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Knowledge that will change your world

Choosing the metabolomics platform

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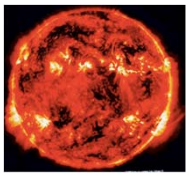
Targeted Metabolomics & Proteomics Laboratory

Challenges

- **Unlike DNA, RNA and proteins, the metabolome is phenomenally chemically diverse**
- **Ranges from a gas (H_2) that prevades the universe and is the principal component of the Sun**

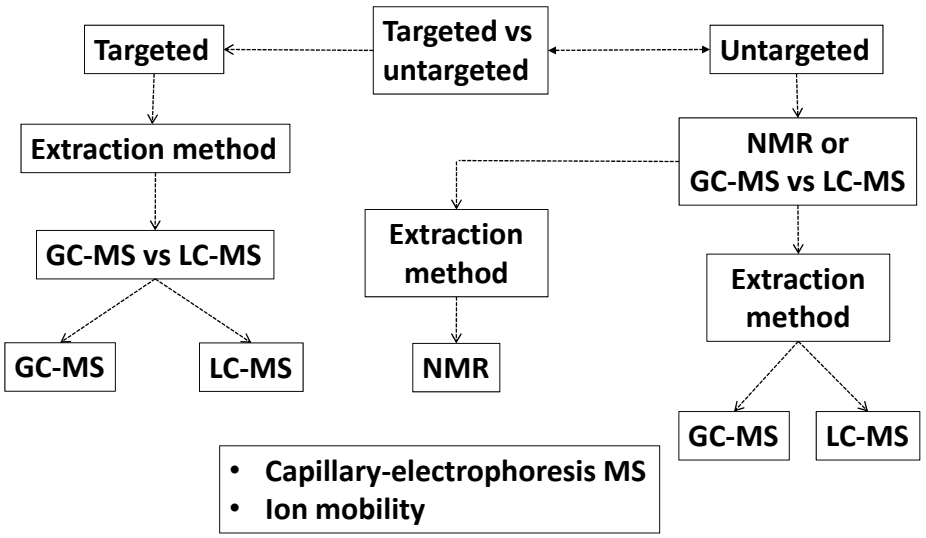
to  

- **Earwax (long chain fatty acids, both saturated and unsaturated, alcohols, squalene, and cholesterol)**
- **No single method of analysis**

Synopsis

- **Decision tree**
- **Gas chromatography-mass spectrometry (GC-MS)**
- **Nuclear Magnetic Resonance (NMR)**
- **Liquid chromatography-mass spectrometry (LC-MS)**
  - nanoLC-MS
  - Rapid flow LC-MS
  - Multiple Reaction Monitoring (MRM)
- **Differential mobility**
- **Imaging mass spectrometry**
  - MALDI-MS
  - DESI-MS
  - Thin layer chromatography (TLC)

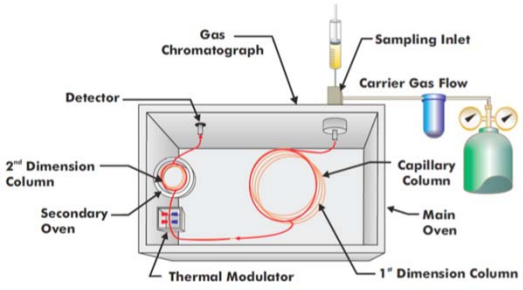
Decision tree



Metabolomics and GC-MS

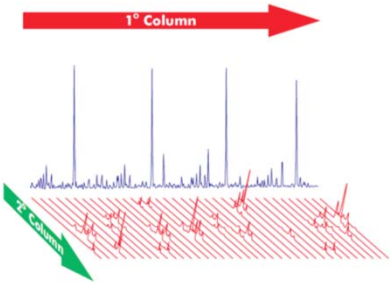
- **PROS**
  - Capillary columns can achieve very high chromatographic resolution
  - Retention times are reproducible
  - Mass spectral libraries are well developed
- **CONS**
  - Not all compounds can be analyzed by GC-MS
  - Although amino acids, sugars, fatty acids, amines and organic acids can be derivatized, complex polyphenol glycosides and polar lipids are too unstable, even when derivatized, at the temperatures used to elute them
  - Approximate mass limit of 400 Da

Two dimensional GC to resolve metabolites



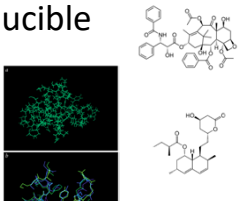
As compounds elute from column 1, they are passed to (cooler) column 2 where they condense. After a period of collection, column 2 is heated so as to separate and elute the compounds.

