Knowledge that will change your world

Ist UAB Metabolomics Workshop
July 22-25, 2013

Future of Metabolomics

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and Proteomics Laboratory

Issues in metabolomics research

- Standards and methods standardization
- Improved databases and MS/MS
- Spatial distribution
  - Imaging mass spectrometry
    - DART
    - Spotting methods
- Localized metabolomics
  - Head space GC-MS
    - Breath and other body odors
    - ?? urine "odor"
  - iKnife-MS
    - Metabolome of the "smoke" created with cauterized surgical knife
- Derivatives and Isotope labeling

Standards

- The majority of compounds that are detected have not been fully characterized and don't exist in pure standard form
- The LipidMaps endeavor (www.lipidmaps.org) has gone a long way to improve the state of that field
- A heavy investment in chemical synthesis of other metabolites is sorely needed

Standardized methods

- Importance of standardized operating procedures and recording keeping of any deviations from them – use of LIMS
- NMR yields absolute, quantitative data, but for a limited number of metabolites
- Whereas LC-MS is very sensitive, quantification is a challenge
- For primary untargeted metabolomics data collection, the Qtof instrument is both the fastest and most robust
  - The UK Phenomics Center is using a single manufacturer's Qtof - harmonization

Capillary electrophoresis-MS and metabolomics

- "*Capillary electrophoresis and MS is a marriage made in heaven, but not on earth*" (Richard Smith)
- Can CE-MS provide for metabolomics what CE has done for DNA deep sequencing?
- CE has many forms and can accommodate a wide variety of even hydrophilic compounds
- The interface is the key component

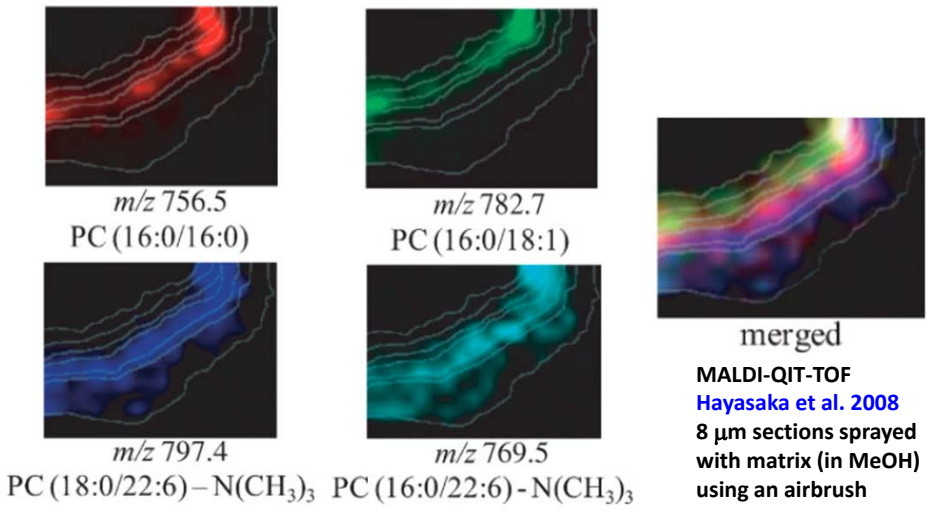
Can we improve the chromatography?

- The sheer numbers of metabolites demands a better chromatographic solution
  - CE-MS is one answer
- Taking a leaf from the gas chromatography community, LC needs to move towards open tubular columns
  - The back pressure of smaller and smaller particles is limiting
  - So, get rid of the particles

Understanding where metabolites are

- When we collect the metabolites from tissue or from cells, we destroy their localization information
- We assume that all metabolites are in contact with each other
- But there as many as 15 distinct compartments in cells
- Some cells rely on neighboring cells for specific metabolites

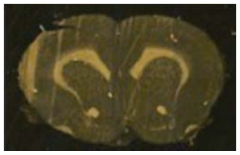
Differential lipid distribution in the retina



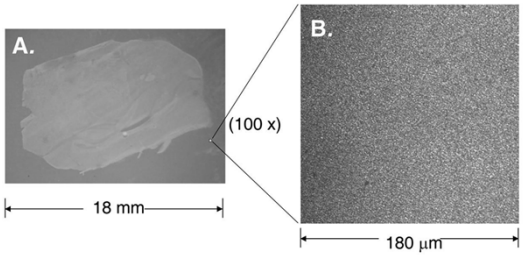
MALDI-QIT-TOF
Hayasaka et al. 2008
8 μm sections sprayed with matrix (in MeOH) using an airbrush

