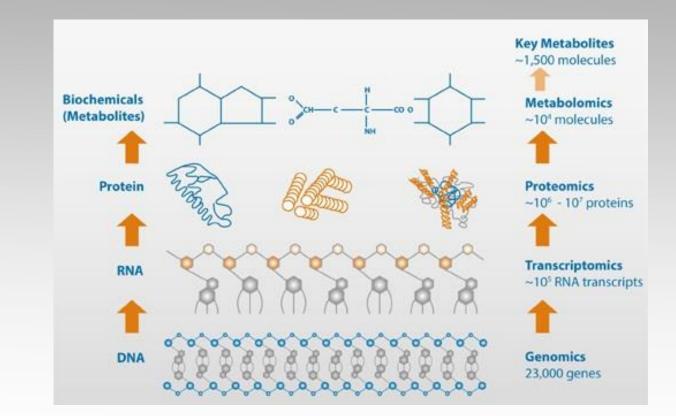
Metabolomics

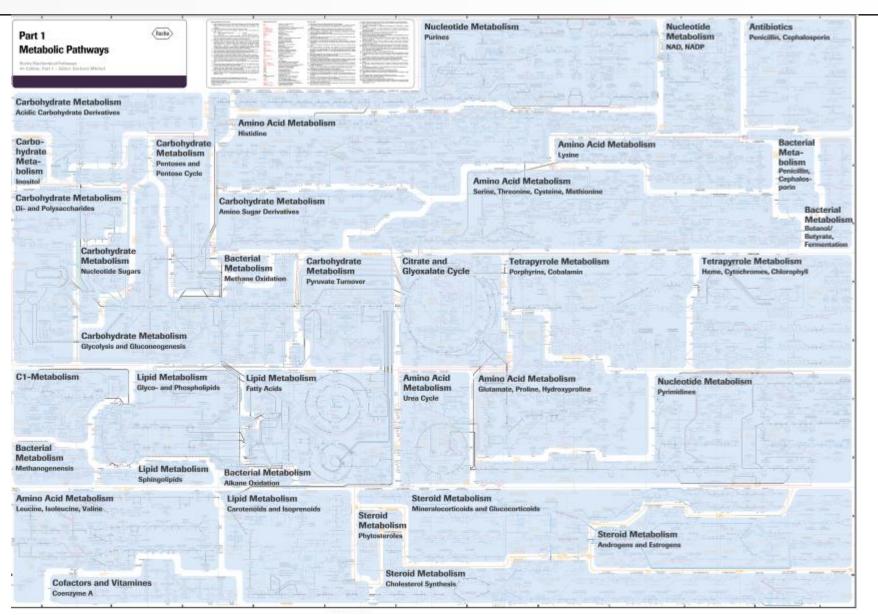




Metabolomics - definition

- 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/

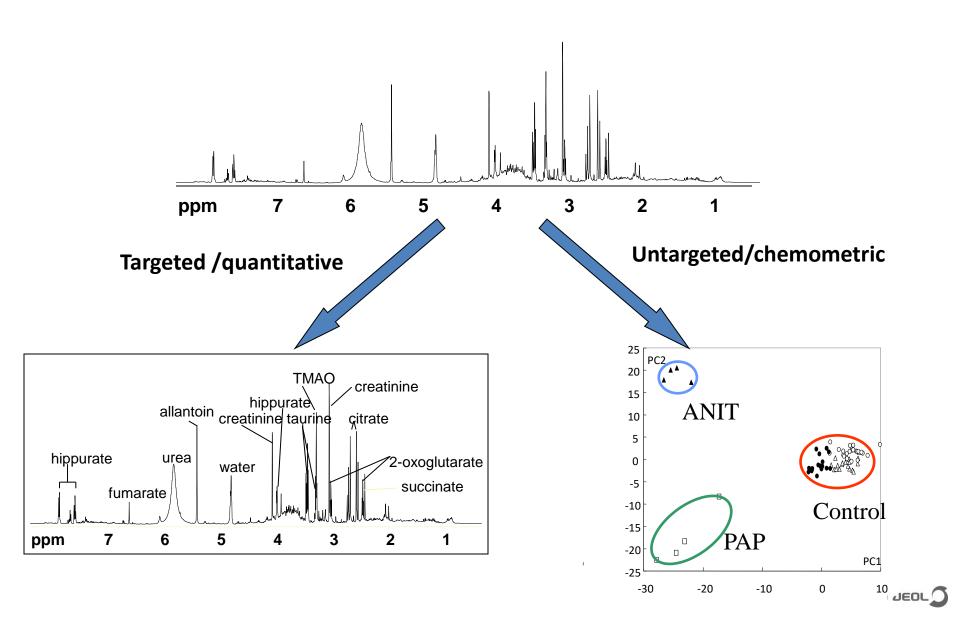
NMR vs MS

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.

NMR	Mass Spectrometry
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.
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.
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.
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
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.
	 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. Using high-resolution magic-angle sample spinning (HRMAS) NMR, it is possible to detect metabolites in tissue samples. Depending on spectral resolution, usually less than 200 metabolites can be unambiguously detected and identified in one measurement. NMR spectroscopy can be used for both targeted and untargeted analyses, but it is not commonly used for targeted analyses. Using magnetic resonance spectroscopy (MRS), in vivo investigation can be carried out most often using nuclei such as ¹H

