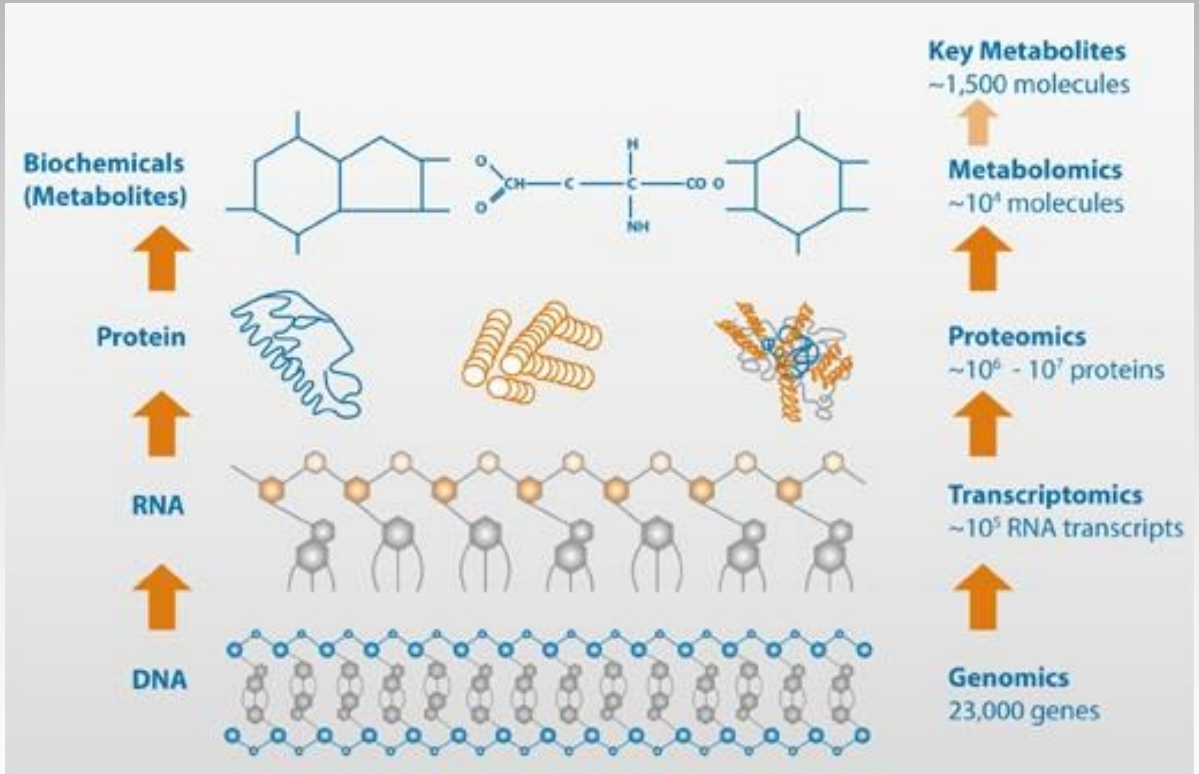
Metabolomics



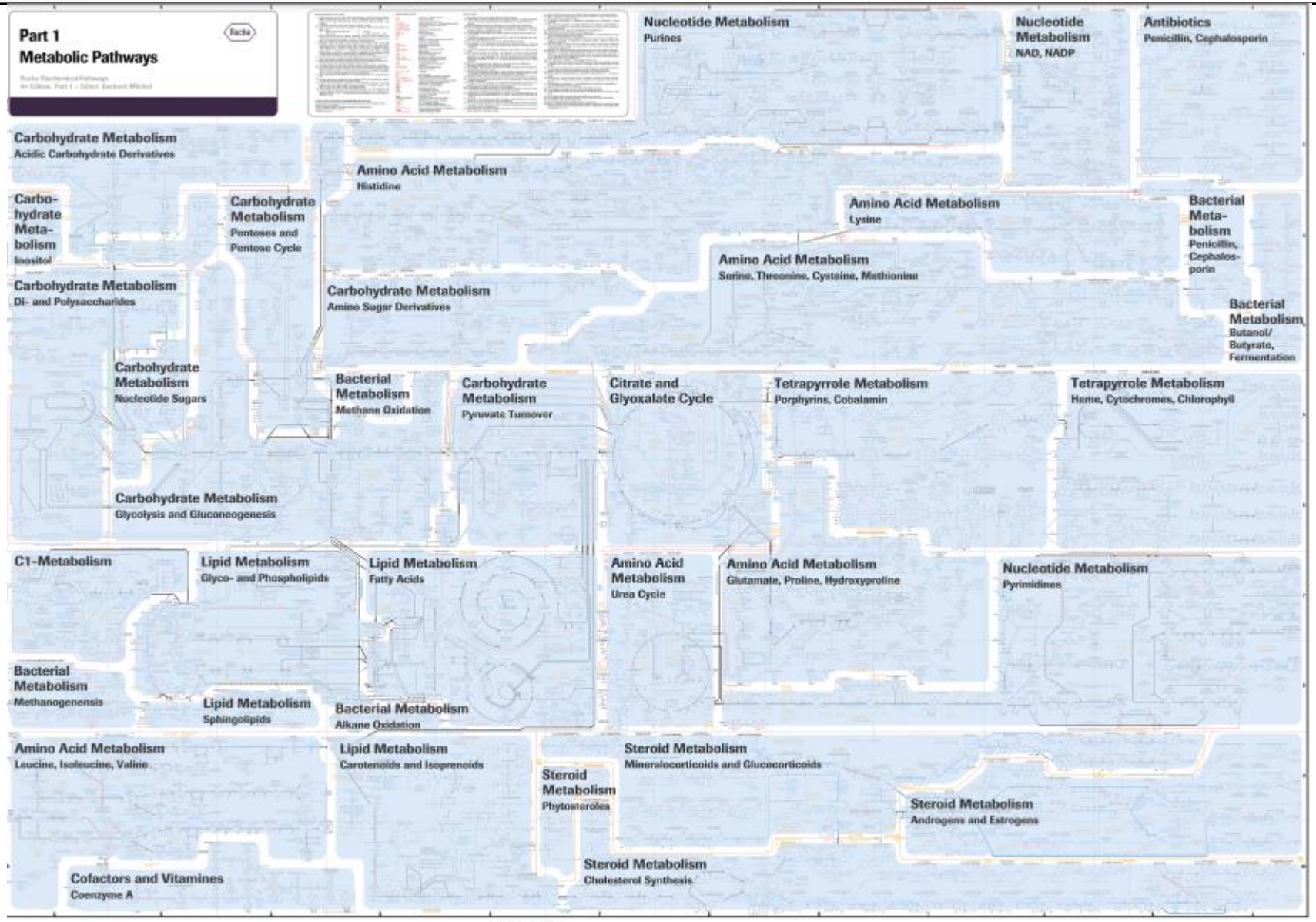
Metabolomics - definition

- Scientific study of chemical processes involving metabolites

- Metabolites
  - Intermediates and products of metabolic reactions
    - Synthesis and breakdown of compounds
  - Lipids, sugars, peptides, volatile compounds, ...
  - Hormones and other signalling molecules
  - Products of metabolism of foreign substances
  - usually < 1500 Da in size

- The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes
  - Very diverse and complex

Metabolic pathways



http://biochemical-pathways.com

Why is Metabolomics Relevant?

- Generate metabolic “signatures”
- Monitor/measure metabolite flux
- Monitor enzyme/pathway kinetics
- Assess/identify phenotypes
- Monitor gene/environment interactions
- Track effects from toxins/drugs/surgery
- Identify functions of unknown genes

NMR vs MS

Table 1. Summary of the most important advantages and limitations of nuclear magnetic resonance (NMR) spectroscopy compared to mass spectrometry (MS) in metabolomics applications.

