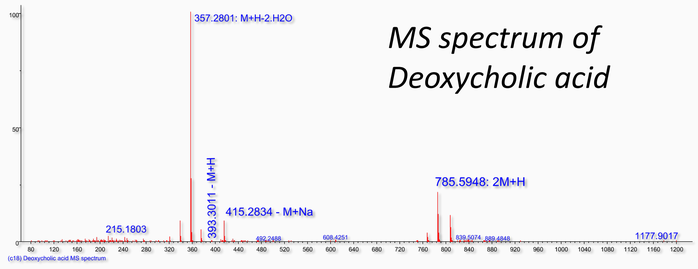
RAMclust/RAMsearch: efficient post-XCMS feature clustering and annotation of MS-based metabolomics datasets.

Corey D. Broeckling and Jessica E. Prenni

Colorado State University, Proteomics and Metabolomics Facility

Introduction:

- Non-targeted profiling by UPLC-MS is a powerful tool for metabolic profiling.
- Electrospray ionization is soft, but many signals are generated for a given metabolite:
  - Isotopes
  - Adducts
  - Multimers
  - In-source fragments
- Most metabolomics processing workflows fail to account for these phenomenon.

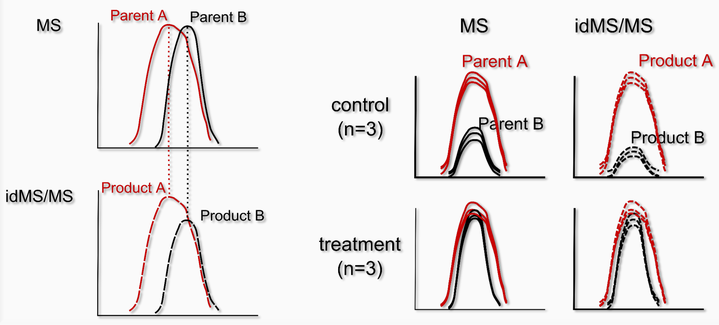


MS spectrum of Deoxycholic acid

- In-source phenomenon collectively result in a mass spectrum of signals representing a compound.
- ***MASS SPECTRA are the most accurate representation of metabolites.***
- Prediction of spectra would require knowledge of structure *and* prediction of fragments.

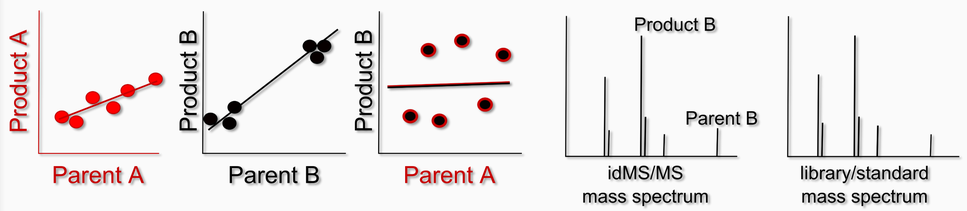
Approach:

- Devise a 'chemically blind' clustering approach to deal with unpredictable phenomena.
- Common MS signals derived from the same compound will both *coelute and covary*.

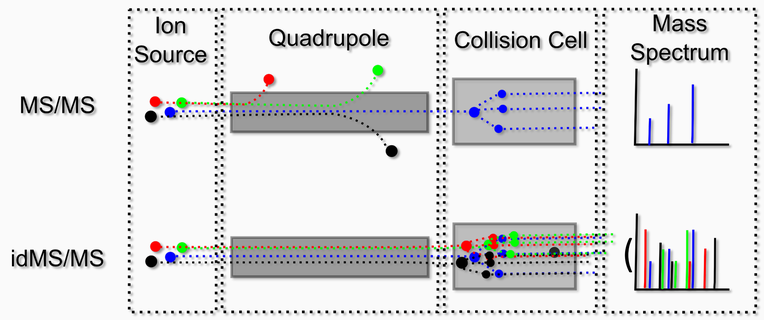


Approach:

- Covariation and coelution of two features represent strong evidence that they derive from same compound → these *features should be grouped together*!