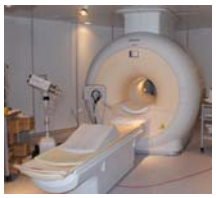
Leco Corp.

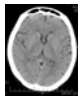


Nuclear Magnetic Resonance (NMR) Spectroscopy

- Detects NMR active nuclei
- Robust and highly reproducible
- Non-destructive
- Quantitative
- Used in
  - Structure elucidation
    - Small molecules
    - Macromolecules (DNA, RNA, Proteins)
  - A number of techniques
    - 1D , 2D, 3D
  - Molecular motion and dynamics
- Similar method used in medical Imaging (MRI, fMRI)







from Wimal Pathmasiri

NMR considerations

- **Sample amount:**
  - Typical 600 MHz instrument requires 0.5 ml plasma/serum
  - Higher field instruments and micro coil detector allow use of 0.1 ml
- **Quality control:**
  - In the UK Phenome Center, all samples are analyzed by NMR
    - This allows for detection of outliers
    - Also found that there is a correlation between the NMR spectrum and whether problems occur in LC-MS analysis
    - NMR analysis used to filter out these samples

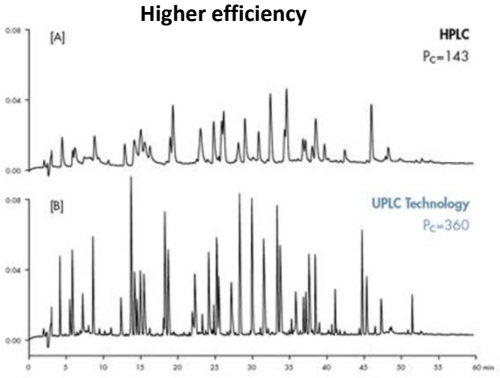
Liquid Chromatography-Mass Spectrometry

- **PROS**
  - **Almost all compounds can be analyzed by LC-MS**
    - Soft ionization, so hydrocarbons do not ionize
  - **Several orders of magnitude increased sensitivity compared to NMR**
  - **Can collect MS, MSMS and ion mobility data**
- **CONS**
  - **Not uniformally quantitative**
  - **Mass spectral libraries are not well enough developed, although improving rapidly**
  - **Chromatographic separation not adequate**
  - **Retention time reproducibility not as good as GC-MS**

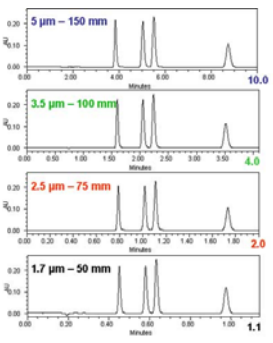
The LC

- **1D-approach**
  - Use of reverse-phase, normal phase and HILIC phase
  - particle size – smaller is more efficient, but back pressure is a problem

Higher efficiency



Same efficiency, but shorter run times



LC flow rate

- **Sensitivity is inversely related to flow rate**
  - Slower flow rates give more sensitivity

normal flow (0.2-0.4 ml/min)