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

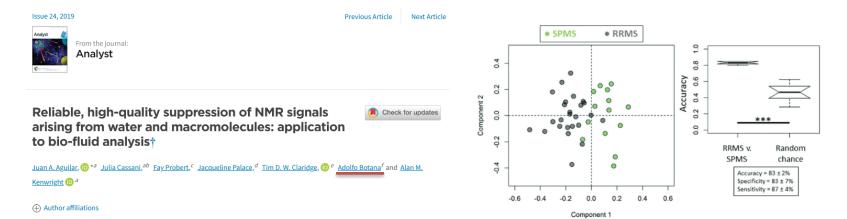
2 Routes to Metabolomics



Targeted vs untargeted metabolomics

- <u>Targeted</u>: 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
- <u>Untargeted</u>: 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



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.

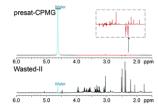


Fig. 6 OPLS-DA results from blood plasma Wasted-II ¹H NMR data discriminating SP from RR multiple sclerosis patients. Left, a representative scores plots illustrating separation between SPMS and RRMS plasma spectra in the multivariate models, and right, the accuracy of the cross-validated ensemble of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

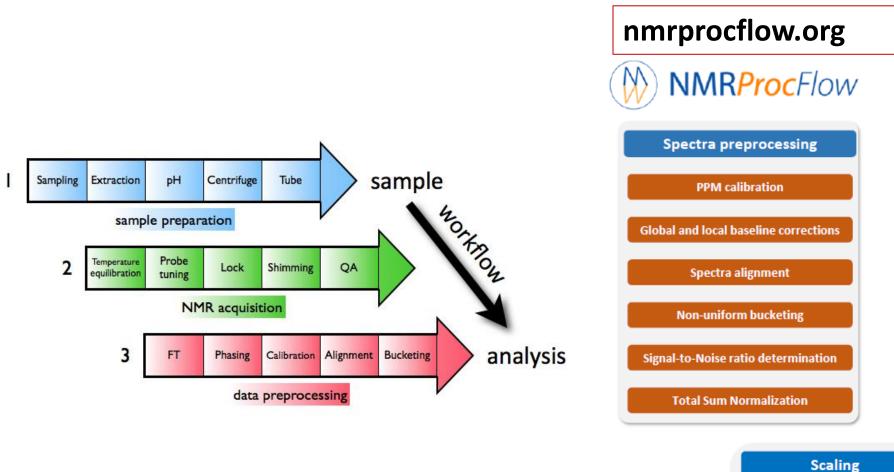
Discrimination between Secondary-Progressive and Relapsing-Remitting Mutiple sclerosis patients