	NMR	Mass Spectrometry
Reproducibility	High reproducibility is one of the fundamental advantages of NMR spectroscopy.	Compared to NMR spectroscopy, MS data are less reproducible.
Sensitivity	Intrinsically low but can be improved with multiple scans (time), higher magnet field strength, cryo-cooled and microprobes, and hyperpolarization methods.	High sensitivity is a major advantage of MS; metabolites with nanomolar concentrations can be readily detected
Selectivity	NMR is generally used for nonselective analysis. Peak overlaps from multiple detected metabolites pose major challenges.	MS is selective. However, in combination with chromatography (such as liquid and gas phase separation), it is a superior tool for targeted analysis.
Sample measurement	Enables relatively fast measurement using 1D ^{1}H-NMR spectroscopy, where all metabolites at a detectable concentration level can be observed in one measurement.	Different ionization methods are required to maximize the number of detected metabolites.

https://www.mdpi.com/2218-1989/9/7/123

NMR vs MS

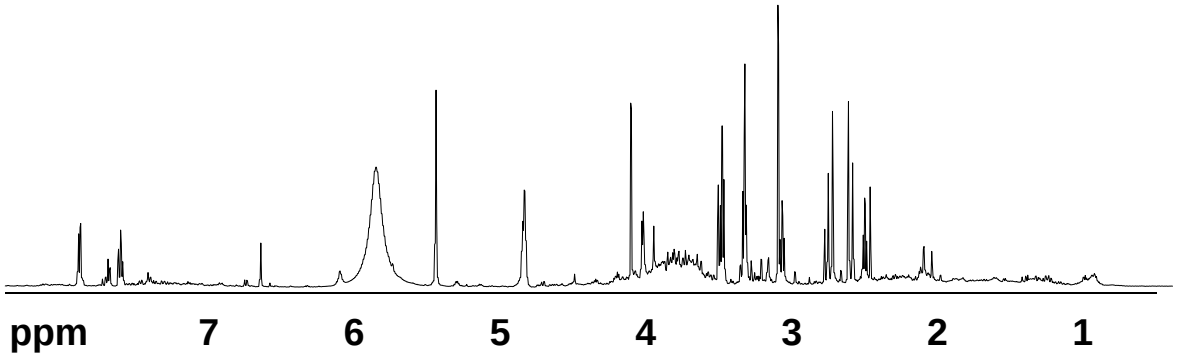
Sample preparation	Involves minimal sample preparation, usually transferring the sample to an NMR tube and adding deuterated locking solvent. Can be automated.	More demanding; requires chromatography; requires sample derivatization for gas chromatography (GC)-MS.
Sample recovery	NMR is nondestructive and, hence, several analyses can be carried out on the same sample. Additionally, the sample can be recovered and stored for a long time.	MS is destructive technique; therefore, the sample cannot be recovered. However, it needs only a small amount of sample.
Quantitative analysis	NMR is inherently quantitative as the signal intensity is directly proportional to the metabolite concentrations and number of nuclei in the molecule.	The intensity of the MS line is often not correlated with metabolite concentrations as the ionization efficiency is also a determining factor.

https://www.mdpi.com/2218-1989/9/7/123

	NMR	Mass Spectrometry
Fluxomics Analysis	NMR permits both in vitro and in vivo metabolic flux analyses. Its inherently quantitative nature also enables precise quantification of precursors and products. Mapping of stable isotope locations and incorporating points in molecules is very easy via NMR.	MS can be used for fluxomics analysis; however, the destructive nature of MS-based methods means it is somewhat more limited than NMR-based fluxomics. In vivo fluxomics is not possible with MS, and isotope mapping is more difficult.
Tissue samples	Using high-resolution magic-angle sample spinning (HRMAS) NMR, it is possible to detect metabolites in tissue samples.	Although some MALDI-TOF approaches can be used to detect metabolites in tissue samples, these approaches are still far from being routine.
Number of detectable metabolites	Depending on spectral resolution, usually less than 200 metabolites can be unambiguously detected and identified in one measurement.	Using different MS techniques, it is possible to detect thousands of different metabolites and identify several hundred.
Targeted analysis	NMR spectroscopy can be used for both targeted and untargeted analyses, but it is not commonly used for targeted analyses.	Both GC-MS and liquid chromatography (LC)-MS are superior for targeted analyses
In vivo studies	Using magnetic resonance spectroscopy (MRS), in vivo investigation can be carried out most often using nuclei such as ^{1}H and ^{31}P.	Although desorption electrospray ionization (DESI) may be a useful way to analyze tissue samples during surgery, MS is not used for in vivo metabolomics studies.

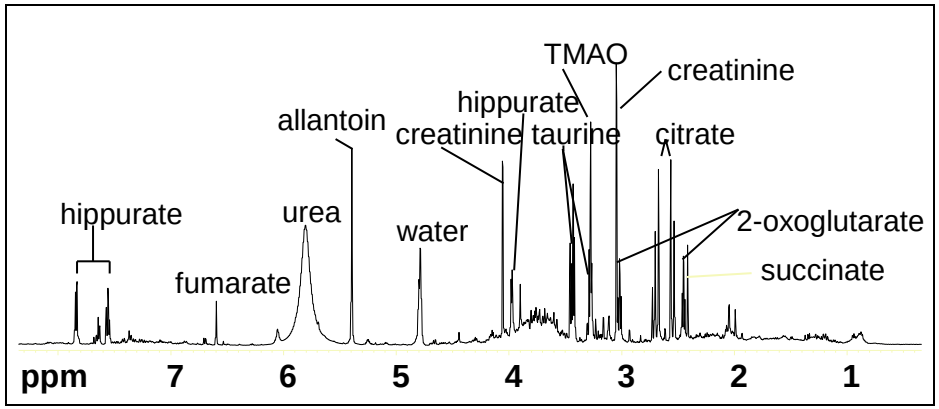
https://www.mdpi.com/2218-1989/9/7/123

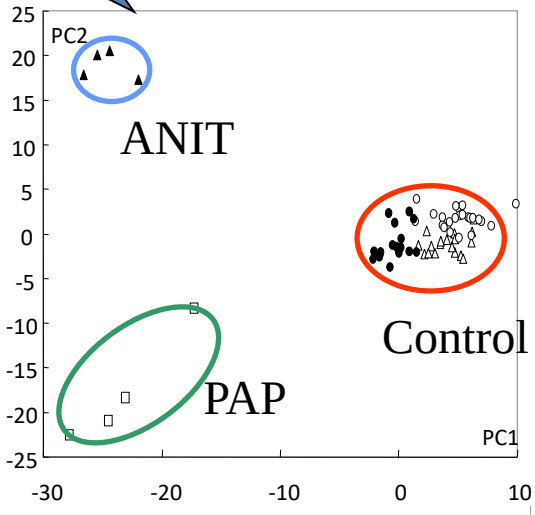
2 Routes to Metabolomics



Targeted /quantitative

Untargeted/chemometric





Targeted vs untargeted metabolomics

- Targeted: Pre-defined set of metabolites to quantify
  - Typically carried out in diagnostics
  - Pros: Cab be used for a wide range of samples, identifies and quantifies compounds
  - Cons: Limited by database size, missing information

- Untargeted: Global analysis of metabolic changes in response to disease, environmental or genetic perturbations.
  - typically carried out for hypothesis generation, followed by targeted profiling for more confident quantification of relevant metabolites.
  - Pros: Unbiased (no selection of metabolites)
  - Cons: Technically challenging (both the analysis and the bioinformatics), risk of getting too many unknowns, limited by training set

New methods

Issue 24, 2019

Previous Article | Next Article

From the journal:
Analyst

Reliable, high-quality suppression of NMR signals arising from water and macromolecules: application to bio-fluid analysis†

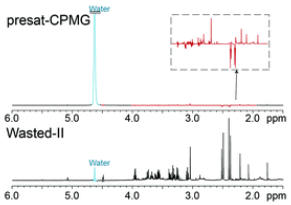
Check for updates

Juan A. Aguilar,[a] Julia Cassani,[ab] Fay Probert,[c] Jacqueline Palace,[d] Tim D. W. Claridge,[e] Adolfo Botana[f] and Alan M. Kenwright[a]

Author affiliations

Abstract

Analysis of metabolites in biofluids using nuclear magnetic resonance often requires the suppression of obscuring signals arising from water and macromolecules. This paper analyses the limitations of the pulse sequence most commonly used to achieve such suppression (presat-CPMG) and proposes new pulse sequences that do not share those limitations. The utility of these improved pulse sequences is demonstrated in a metabolomic study of multiple sclerosis (MS) patients.