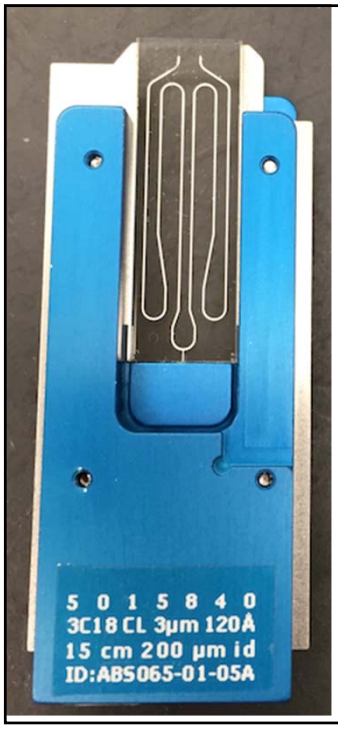
microflow/capillary (5-50 µl/min)

nanoflow (0.3-5 µl/min)

Optimizing nanoLC for metabolomics

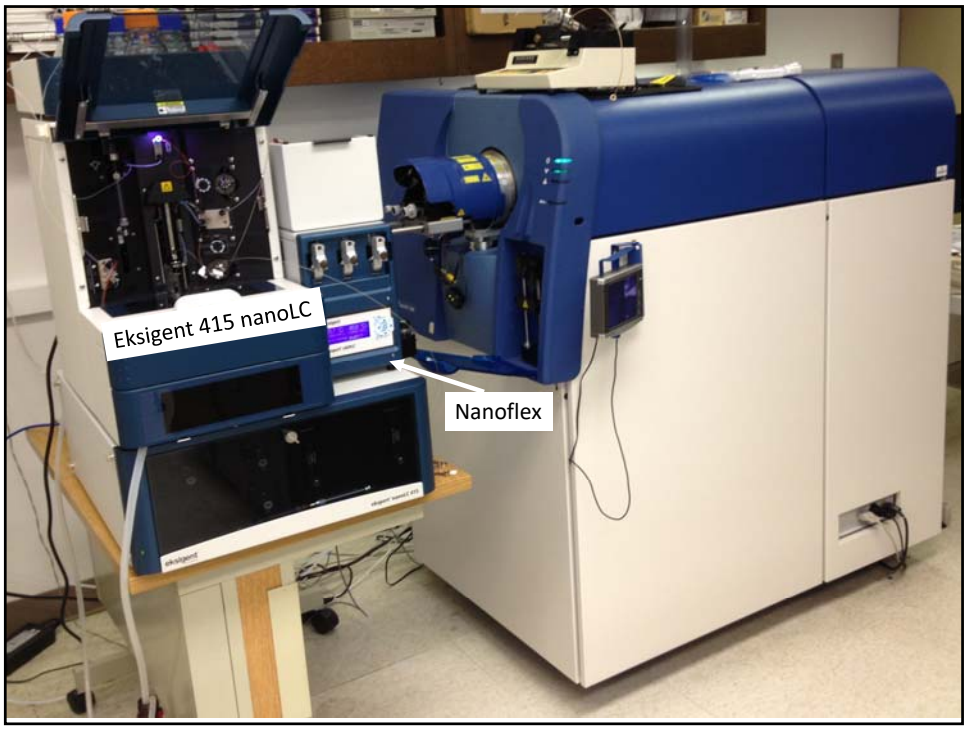
- **Objective is to develop metabolomics for small animal model systems**
  - ***D. melangaster***
  - ***C. elegans***
  - ***D. rerio***

- **A single zebrafish yields about 1 µl of plasma**
- **Need to move down to the nanoscale**
- **Important to maintain consistency and quantitation**
  - **Reproducible columns and temperature**



Close up of a nanochipLC cartridge (15 cm x 0.2 mm ID).

- **Each long section of the column is ~2.5 cm (1 inch).**
- **Can be machined to a better tolerance.**
- **Simpler connections to the liquid stream.**
- **Can be placed in a temperature-controlled environment**



The mass spectrometer

- **For untargeted analysis it is important to have high mass resolution and accuracy**
  - Initial data analysis is performed on the molecular ions
  - Each metabolite ion has a unique mass (*m/z*) and in practice forms adducts and has isotopic ions
    - ion features ≠ metabolites (see **Corey Broeckling's talk** – Thursday)
  - Nonetheless, a particular mass, however exact, is not necessarily a unique metabolite, only a particular empirical formula
- **Fourier transform-ion cyclotron resonance and Orbitrap instruments have the greatest mass accuracy**
  - However, their performance is time-dependent and is degraded significantly by short acquisition times (<100 ms)
  - They are best used for follow up experiments