Imaging of lipids in tissue

Fresh frozen tissue



Frozen tissue sectioning
Cut and transfer 10-20 micron section to conductive glass slide



DHB (MALDI matrix) is transferred by sublimation.
This produces a very uniform coating (as shown in B) and high resolution images

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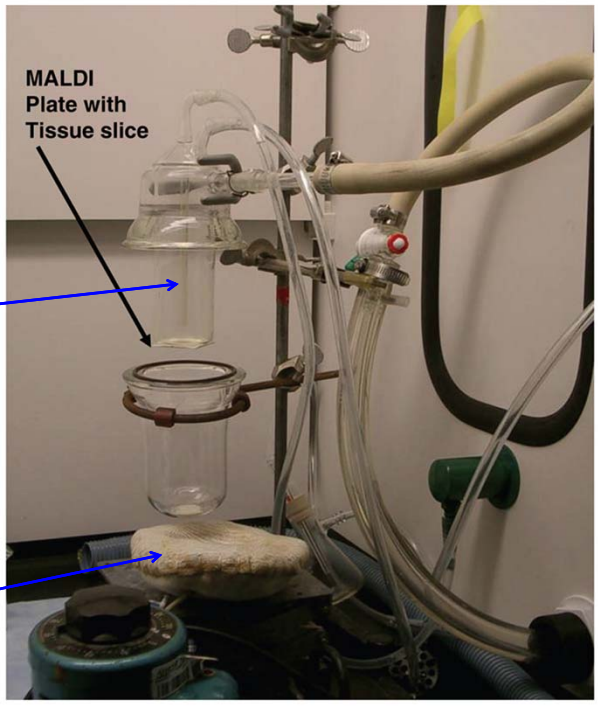
Sublimation method for applying MALDI matrix to the tissue slice

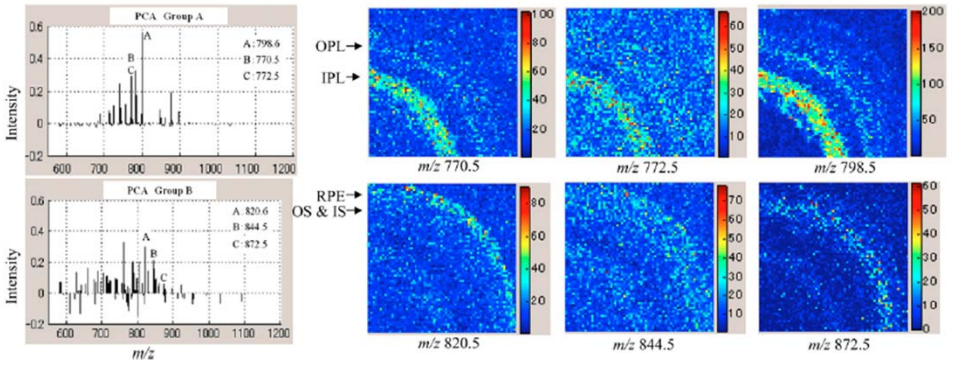
MALDI plate is upside down – ice is placed inside the glass "finger" to condense the DHB

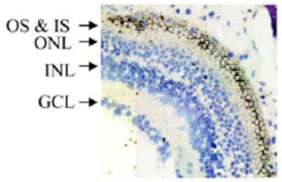
A cold trap (not shown) is needed after the heated chamber to protect the vacuum pump

Sand bath to heat the DHB



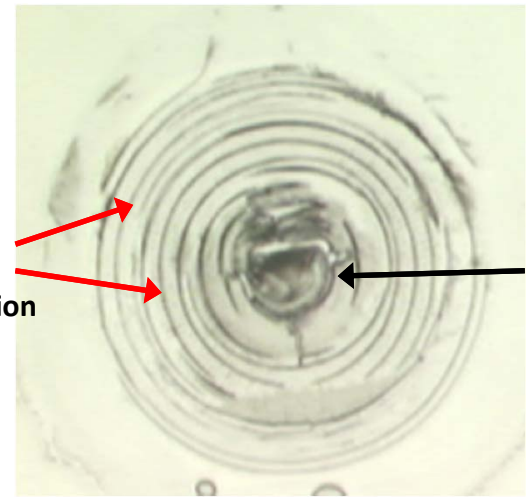
Using a higher resolution approach





The same Japanese group used an "evaporation" method to apply the matrix – sublimation.
The resolution is at the 5 μm level, i.e., single cells