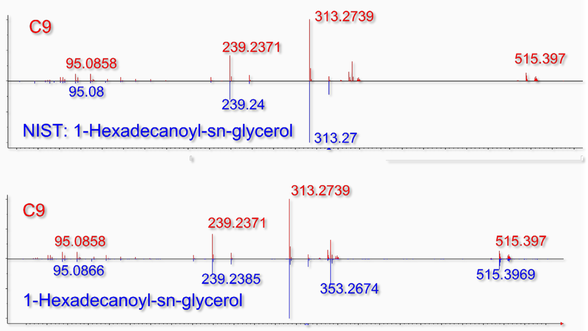
- ***Feature groups (including signal intensities) are representative of compound spectra that can be used for spectral matching to facilitate compound annotation.***

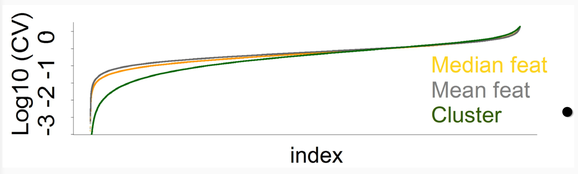


- (OPTIONALLY) Utilize indiscriminant MS/MS data to generate fragmentation data for every feature.
- Feature similarity scores are based on product of correlational (covariance) and retention time (coelution) similarity.

Results (RAMclustR):

- Feature clustering enables confident recreation of mass spectra from XCMS output.
- Spectra can be used for compound annotation.

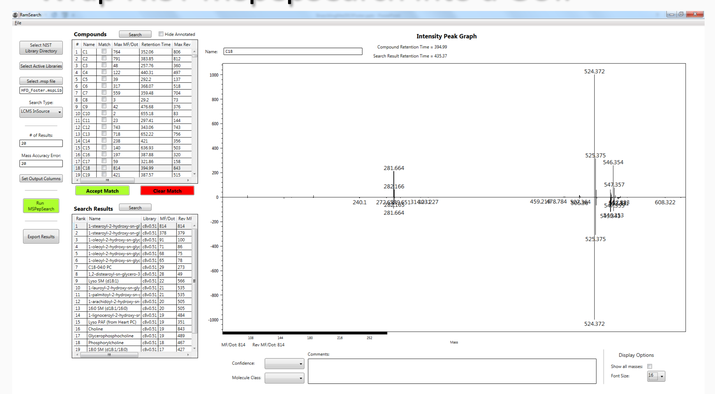




- Simultaneous reduction in complexity
- Reduction in dataset wide variance

Results (RAMsearch):

- Feature groups export as MS spectra in *.msp format.
- Enables efficient spectral searching.
- Wrap NIST MSpepSearch into a GUI.



Enables:

- ✓ Batch searching spectral libraries
- ✓ rapid manual validation of spectral matches
- ✓ Assignment of annotation confidence scores (MSI)
- ✓ Export of evidence, including match spectra, for import into RAMclustR

Conclusions:

More realistic - features are derived from compounds and feature clusters (spectra) fully represent compounds.

More streamlined - ~ 5-10 fold reduction of features (RAMclustR). Batch searching is enabled (RAMsearch).

More sensitive - Aggregation of features into spectra reduces analytical variance.

More confidence - Several mass spectral signals offer more annotation confidence than a single accurate mass alone.

Open source:

https://github.com/cbroeckl/RAMClustR

Anal. Chem., **2014**, *86* (14), pp 6812–6817

Acknowledgements:

Asa Ben-Hur, and Fayyaz A Afsar helped to develop ramclustR algorithms.
Steffen Neumann helped package ramclustR
NIST (Steve Stein, David Sparkman, Dmitri Tchekhovskoi)
Kevin Brown and Ben Sutton helped develop RAMsearch