Metabolomics methodology





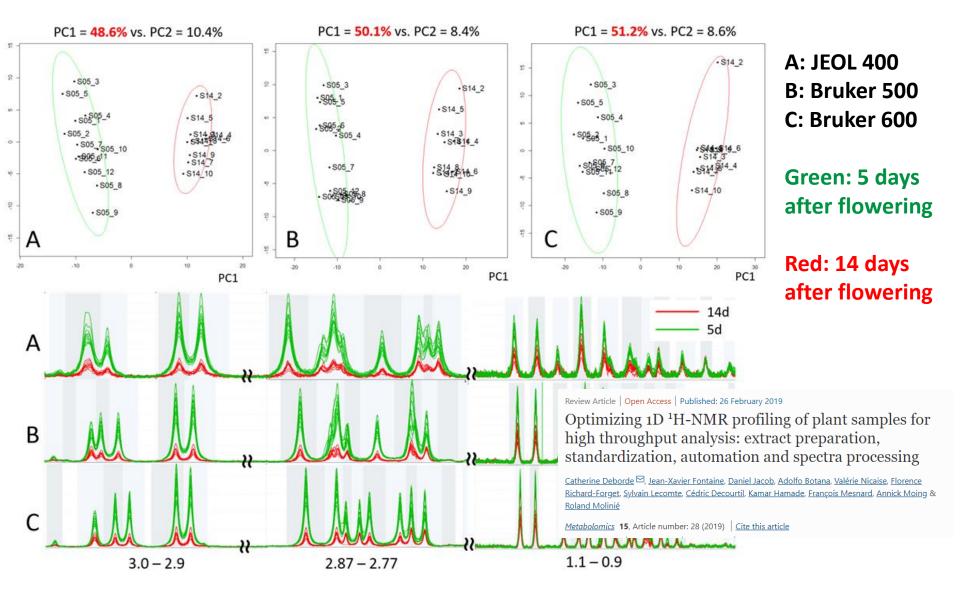




PCA of 22 wheat samples

inter-laboratory study

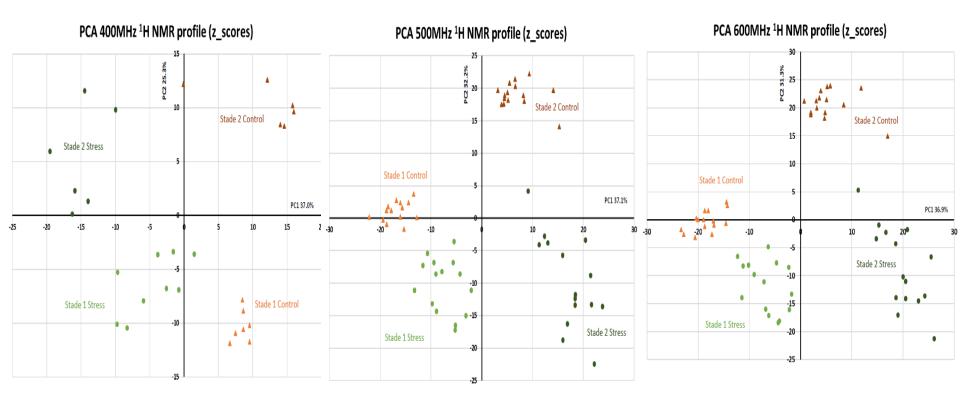






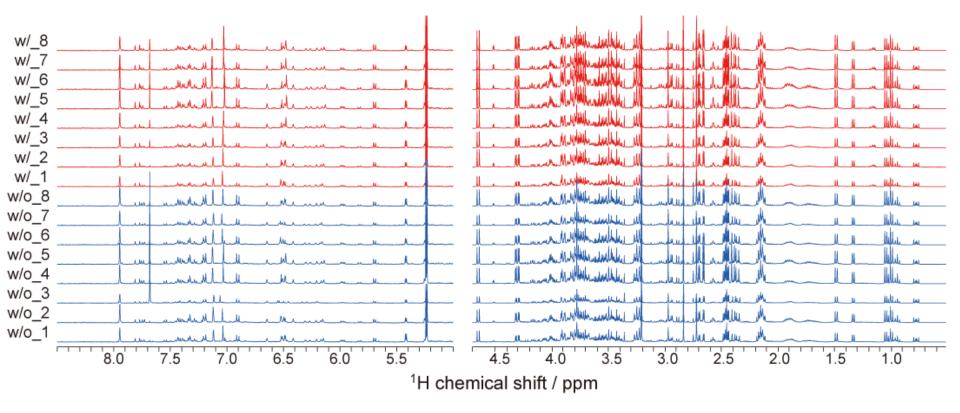


- two stages: 5 and 14 days after flowering
- two culture conditions: control and stress



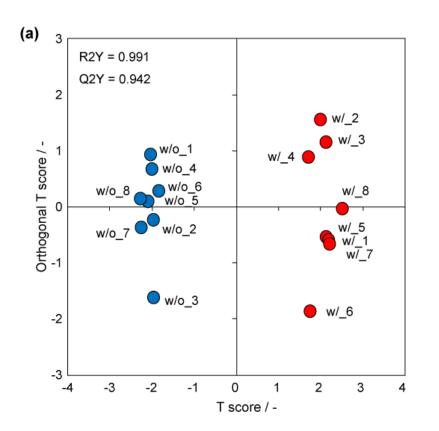
Metabolomics – Broccoli sprouts

What is the difference between these spectra?



Acquired with ECZ-S 400 spectrometer with a ROYAL probe

Metabolomics – Broccoli sprouts



OPLS-DA using ¹H 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				Ass
	HO OH	14		No.
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~	ĭ∕_i∕u∕	×13		2
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HO 6 50~	40 3 0 16			16
no	4 0			4
0				40
17				5
				17
Compound In	iformation			- 7
				8
Name	cowaxanthone A			
Molecular Formula	C20H2006	Molecular Weight	356.4	9
		-		90
NP NO.	12421	Spectral Key	12-421	50
Source	Garcinia cowa			11
				12
Remarks	fruits			13
Characteristic	xanthone			14
CAS Registry No.	861886-17-1			
Solvent	CDCl3			