Mass analyzer of choice for untargeted metabolomics

- **Quadrupole-orthogonal time-of-flight (Q-TOF)**

Agilent 6500

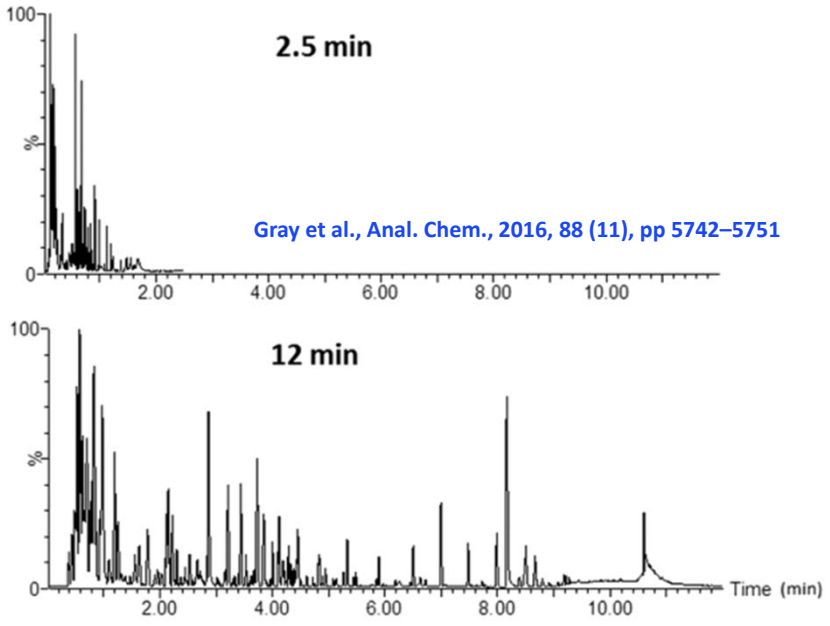
Waters Synapt G2Si/HMDS

Bruker

Sciex TripleTOF 6600

Current models have 40-80,000 mass resolution and 1-3 ppm mass accuracy

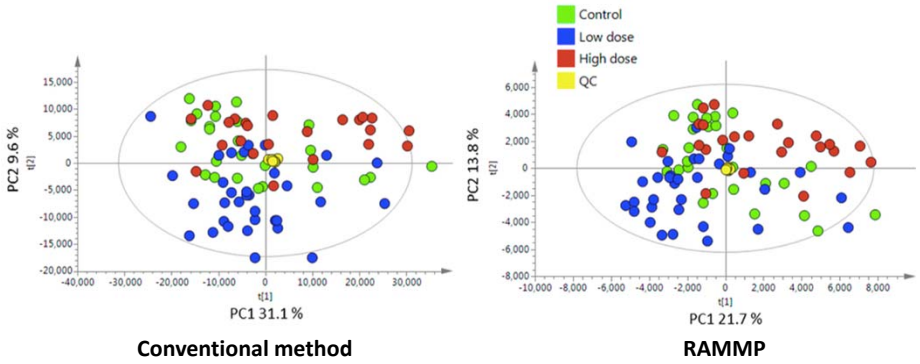
RAMMP, speeding up metabolomics



Gray et al., Anal. Chem., 2016, 88 (11), pp 5742–5751

RAMMP

- **There was a reduction in independent features**
  - 19,000 by conventional method
  - 6,000 by RAMMP



Conventional method **RAMMP**

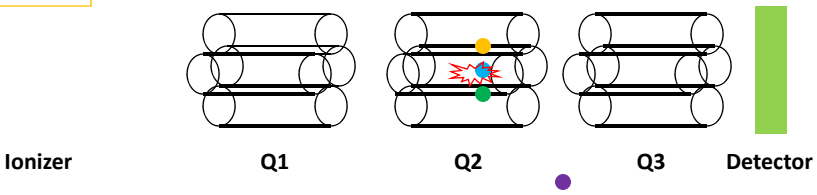
Gray et al., Anal. Chem., 2016, 88 (11), pp 5742–5751

Targeted vs untargeted methods

- If we know what the metabolites to be measured are (from previous untargeted analyses, or prior knowledge), then a multiple reaction monitoring (MRM) approach is the best way to go since it allows quantitative analysis of possibly 100s of metabolites
- If there is no hypothesis, but instead you want to generate hypotheses, then the untargeted approach is better.