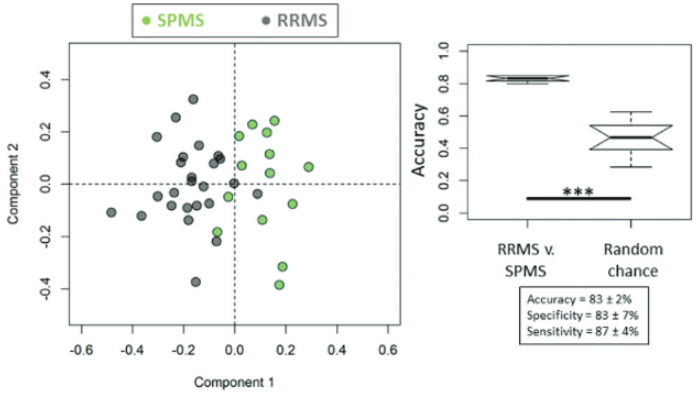
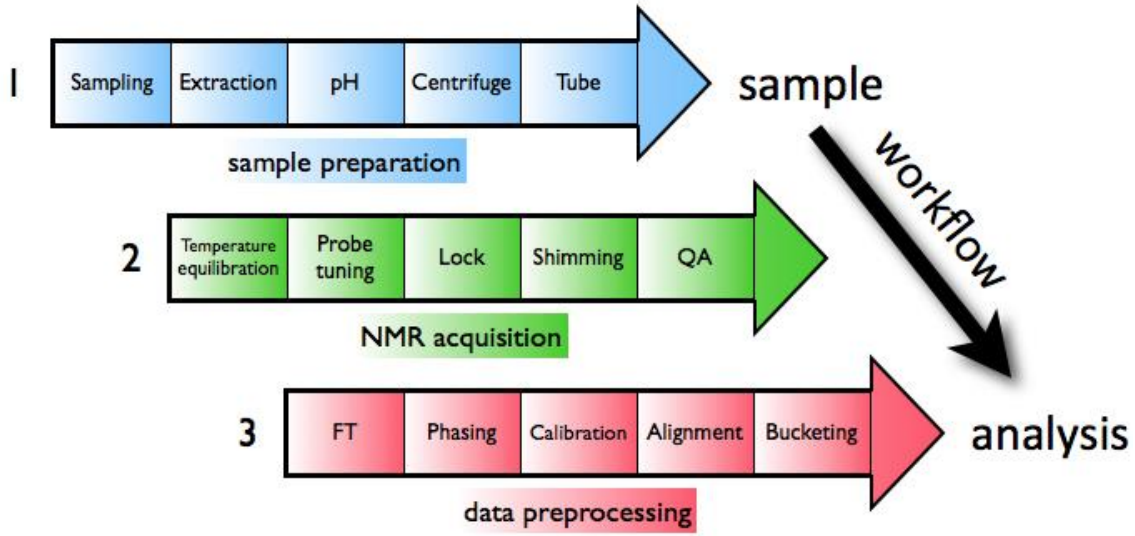


Fig. 6 OPLS-DA results from blood plasma Wasted-II ^{1}H NMR data discriminating SP from RR multiple sclerosis patients. Left, a representative scores plots illustrating separation between SPMS and RRMS plasma spectra in the multivariate models, and right, the accuracy of the cross-validated ensemble of OPLS-DA models is significantly better than random chance. Kolmogorov–Smirnov test p-values <0.001 are represented by ***.

Discrimination between Secondary-Progressive and Relapsing-Remitting Mutiple sclerosis patients

Metabolomics methodology



nmrprocflow.org

NMRProcFlow

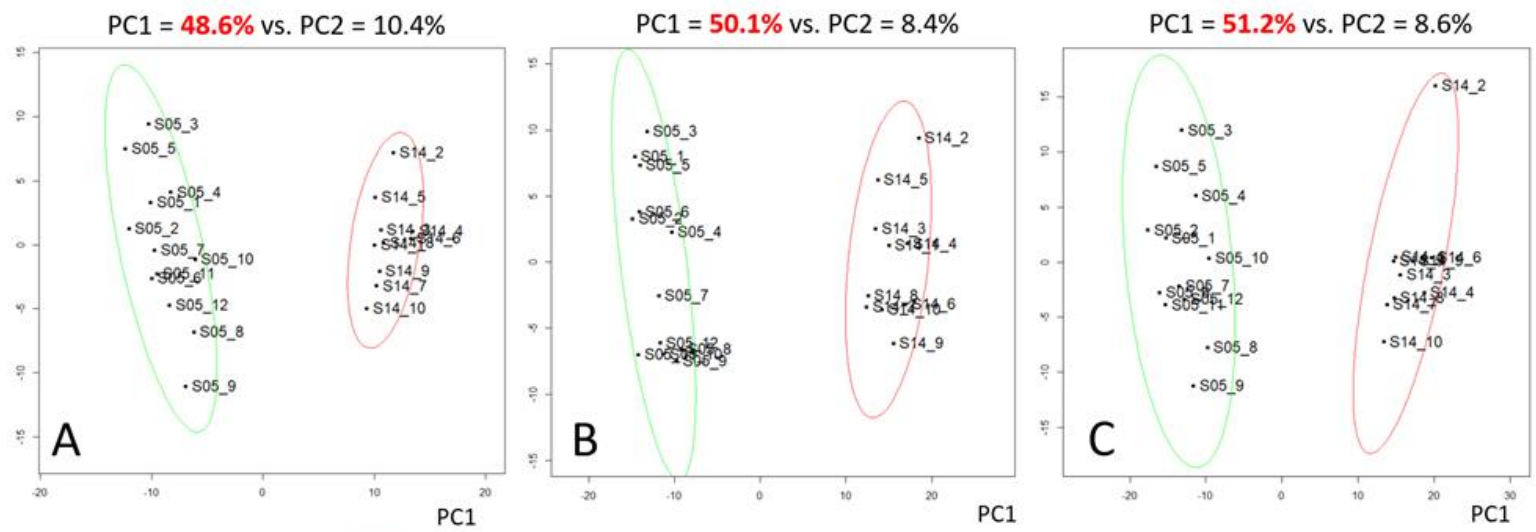
- Spectra preprocessing
  - PPM calibration
  - Global and local baseline corrections
  - Spectra alignment
  - Non-uniform bucketing
  - Signal-to-Noise ratio determination
  - Total Sum Normalization

BioStatFlow

biostatflow.org

- Scaling
  - Z-score
- Statistical analysis
  - Principal Component Analysis

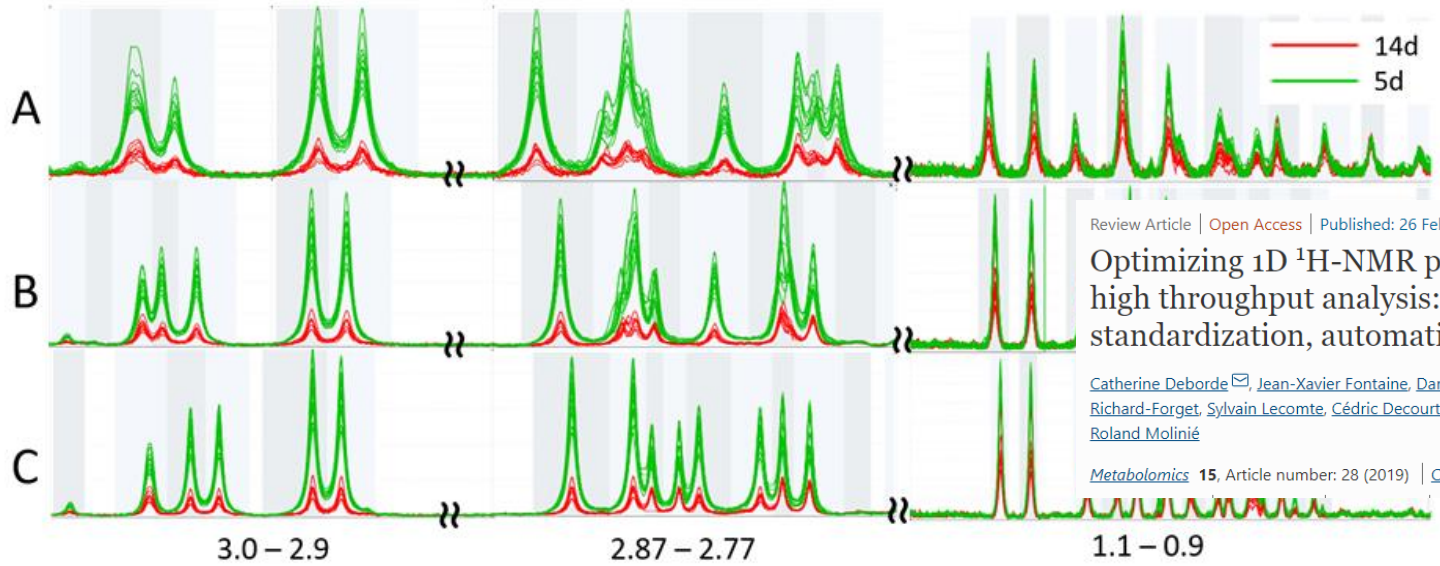
PCA of 22 wheat samples inter-laboratory study



A: JEOL 400
B: Bruker 500
C: Bruker 600

Green: 5 days after flowering

Red: 14 days after flowering



Review Article | Open Access | Published: 26 February 2019

Optimizing 1D ^{1}H-NMR profiling of plant samples for high throughput analysis: extract preparation, standardization, automation and spectra processing