Frozen, thin section of zebrafish lens

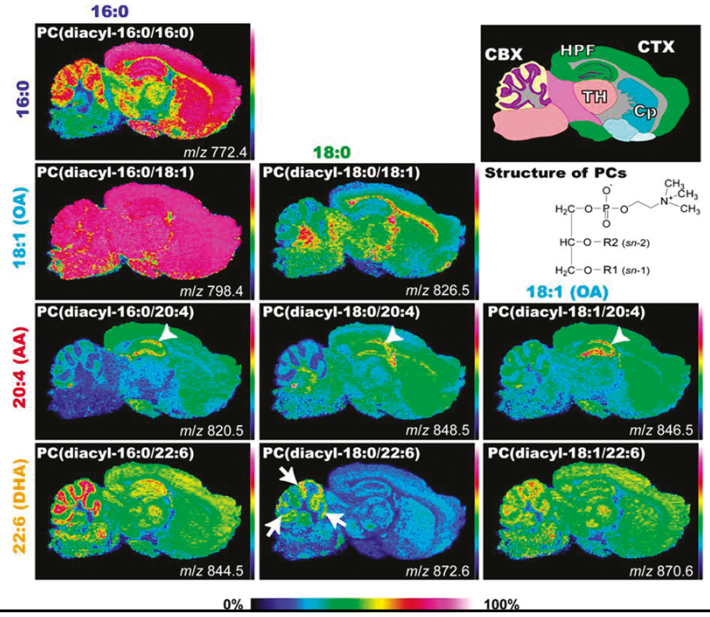


Concentric rings in the cortical region

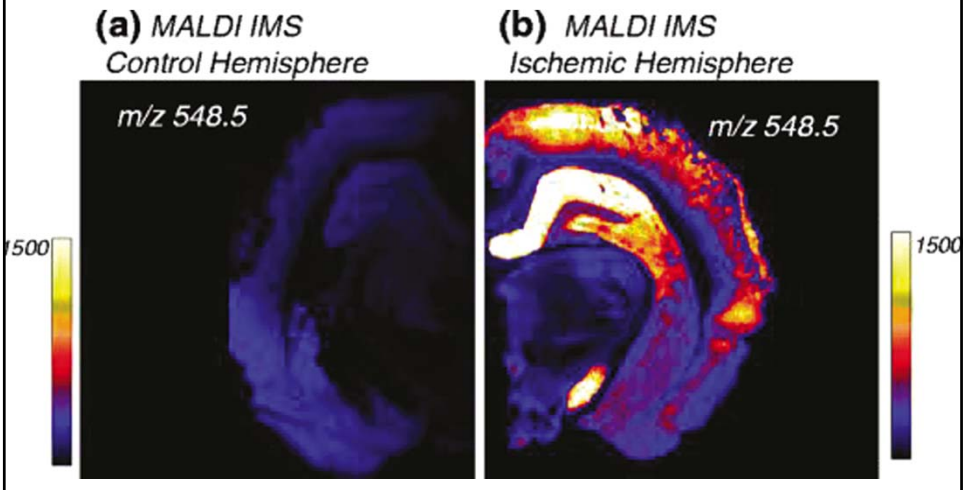
Nuclear zone

Miranda Collier, 2013

PC species distribution in the brain



Ceramides and ischemia by IMS



The ion was determined to be Cer 18:0/18:1 (-H_2O)