Multiple reaction ion monitoring

Quantitative analysis of metabolites in a complex mixture carried out using a triple quadrupole instrument



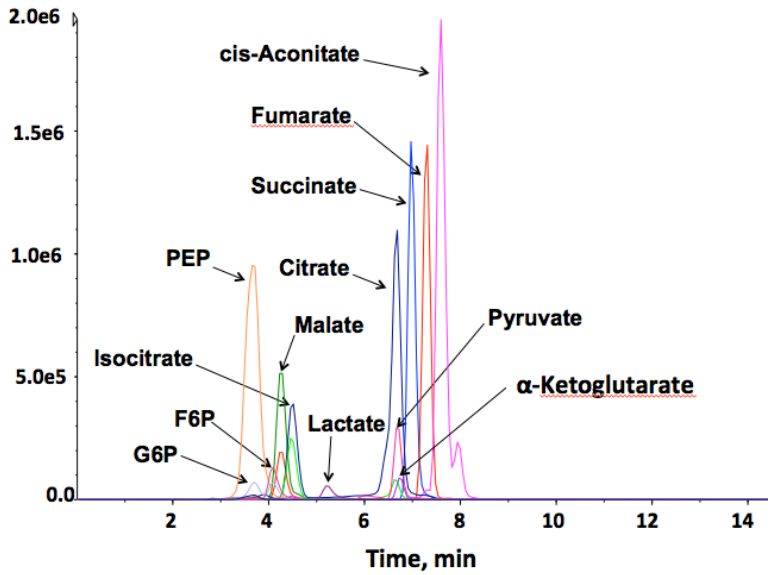
Based on precursor ion/product ion pair(s)

Courtesy, John Cutts

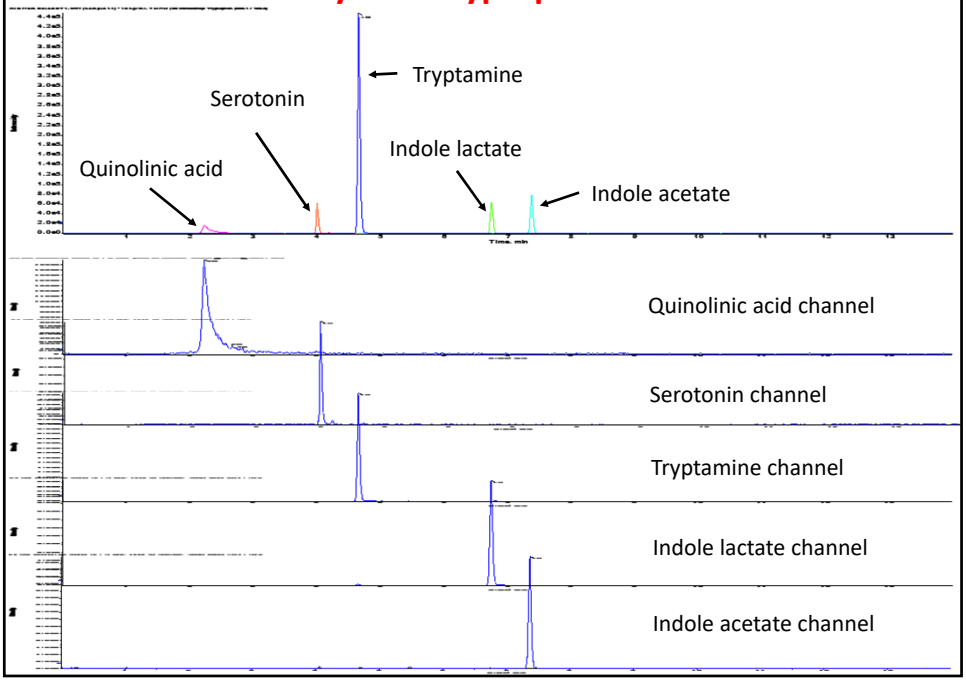
How many MRM transitions?