¹ H Frequency	500 MHz	Shift Ref.	TMS	
Chemical Name		nethoxy-2-(3-methyl-2-buten)		
Reference		<u>vtochemistry 67, 999 (2006)</u>		
•••••••••••••••••••••••••••••••••••••••	•••••			

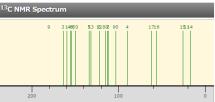
			Ass	signment	List		
	14		No.	¹³ C Shift /ppm	Carbon Type	¹ H Shift /ppm	¹ H Pattern /Hz
			1	159.67	С	12.98	s OH
×11	13		2	112.27	С		
1 12	15		3	164.06	С		
3 0~16			16	55.97	CH3	3.95	s
0			4	89.80	CH	6.48	5
			40	155.72	С		
			5	133.63	С		
			17	61.94	CH3	4.12	5
m			6	154.23	С	6.58	br s OH
			7	112.35	CH	6.99	d 8.7
hone A			8	121.92	CH	7.93	d 8.7
			80	115.14	С		
	Molecular Weight	356.4	9	180.06	С		
	_		90	103.17	С		
	Spectral Key	12-421	50	149.57	С		
			11	21.32	CH ₂	3.37	d 6.9
towa			12	122.03	CH	5.24	mt 6.9
			13	131.93	С		

5

s

17.79 CH₃ 1.82

25.79 CH₃ 1.70



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 Download 13C NMR data in Delta format

 𝔅 C 𝔅 CH 𝔅 CH₂ 𝔅 CH₃ 𝔅 APT □
 Show ¹H NMR shifts



JEOL CH-NMR-NP Database

- 30500 molecules published between 2002 and 2014
- ¹³C and ¹H data
- Compiled by Prof. K. Hayamizu
- Search by mass, family, δ_c , mol file ...