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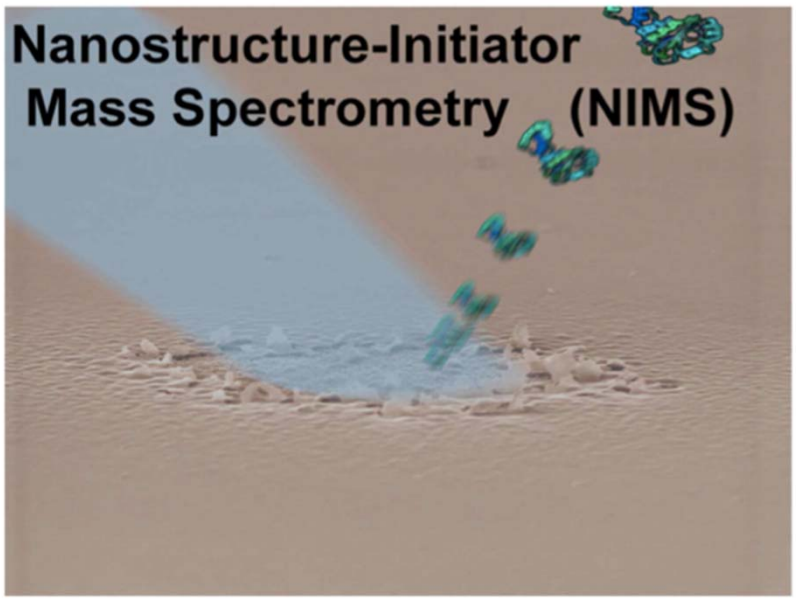
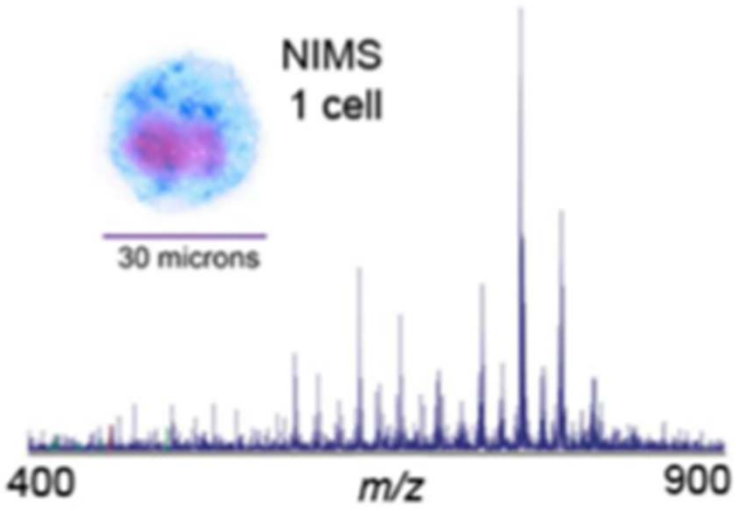


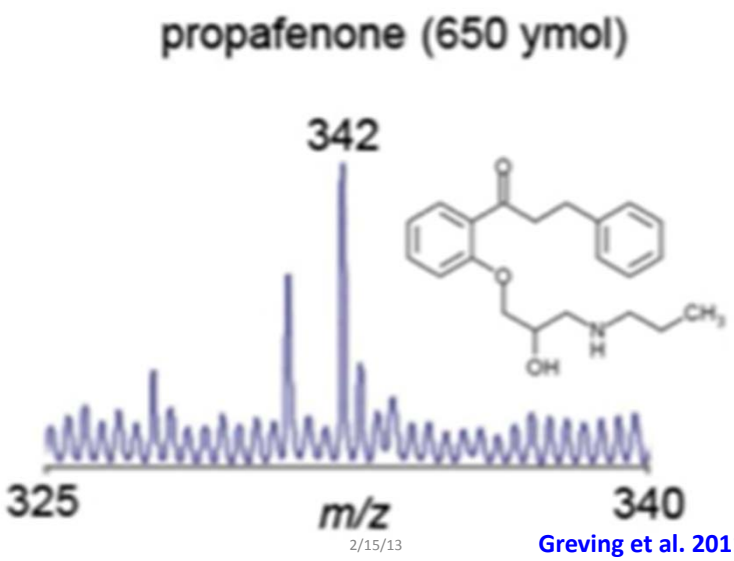
2/15/13 Greving et al. 2011

Spectrum from a single cell



2/15/13 Greving et al. 2011

Extreme sensitivity



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Greving et al. 2011

Metabolism and time

- Not only should metabolites appear in the right place, there is also the question of the importance of the timescale
- Metabolism defects in the heart may be only seconds away from death – rogue wave in metabolism??
- Irreversible damage to the brain may occur in minutes
- Go/No-go decisions for a cell to divide or apoptose may occur in a similar timescale

Can existing techniques provide answers?

- Mass spec is mostly a destructive method
  - However, it can measure volatiles rapidly on a sec timescale (as we'll see shortly)
- NMR can provide spatial and descriptive information on living or unextracted materials
  - However, sensitivity and speed of data acquisition are extremely limiting
- Future in other spectroscopic techniques?