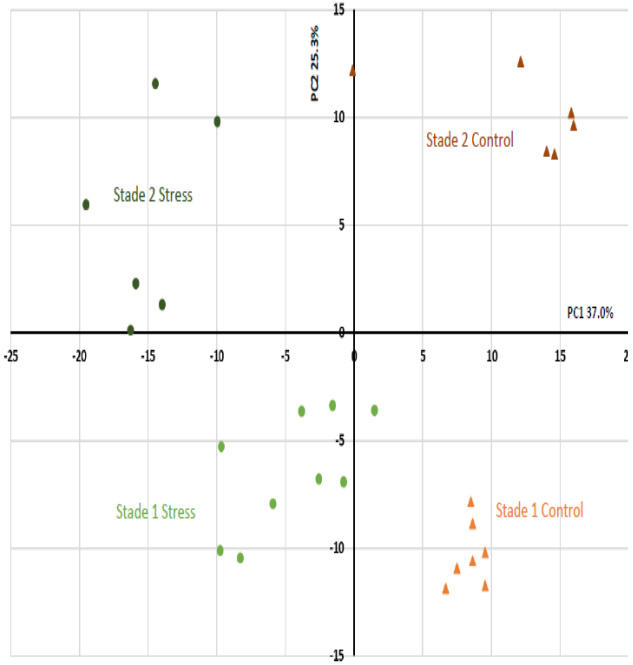
Catherine Deborde, Jean-Xavier Fontaine, Daniel Jacob, Adolfo Botana, Valérie Nicaise, Florence Richard-Forget, Sylvain Lecomte, Cédric Decourtil, Kamar Hamade, François Mesnard, Annick Moing & Roland Molinié

Metabolomics **15**, Article number: 28 (2019) | Cite this article

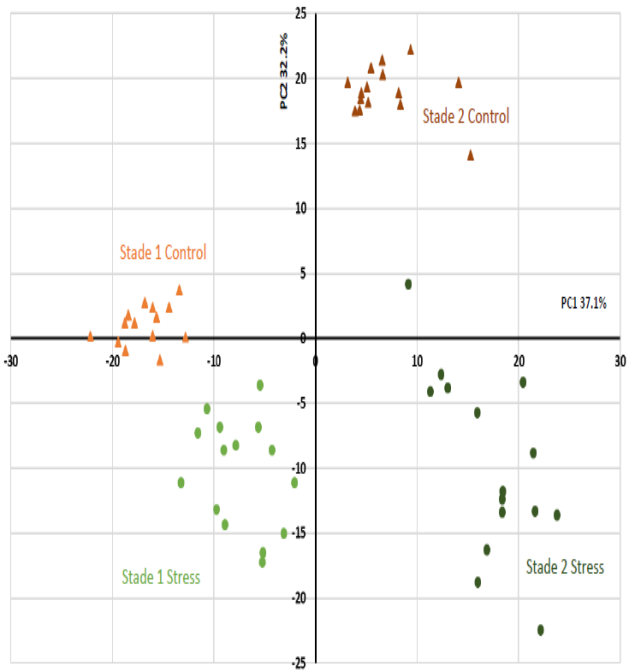
PCA of 22 wheat samples inter-laboratory study

- two stages: 5 and 14 days after flowering
- two culture conditions: control and stress

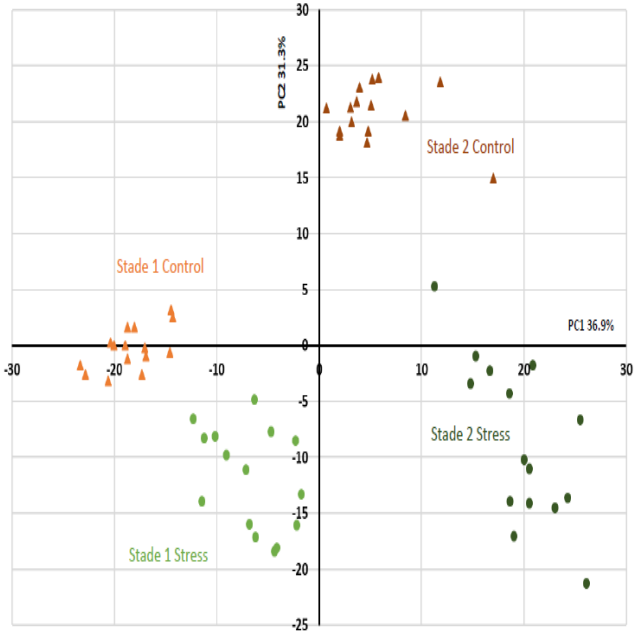
PCA 400MHz ^{1}H NMR profile (z_scores)



PCA 500MHz ^{1}H NMR profile (z_scores)

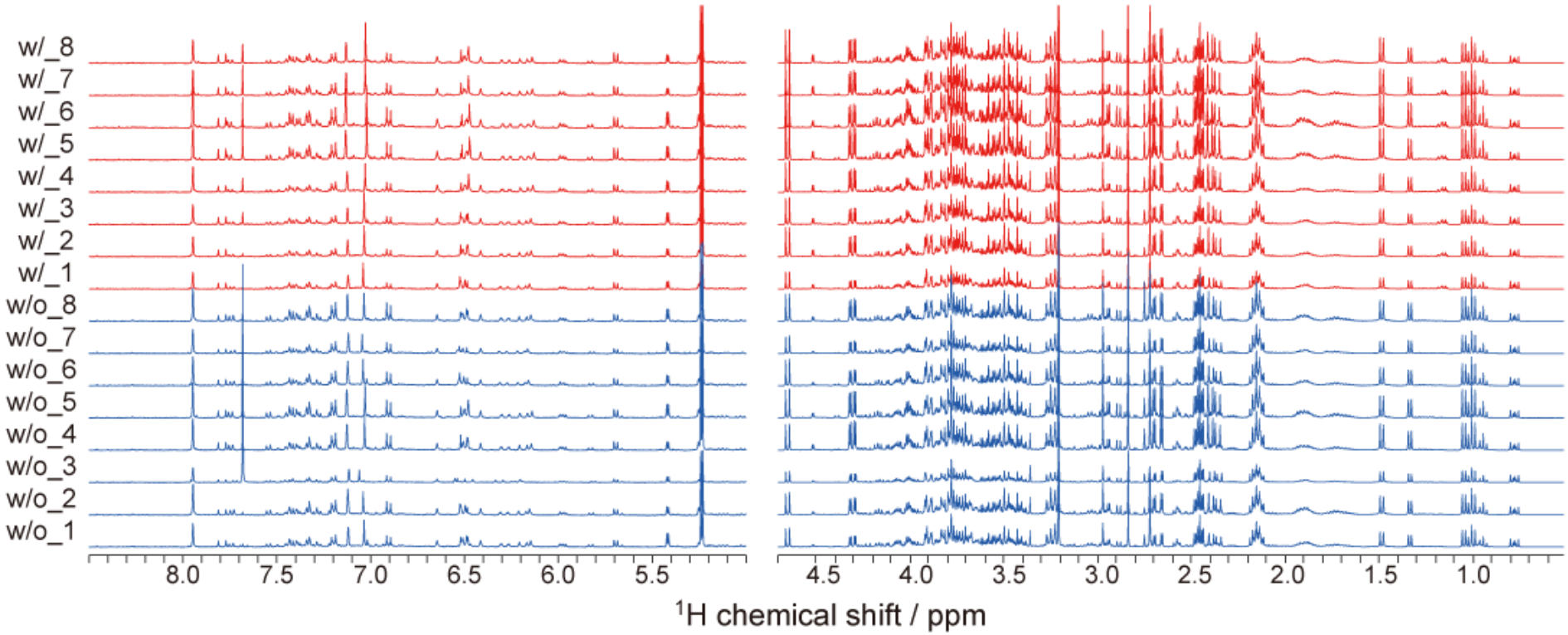


PCA 600MHz ^{1}H NMR profile (z_scores)



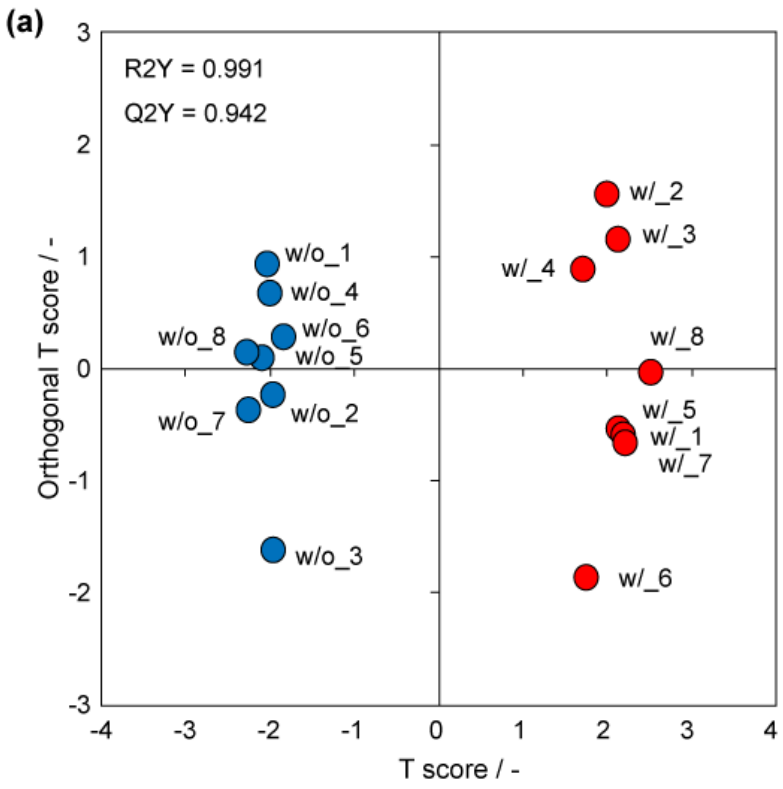
Metabolomics – Broccoli sprouts

What is the difference between these spectra?



