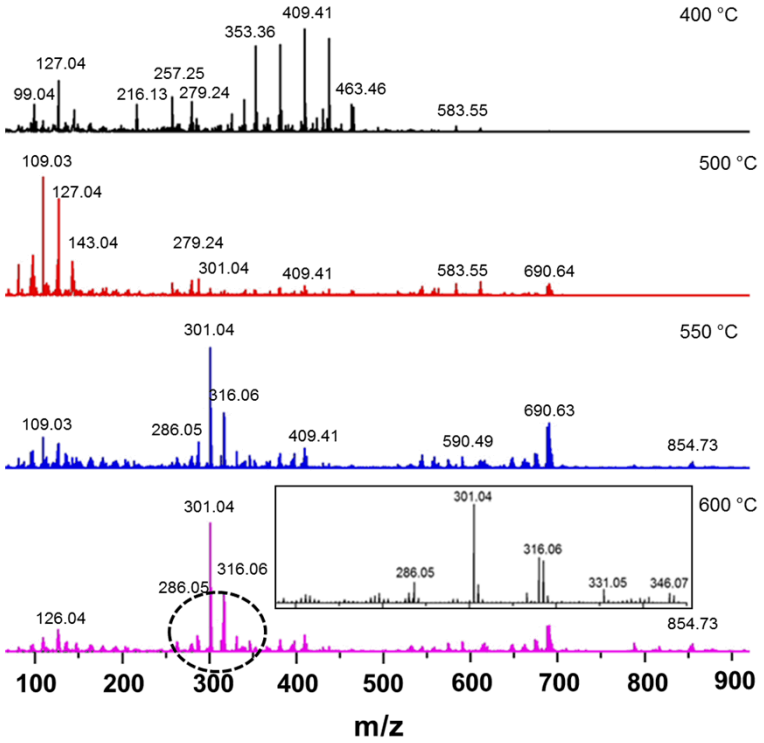
ASAP of Whole Pollen Grains

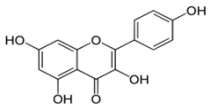
- The thermal desorption of pollens produces similar and dis-similar products depending on temperature and pollen type
- The products can be changed with the temperature
- Identified flavonoid products

Populus nigra tree pollens



Colorado bee pollens





Flavonoid and isomers

Xiao, Miller, Hochrein et al. Manuscript submitted

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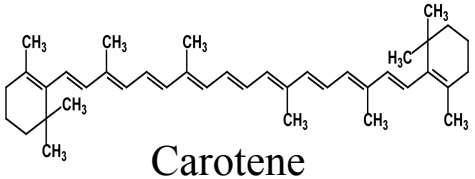
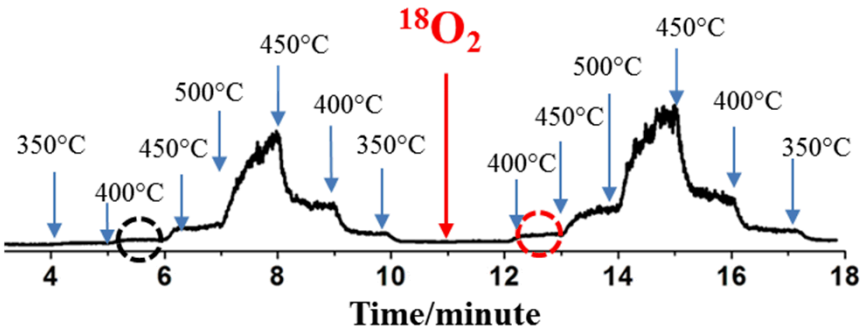
ASAP for Real-time Reaction Mechanisms