Components of breath

- Two types of components
  - Droplets in exhaled breath
    - Can be condensed (proteins, lipids, metabolites)
    - LC-MS analysis
  - True volatiles (requires GC-MS)
    - Non-condensable gases
      - H_2, N_2, O_2, CO_2
    - Organics
      - Alcohols, aldehydes, alkanes

Detecting smells

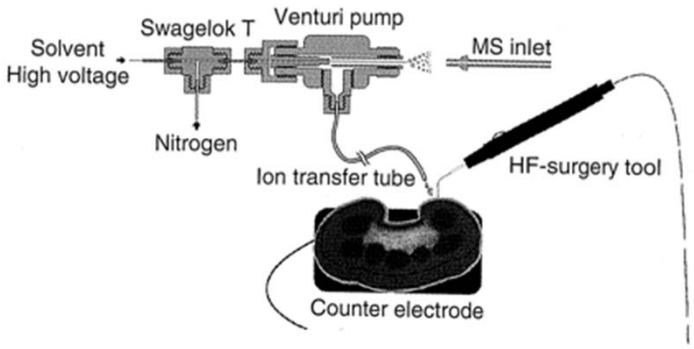
- Unlike dogs, humans have relatively few olefactory receptors (dogs have lousy taste!)
  - We can detect certain "bad smells" (and cover these by washing and deodorants and fragrances)
- The reality is that we all emit gases that can be diagnostic
  - GC-MS using a headspace technique can be used
    - Breath hydrogen is diagnostic for lactase insufficiency

Portable Metabolomics Devices

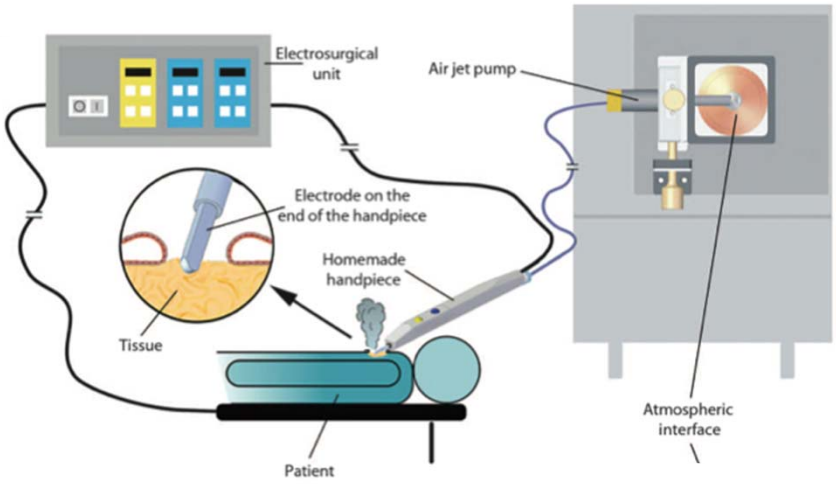
David Wishart

iKnife mass spectrometry

Electrospray post ionization mass spectrometry of electrosurgical aerosols. **Guenther et al., JASMS 2011**