Acquired with ECZ-S 400 spectrometer with a ROYAL probe

Metabolomics – Broccoli sprouts



OPLS-DA using ^{1}H NMR

- **Blue: Grown without light**
- **Red: grown with light**

https://www.jeol.co.jp/en/applications/detail/1634.html

https://www.jeol.co.jp/en/applications/detail/1577.html

https://www.jeol.co.jp/en/applications/detail/1575.html

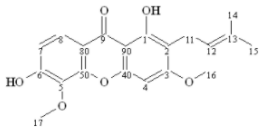
Metabolomics courses

https://www.emsl.pnnl.gov/learn/summer-school/
https://bioinformaticsdotca.github.io/

Natural products database

cowaxanthone A

Structure



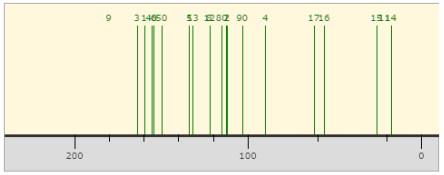
Compound Information

Name	cowaxanthone A		
Molecular Formula	$C_{20}H_{20}O_6$	Molecular Weight	356.4
NP No.	12421	Spectral Key	12-421
Source	Garcinia cowa		
Remarks	fruits		
Characteristic	xanthone		
CAS Registry No.	861886-17-1		
Solvent	$CDCl_3$		
^1H Frequency	500 MHz	Shift Ref.	TMS
Chemical Name	1,6-dihydroxy-3,5-dimethoxy-2-(3-methyl-2-butenyl)xanthone		
Reference	Panthong, K., et al. Phytochemistry 67, 999 (2006)		

Assignment List

No.	^{13}C Shift /ppm	Carbon Type	^1H Shift /ppm	^1H Pattern /Hz
1	159.67	C	12.98	s OH
2	112.27	C		
3	164.06	C		
16	55.97	CH_3	3.95	s
4	89.80	CH	6.48	s
40	155.72	C		
5	133.63	C		
17	61.94	CH_3	4.12	s
6	154.23	C	6.58	br s OH
7	112.35	CH	6.99	d 8.7
8	121.92	CH	7.93	d 8.7
80	115.14	C		
9	180.06	C		
90	103.17	C		
50	149.57	C		
11	21.32	CH_2	3.37	d 6.9
12	122.03	CH	5.24	mt 6.9
13	131.93	C		
14	17.79	CH_3	1.82	s
15	25.79	CH_3	1.70	s

^{13}C NMR Spectrum



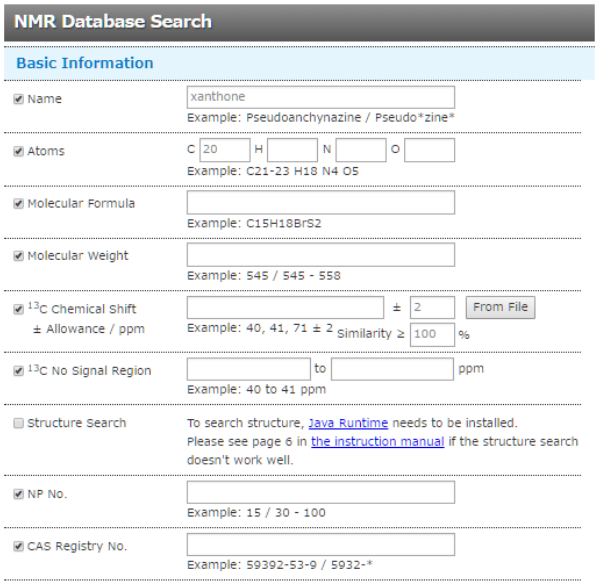
View Range : 240 to -10 ppm Reset Download 13C NMR data in Delta format

C CH CH_2 CH_3 APT Show ^1H NMR Shifts

JEOL CH-NMR-NP Database

- 30500 molecules published between 2002 and 2014
- ^{13}C and ^{1}H data
- Compiled by Prof. K. Hayamizu

- Search by mass, family, δ_C, mol file …

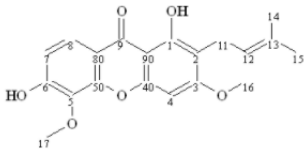


https://www.j-resonance.com/en/nmrdb/

CH-NMR-NP: entry preview

cowaxanthone A

Structure



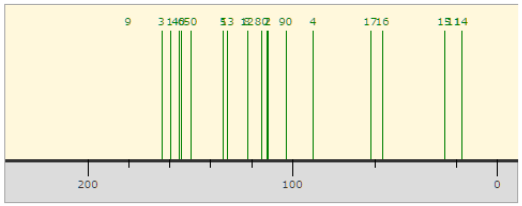
Compound Information

Name	cowaxanthone A		
Molecular Formula	$C_{20}H_{20}O_6$	Molecular Weight	356.4
NP No.	12421	Spectral Key	12-421
Source	Garcinia cowa		
Remarks	fruits		
Characteristic	xanthone		
CAS Registry No.	861886-17-1		
Solvent	$CDCl_3$		
^1H Frequency	500 MHz	Shift Ref.	TMS
Chemical Name	1,6-dihydroxy-3,5-dimethoxy-2-(3-methyl-2-butenyl)xanthone		
Reference	Panthong, K., et al. Phytochemistry 67, 999 (2006)		

Assignment List

No.	^{13}C Shift /ppm	Carbon Type	^1H Shift /ppm	^1H Pattern /Hz
1	159.67	C	12.98	s OH
2	112.27	C		
3	164.06	C		
16	55.97	CH_3	3.95	s
4	89.80	CH	6.48	s
40	155.72	C		
5	133.63	C		
17	61.94	CH_3	4.12	s
6	154.23	C	6.58	br s OH
7	112.35	CH	6.99	d 8.7
8	121.92	CH	7.93	d 8.7
80	115.14	C		
9	180.06	C		
90	103.17	C		
50	149.57	C		
11	21.32	CH_2	3.37	d 6.9
12	122.03	CH	5.24	mt 6.9
13	131.93	C		
14	17.79	CH_3	1.82	s
15	25.79	CH_3	1.70	s

^{13}C NMR Spectrum



View Range : 240 to -10 ppm Reset Download 13C NMR data in Delta format

C CH CH_2 CH_3 APT Show ^1H NMR Shifts

Natural extracts study

Algorithm for automated
peak matching of spectra vs database,
and scoring / ranking of results

Mangosteen extract

Pascal Richomme
Antoine Bruguiere
Severine Derbre
Joël Dietsch

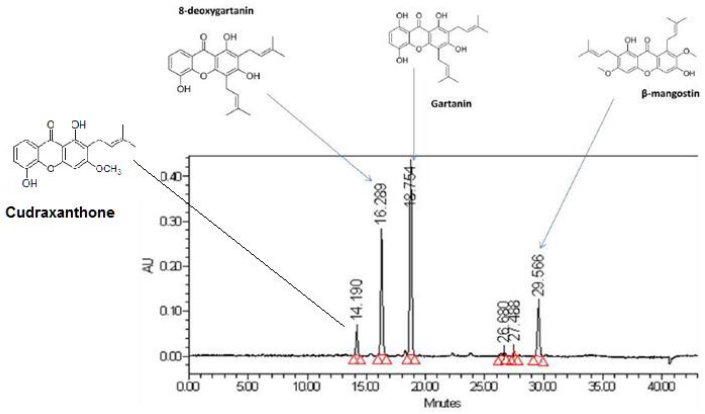
Natural extracts study

Some results from mangosteen extract using the CH-NMR-NP

	Score	Rank
Beta-mangostin	0.96	3
Cudraxanthone	0.84	27*
8-Desoxygartanine	0.96	1
Gartanine	0.96	2

**Cudraxanthone is described in the literature under different solvent*

HPLC confirmation:



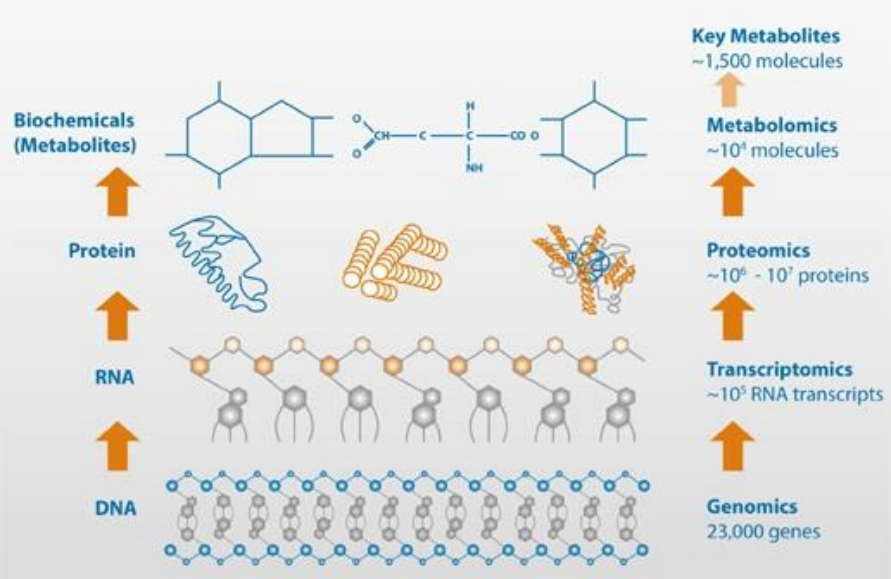
Other databases

- https://nmrshiftdb.nmr.uni-koeln.de/
- https://bmrb.io/
- https://hmdb.ca/

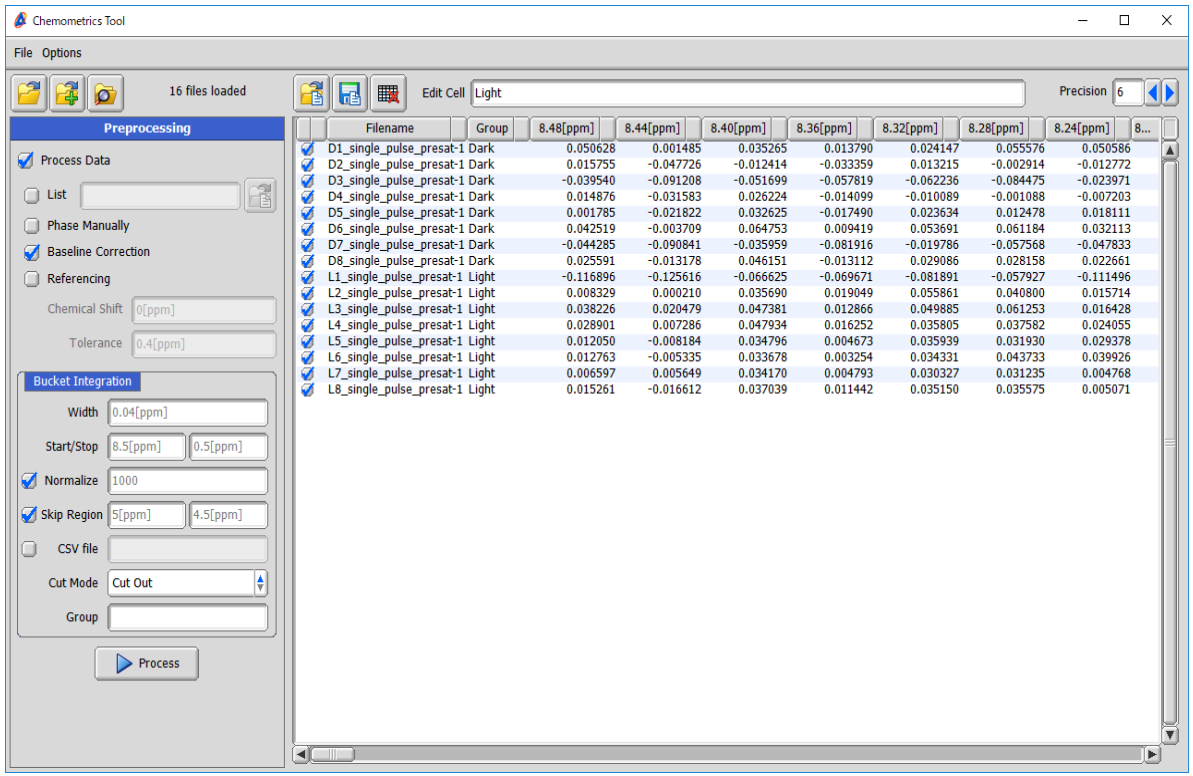
Lipoproteins

- https://doi.org/10.1194/jlr.M092643
- https://doi.org/10.1021/acs.analchem.7b04148

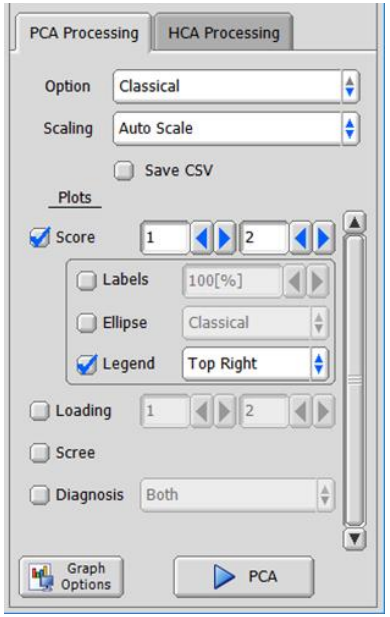
Metabolomics in Delta



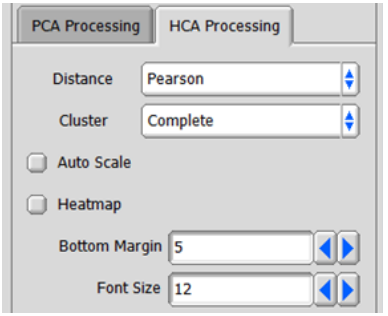
New chemometrics tool (delta 6.0)