- **Acquisition time can be as little as 2 msec, but acquisition time also determines sensitivity**
  - Fast switching electronics can measure as many as 500 different mass transitions per second
- **Since measuring the area under a peak requires 10 data points, the number of mass transitions measured has to be matched against the shape and width of the chromatographic peaks**
  - to be discussed in more detail later today

Combined channels for Krebs cycle



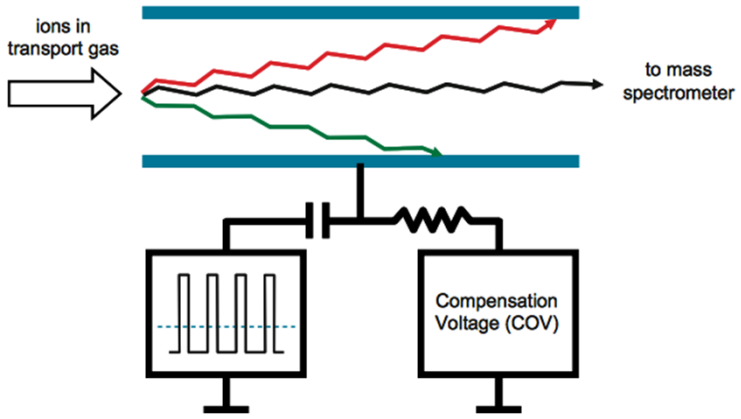
LC-MS analysis of tryptophan metabolites