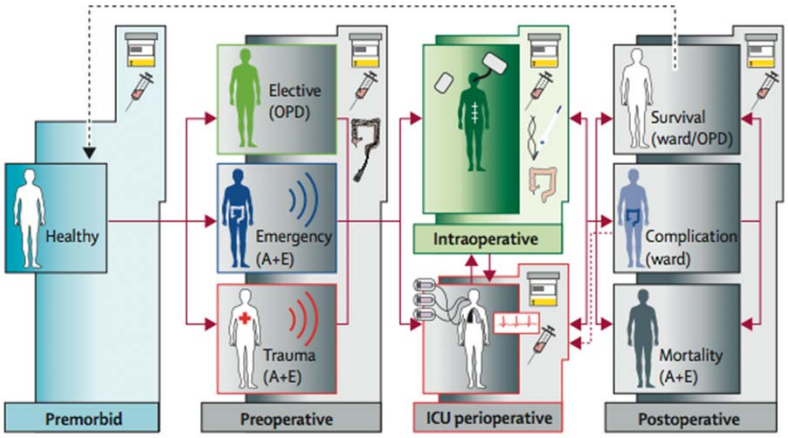


The iKnife – real time metabolomics



Balog et al. Nature Med 5; 194r93

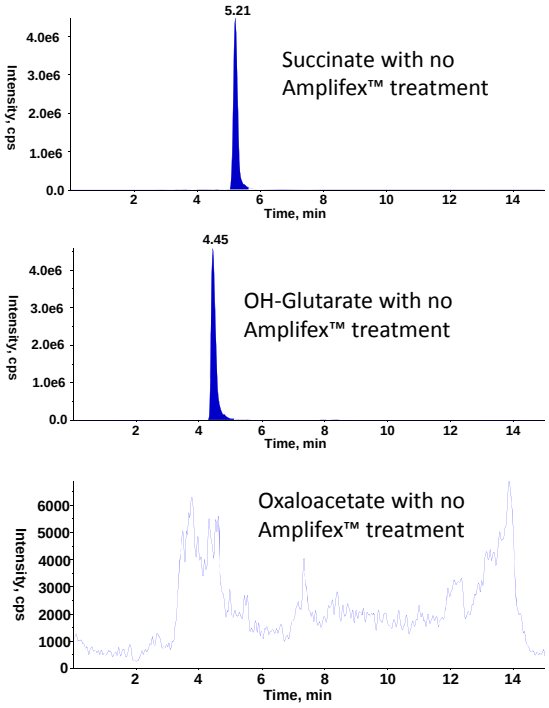
Clinical applications of metabolomics



Concepts of Jeremy Nicholson

Back to derivatization in metabolomics

- A great advantage of LC-MS is its ability to measure compounds “as is”
- However, there are many compounds that either weakly form ions or are found in multiple chemical forms (e.g., steroids, ketosteroids, keto acids, fatty acids)
- The reagents used to overcome this problem can be isotopically labeled (iMetab)



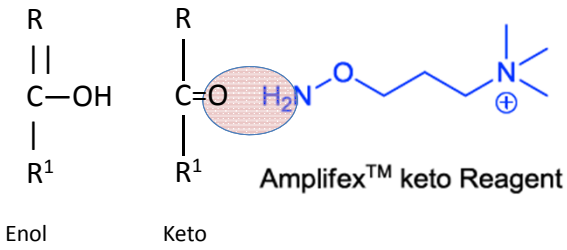
Individual MRM channels for citric acid cycle intermediates

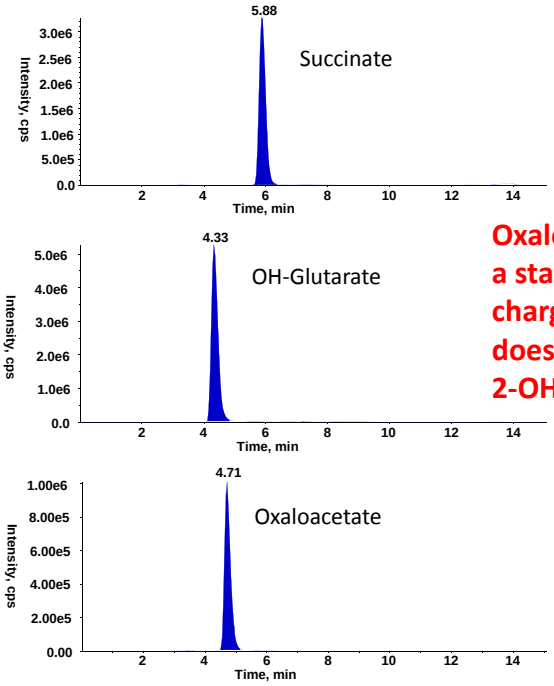
For most compounds, the MRM signal yields a single peak

Note that the signal for oxaloacetate is weak and "ugly" – keto acids can undergo keto-enol tautomerism

Modifiers to the keto group

- **Methoxylamine – CH_3ONH_2 (from GC-MS)**
- **Biotin hydrazide – $RN.NH_2$**
- **Amplifex™-Keto Reagent (ketosteroids)**





Oxaloacetate is converted to a stabilized, positively charged oxime The reagent does not alter succinate or 2-OH glutarate

Isotopic derivatization

- investigators are exploring reagents that can be coupled to $-NH_2$ and –COOH groups
  - c.f., ICAT, iTRAQ and TMT reagents for proteomics
- These may allow one sample of a pair to be labeled with a "light" reagent and another sample with the same reagent, but one incorporating ^{2}H, ^{13}C or ^{15}N atoms and therefore being "heavy".
  - The two samples can then be mixed and run at the same time
  - Multiplexed reagents are also possible
- Software to sort this out has not yet been written