Basic Information	
✓ Name	xanthone Example: Pseudoanchynazine / Pseudo*zine*
✓ Atoms	C 20 H N O Example: C21-23 H18 N4 O5
Molecular Formula	Example: C15H18BrS2
Molecular Weight	Example: 545 / 545 - 558
 ¹³C Chemical Shift ± Allowance / ppm 	
☑ ¹³ C No Signal Region	Example: 40 to 41 ppm
Structure Search	To search structure, <u>Java Runtime</u> needs to be installed. Please see page 6 in <u>the instruction manual</u> if the structure sear doesn't work well.
✔ NP NO.	Example: 15 / 30 - 100
CAS Registry No.	Example: 59392-53-9 / 5932-*

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

CH-NMR-NP: entry preview

d 6.9

mt 6.9

s

s

cowaxanthone A

Structure	Assignment List						
	्र ्म	14		No.	¹³ C Shift /ppm	Carbon Type	¹ H Shif /ppm
				1	159.67	С	12.98
7 8 30 5	90 1 11	>13		2	112.27	С	
ÍÎ	ji 1	15		3	164.06	С	
HO 6 50~0	2 ⁴⁰ 3 0 16			16	55.97	CH3	3.95
10 5 1	4			4	89.80	CH	6.48
_0				40	155.72	С	
17				5	133.63	С	
				17	61.94	CH3	4.12
Compound In	formation			6	154.23	С	6.58
				7	112.35	CH	6.99
Name	cowaxanthone A			8	121.92	CH	7.93
				80	115.14	С	
Molecular Formula	C20H20O6	Molecular Weight	356.4	9	180.06	С	
				90	103.17	С	
NP NO.	12421	Spectral Key	12-421	50	149.57	С	
Source	Garcinia cowa			11	21.32	CH ₂	3.37
				12	122.03	CH	5.24
Remarks	fruits			13	131.93	С	
				14	17.79	CH3	1.82
Characteristic	xanthone			15	25.79	CH3	1.70
CAS Registry No.	861886-17-1						
Solvent	CDCl3						
¹ H Frequency	500 MHz	Shift Ref.	TMS				
Chemical Name	1,6-dihydroxy-3,5-dir	nethoxy-2-(3-methyl-2-buten)	/l)xanthone				
Reference	Panthong, K., et al, P	hytochemistry 67, 999 (2006)					

		¹³ C NMR Spectrum
¹ H Shift /ppm	¹ H Pattern /Hz	9 314650 53 122802 90
12.98	s OH	
3.95	S	
6.48	S	
4.12	s	
6.58	br s OH	200 100
6.99	d 8.7	View Range : 240 to -10 ppm Reset Downloa
7.93	d 8.7	C CHCHCH2 CH3 APT Show ¹ H NMR Shi