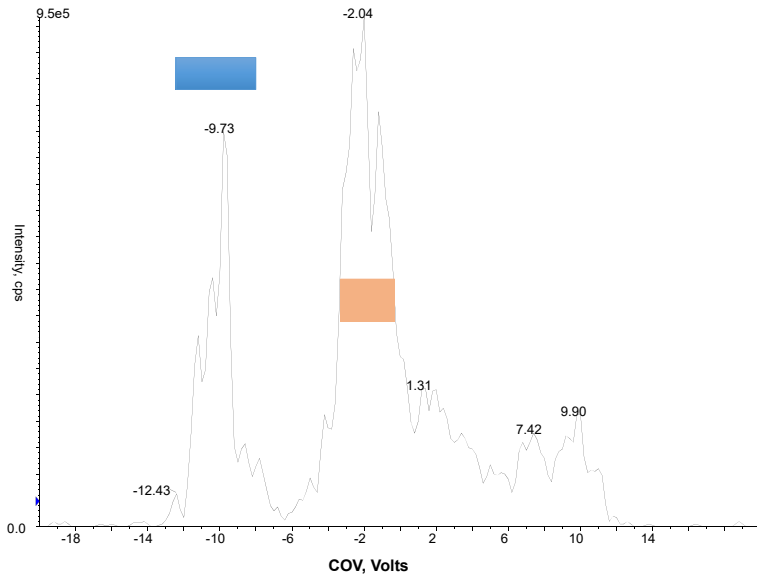
Differential mobility MS is an answer

No chromatography

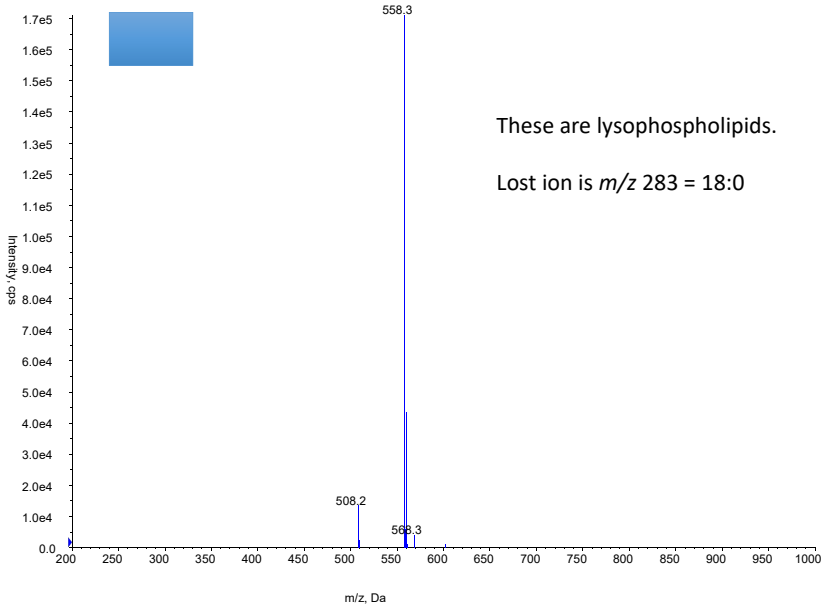
Innovative Planar Design; SelexION™ Ion Mobility Cell.



TIC of Precursors of *m/z* 283.0 (18:0)
Separation of lipids with FA 283 in negative ion mode



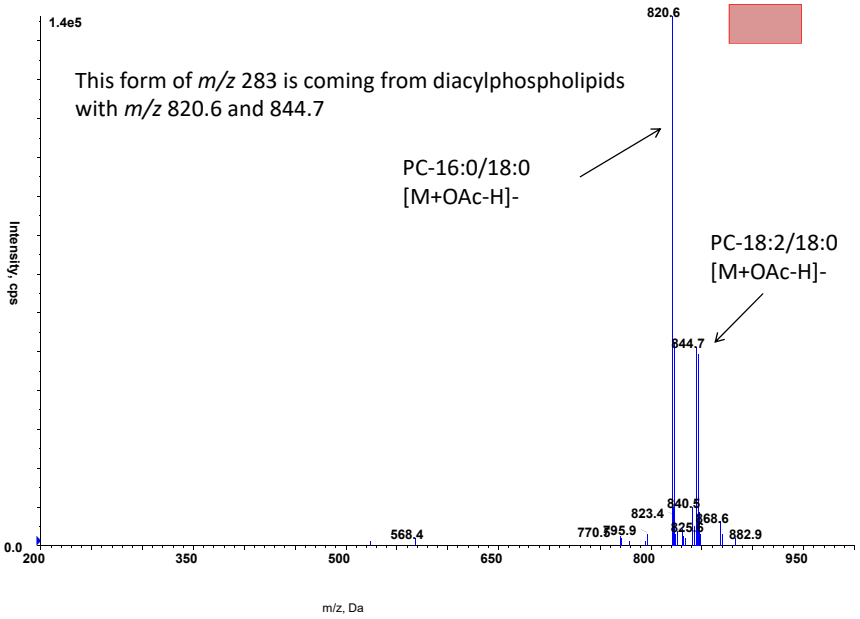
Precursors of *m/z* 283.0 – CoV from -11.8 to -8.6 V



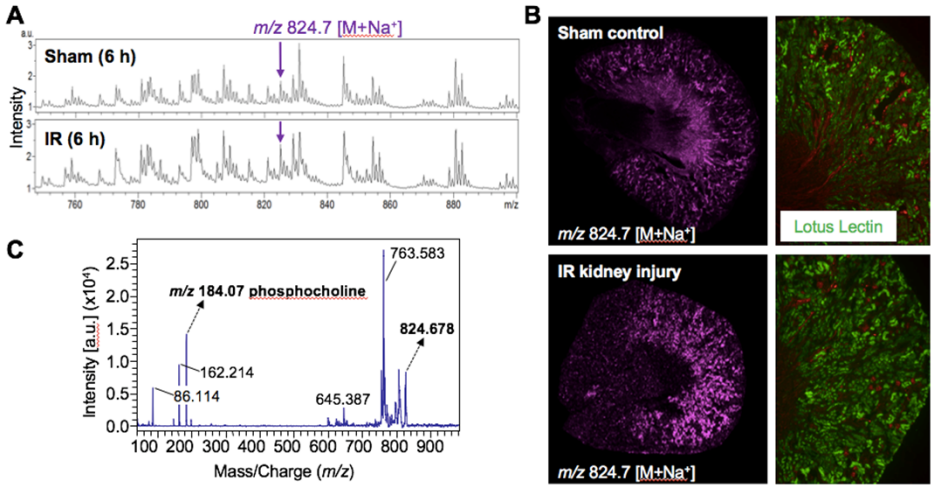
These are lysophospholipids.