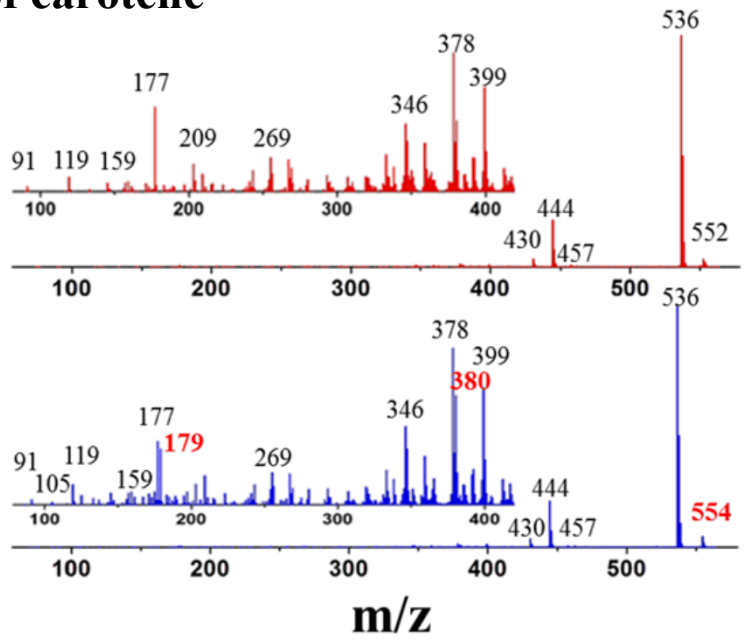
Carotene



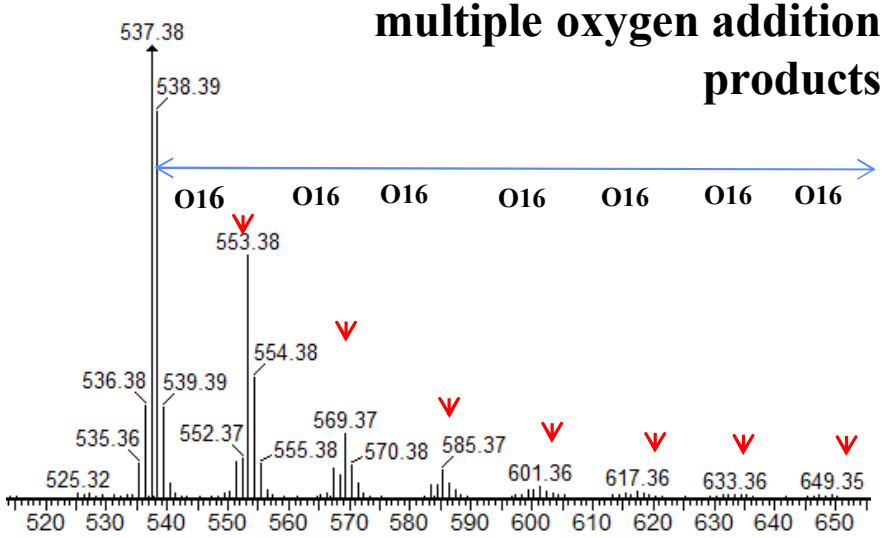
The reaction chamber can be modulated with:

Gas,
Solvent vapor,
Isotopically enriched chemicals

Oxygen effect on thermo-degradation of carotene



First-time observation of multiple oxygen addition products



Xiao, Miller, Hochrein et al. Manuscript submitted

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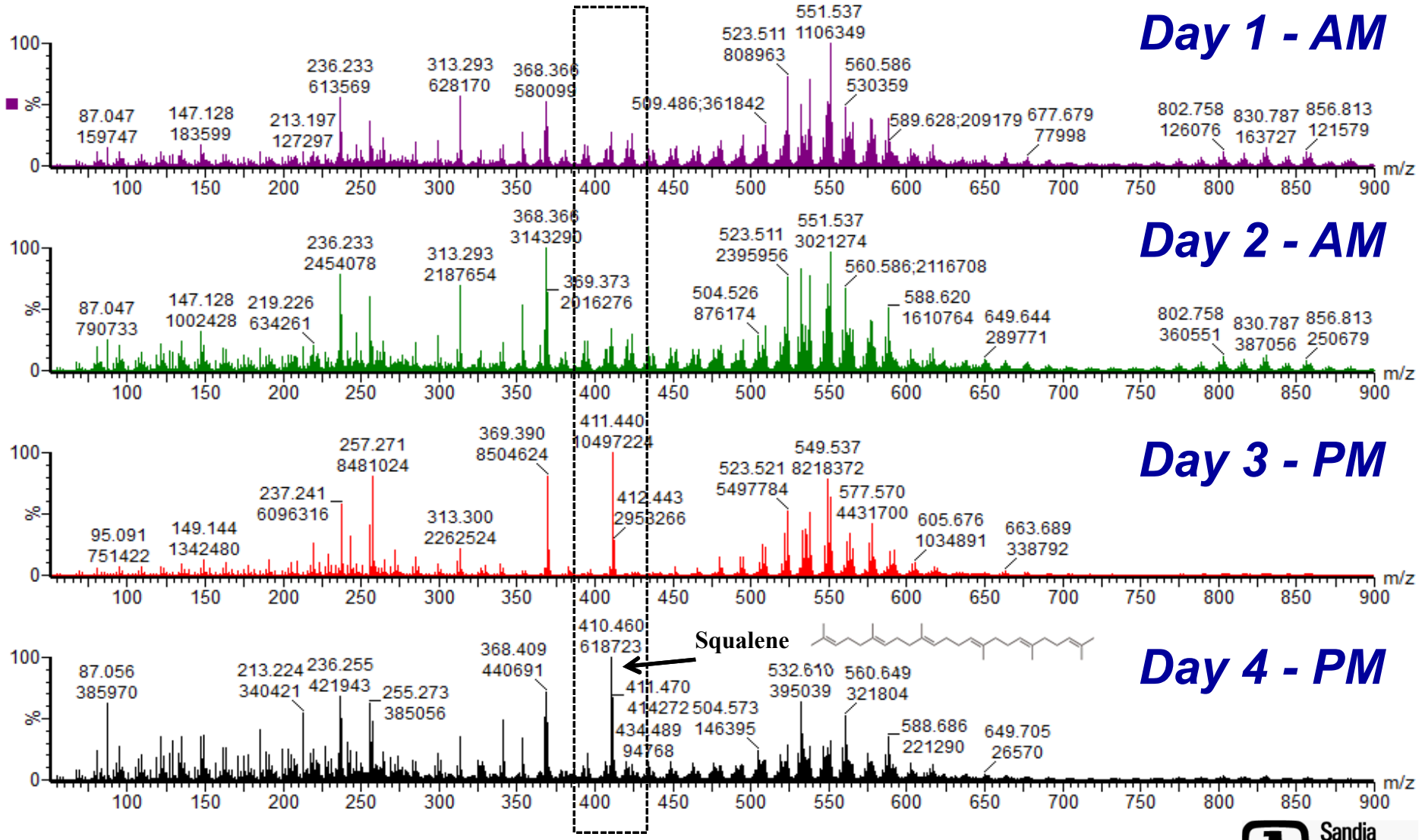
ASAP of Skin Oil Showing Temporal Changes



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Progenesis Software for Metabolomics and PCA

File | Import Data | Review Alignment | Experiment Design Setup | Peak Picking | Review Deconvolution | Identify Compounds | Review Compounds | Compound Statistics

nonlinear DYNAMICS — A Waters Company

Question:

Are there any outliers in my data?
Does my data cluster according to my experimental conditions?

What's this?

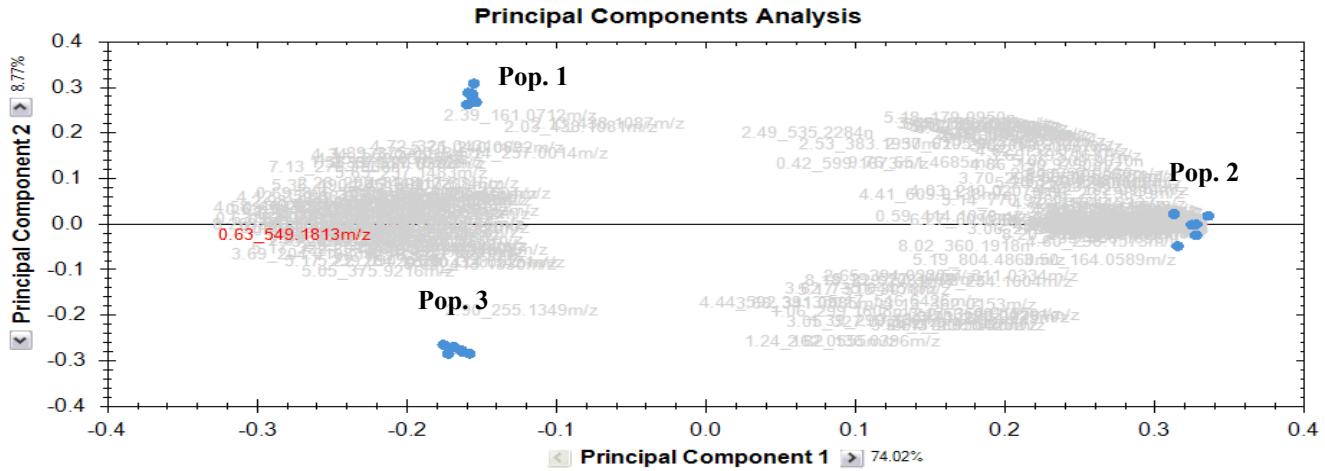
Principal Components Analysis produces a simplified, graphical representation of your multidimensional data.
More...