1716 1914

d 13C NMR data in Delta format ifts



Natural extracts study

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

Mangosteen extract



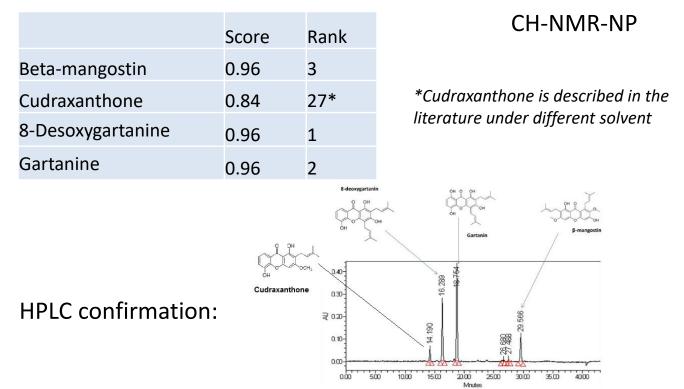


Pascal Richomme Antoine Bruguiere Severine Derbre Joël Dietsch

Natural extracts study



Some results from mangosteen extract using the





Other databases

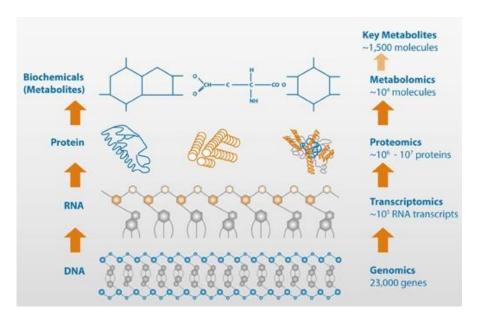
- <u>https://nmrshiftdb.nmr.uni-koeln.de/</u>
- <u>https://bmrb.io/</u>
- https://hmdb.ca/

Lipoproteins

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



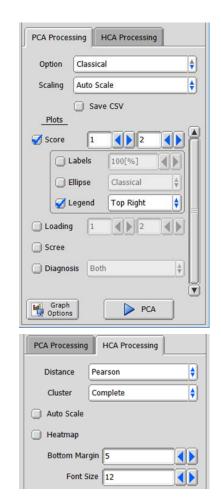
Metabolomics in Delta





New chemometrics tool (delta 6.0)

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PCA

HCA

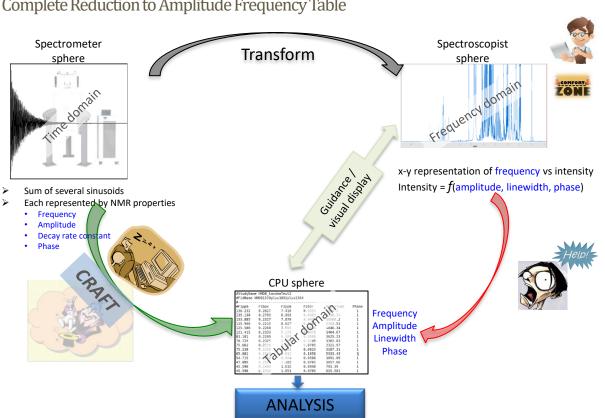


CRAFT





What is CRAFT?



Complete Reduction to Amplitude Frequency Table

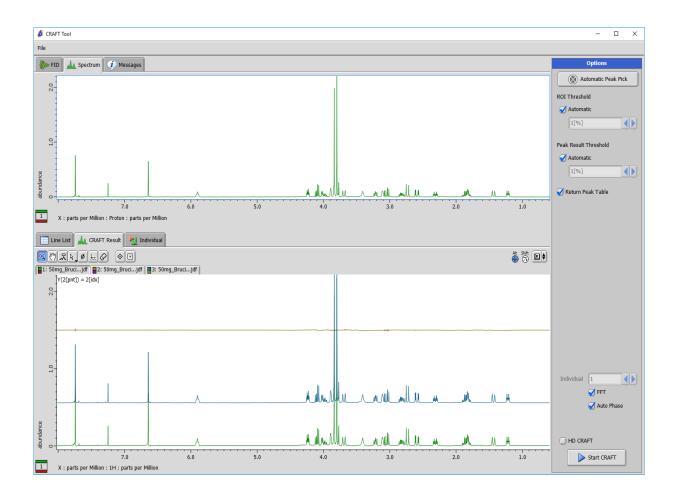


JEOL Delta with CRAFT

- 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)