Lost ion is *m/z* 283 = 18:0

Precursors of *m/z* 283.0 CoV from -3.4 to -0.4 Volts

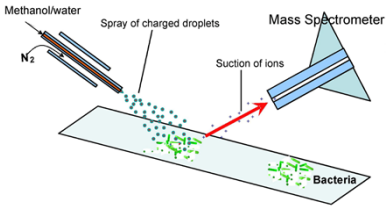


Imaging mass spectrometry



Generated by Janusz Kabarowski – will talk to you on Friday

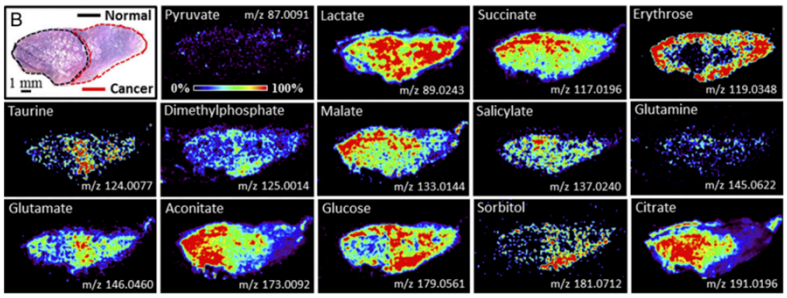
Imaging by DESI-MS



No matrix needed, but lower spatial resolution

DESI Experiment

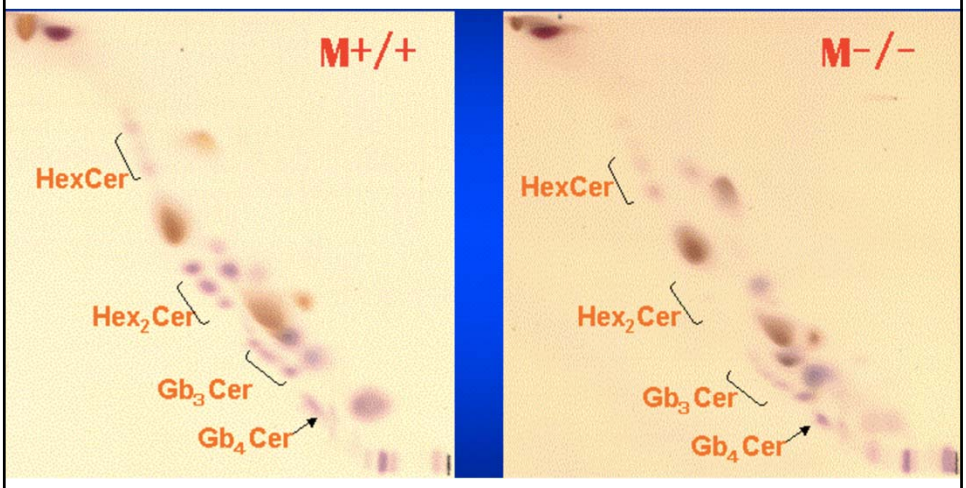
http://www.chem.purdue.edu/NewsFeed/newsstory.asp?itemID=207



Banerjee et al., PNAS 114: 3334, 2017

2D-Thin layer chromatography of lipids

KO of cerebrosidesulfatase in kidney



These plates can be studied by direct electrospray ionization (DESI)

Questions?