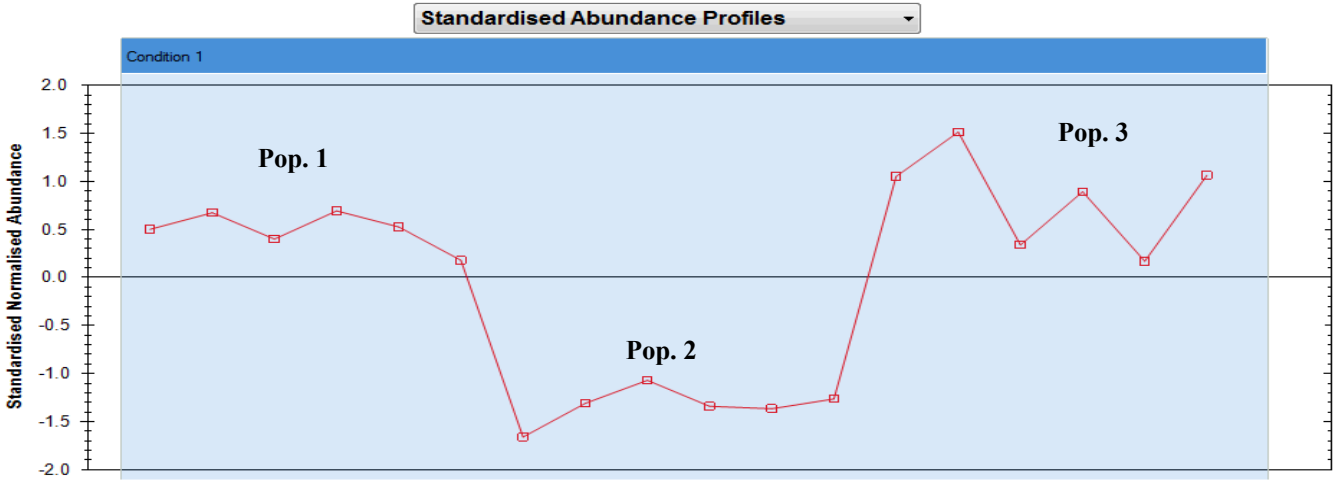
Ask another question

Tag filter applied
compounds may be hidden
Edit...

Compour	Anova	q Value	Power	Tag	Clust
0.42_599...	---	---	---		
0.59_643...	---	---	---		
0.59_414...	---	---	---		
0.60_661...	---	---	---		
0.60_609...	---	---	---		
0.63_320...	---	---	---		
0.63_549...	---	---	---		
0.63_821...	---	---	---		
0.63_557...	---	---	---		
0.63_813...	---	---	---		
0.63_536...	---	---	---		
0.63_511...	---	---	---		
0.64_584...	---	---	---		
0.64_576...	---	---	---		
0.66_585...	---	---	---		
0.68_348...	---	---	---		
0.68_407...	---	---	---		
0.69_806...	---	---	---		
0.69_375...	---	---	---		
0.70_505...	---	---	---		

Experiment design
Review your data from a different perspective:
Current design: nogroup





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Conclusion

- Our capabilities provide analysis of a wide range of biomolecules
  - Samples can be:
    - Intact cells
    - Isolated proteins, peptides and lipids
    - Biological solids and fluids
  - Can detect, identify, quantitate and possibly even image:
    - Proteins
    - Peptides
    - Lipids
    - Metabolites
    - Potentially any Biomarker under the right conditions