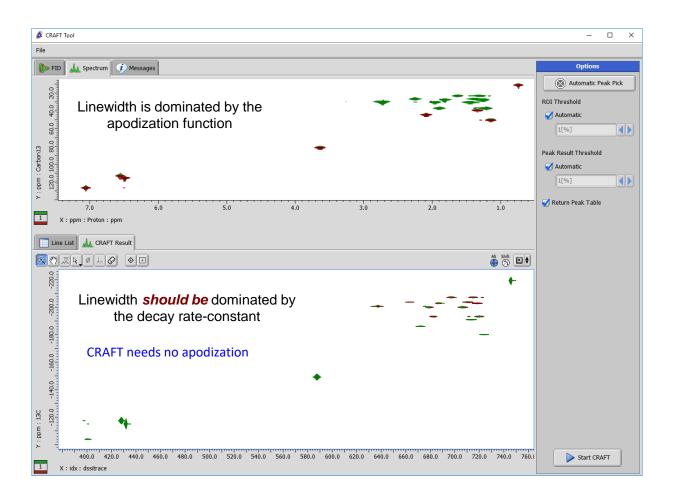


CRAFT of Brucine, results

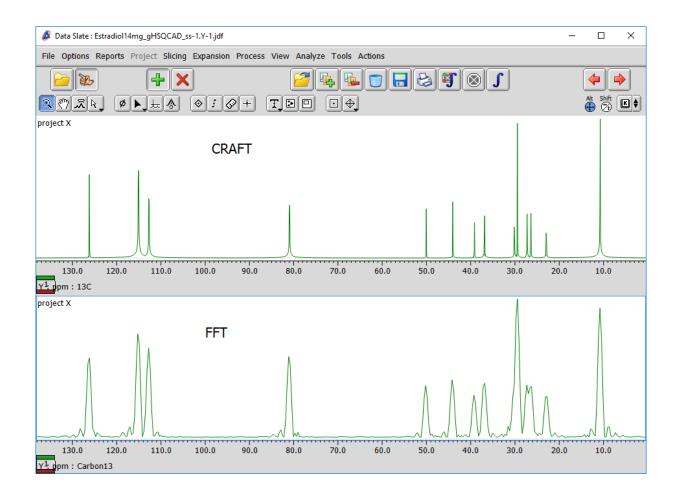


Solutions for Innovation JEOL

2D CRAFT of estradiol



2D CRAFT of estradiol - Projections



Solutions for Innovation JEOL

CRAFT and chemometrics

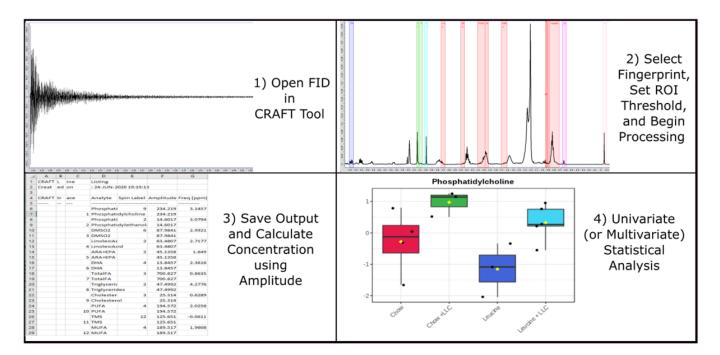


FIGURE 1 CRAFT workflow for NMR-based lipidomics research. The FID is first opened in the CRAFT tool, an appropriate fingerprint is selected for targeted analysis, and an ROI threshold is set for calculating the amplitude–frequency table. The amplitudes are then converted into lipid concentrations, and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

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



CRAFT and chemometrics

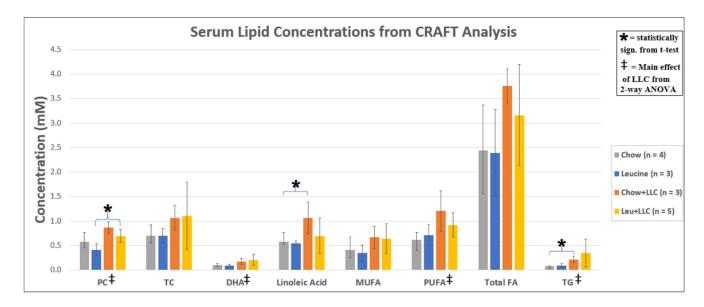


FIGURE 3 Serum lipid profile and statistical differences between healthy and LLC mice on different diets (chow, leu) noted from *t*-tests and two-way ANOVA (p < 0.05). LLC inoculation demonstrated serum hyperlipidemia with main effects noted for PC, DHA, PUFA, and TG. LLC inoculation was associated with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

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