PCA

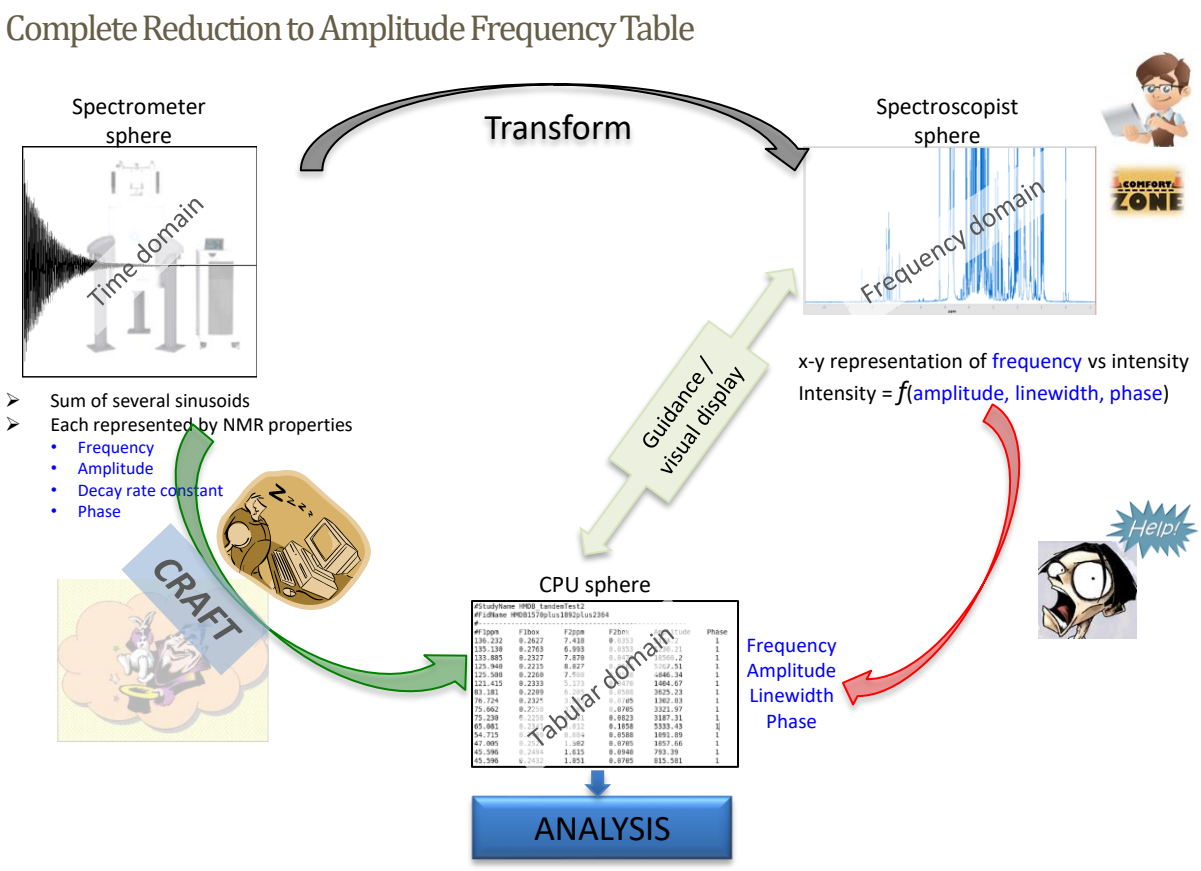


HCA



CRAFT

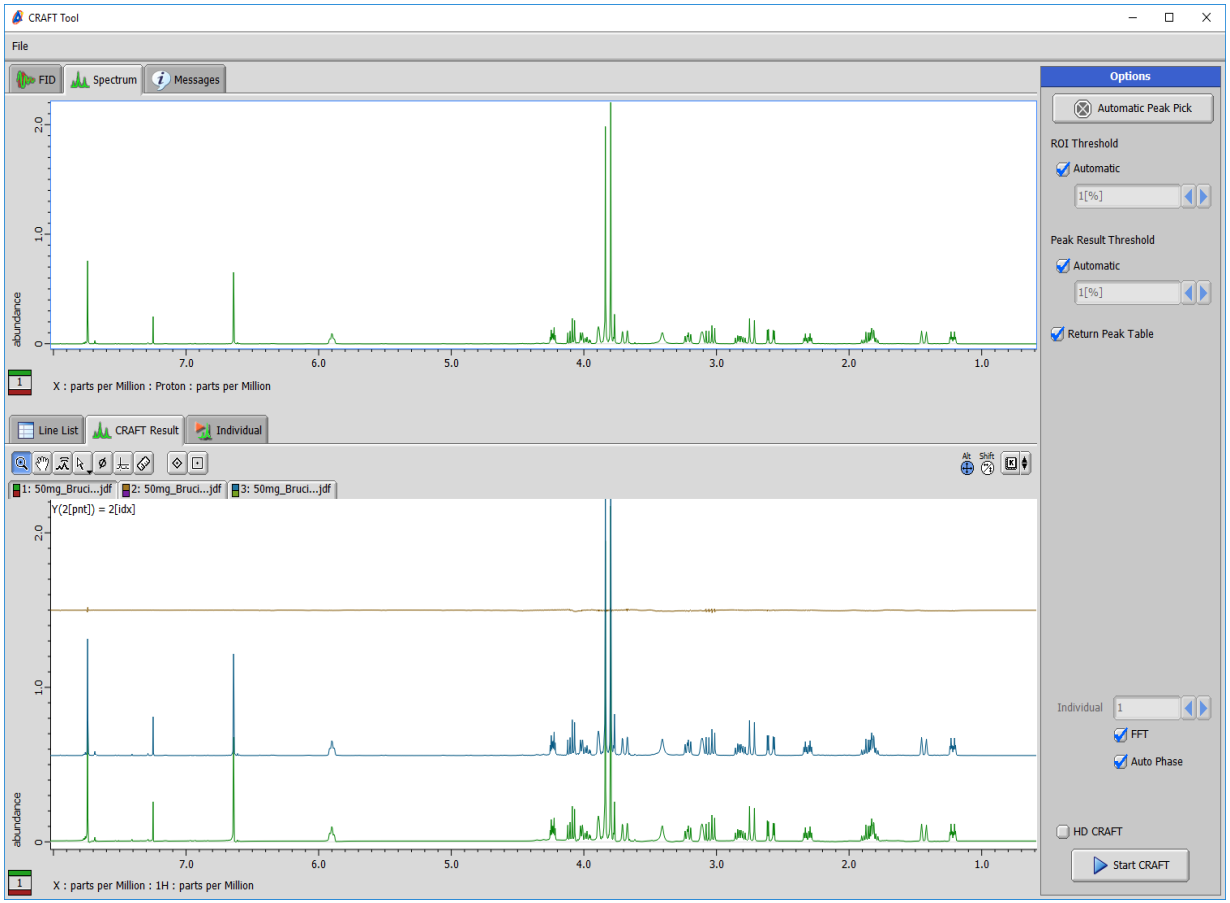
What is CRAFT?



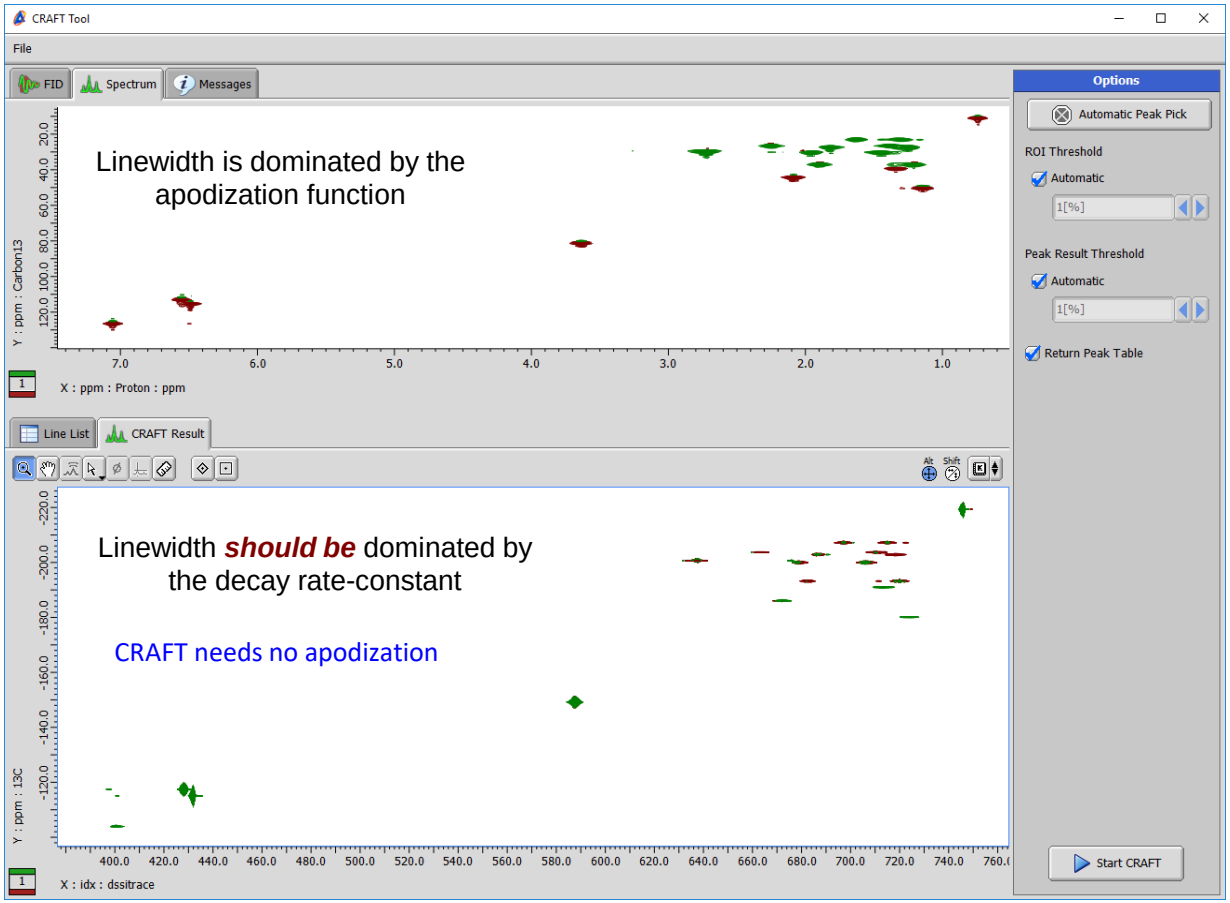
JEOL Delta with CRAFT

- Working with
  - Krish Krishnamurthy, Chempacker LLC
  - Dan Iverson, OpenVNMRJ
- Direct access from Delta to CRAFT processing and back to Delta
- CRAFT Supports:
  - 1D NMR Data
  - High Dynamic Range Data
  - 2D NMR Data
- CRAFT (complete reduction to amplitude frequency table) – robust and time-efficient Bayesian approach for quantitative mixture analysis by NMR, Krish Krishnamurthy*, Magn. Reson. Chem*., 51, p 821-829, (2013)

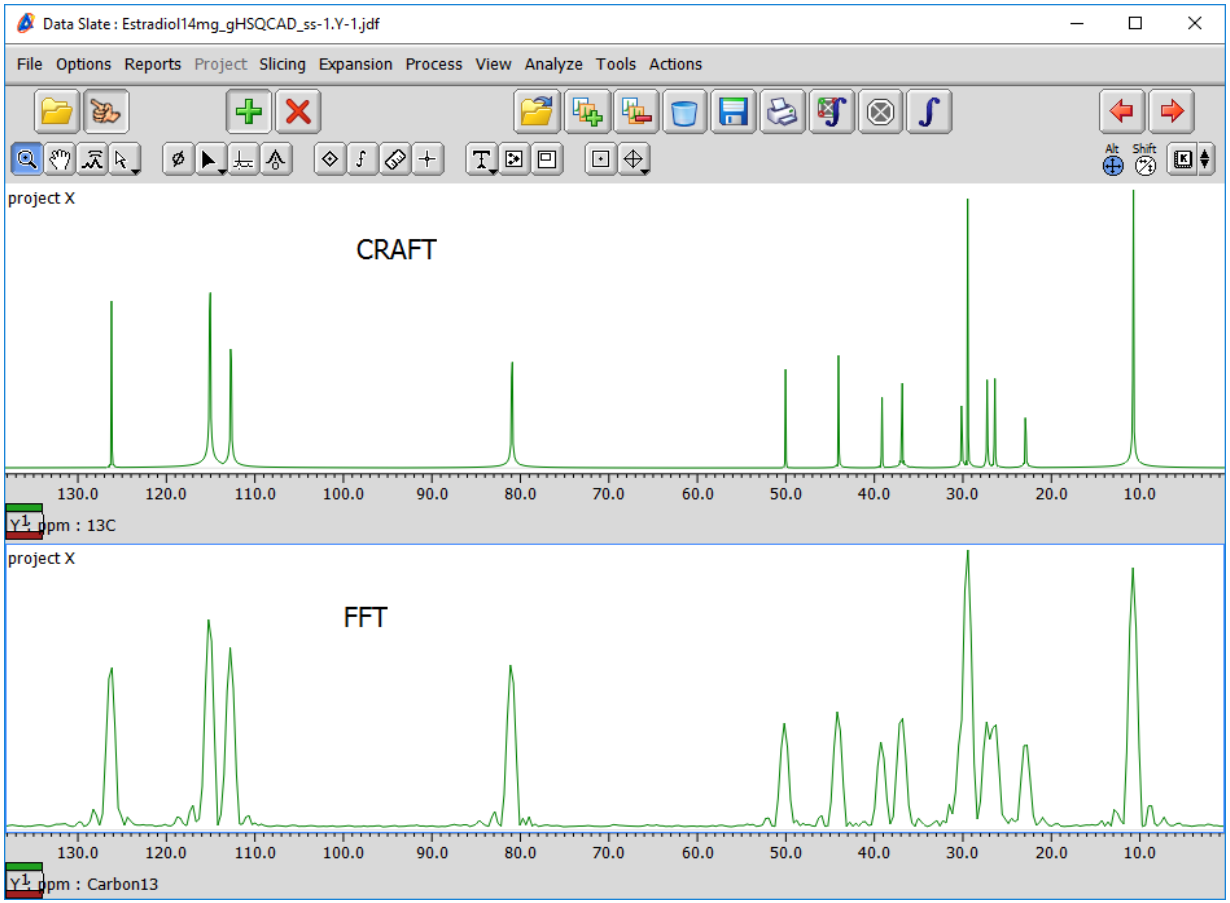
CRAFT of Brucine, results



2D CRAFT of estradiol



2D CRAFT of estradiol - Projections



CRAFT and chemometrics

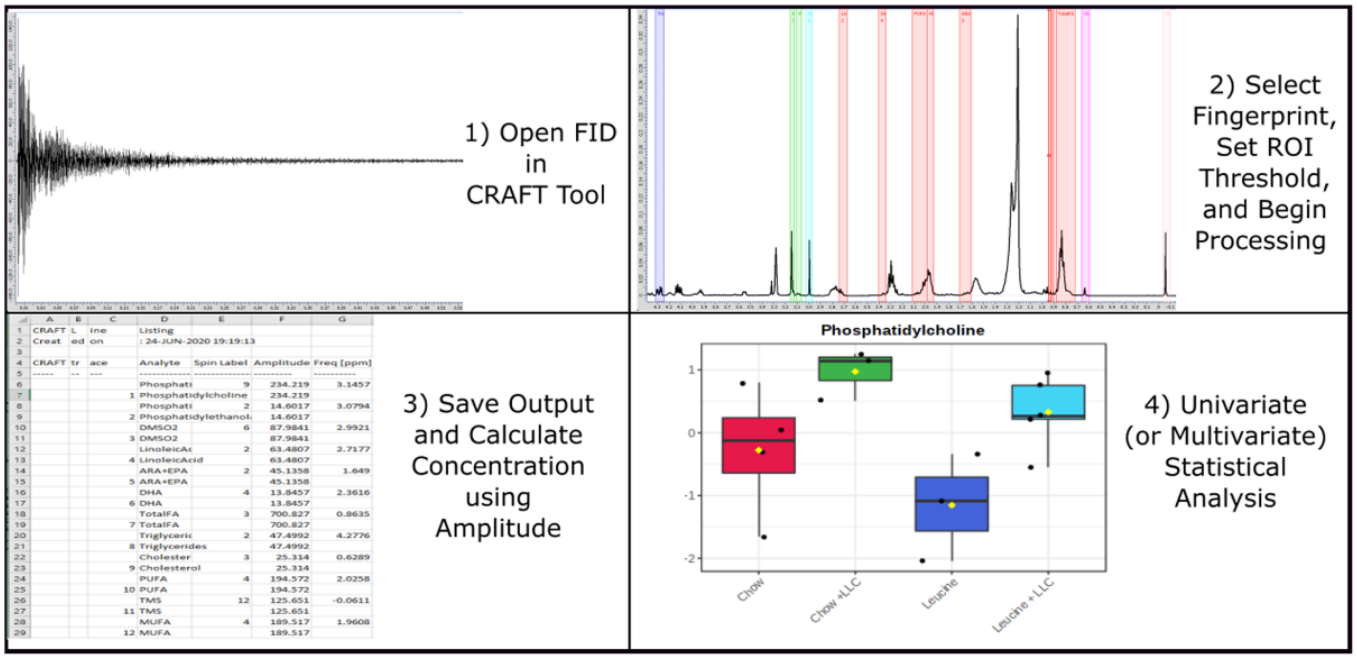


FIGURE 1 CRAFT workflow for NMR-based lipidomics research. The FID is first opened in the CRAFT tool, an appropriate fingerprint is selected for targeted analysis, and an ROI threshold is set for calculating the amplitude–frequency table. The amplitudes are then converted into lipid concentrations, and statistical analysis is performed to reveal differences and interactions between variables and subgroups

https://doi.org/10.1002/mrc.5092

CRAFT and chemometrics

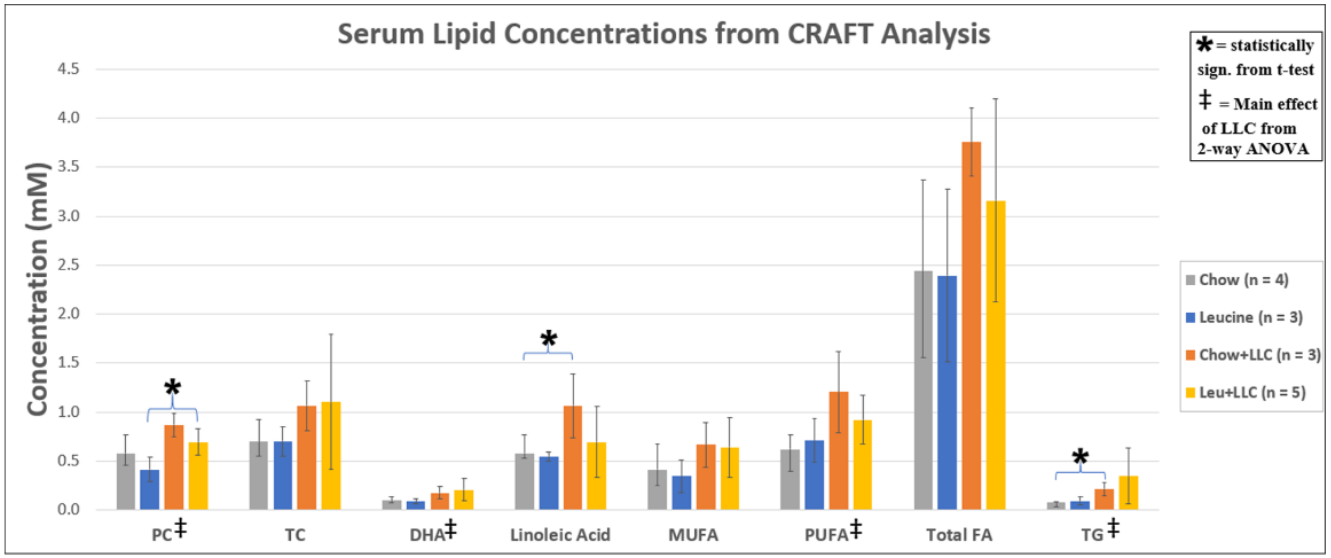


FIGURE 3 Serum lipid profile and statistical differences between healthy and LLC mice on different diets (chow, leu) noted from *t*-tests and two-way ANOVA ($p < 0.05$). LLC inoculation demonstrated serum hyperlipidemia with main effects noted for PC, DHA, PUFA, and TG. LLC inoculation was associated with increased linoleic acid and TG in chow-fed mice and increased PC in leucine-supplemented mice

https://doi.org/10.1002/mrc.5092