Biomarker komoponensek szelektív kvantitatív elemzése lovak szérum mintáinak ^{1}H-NMR spektrumában a CRAFT szoftver segítségével

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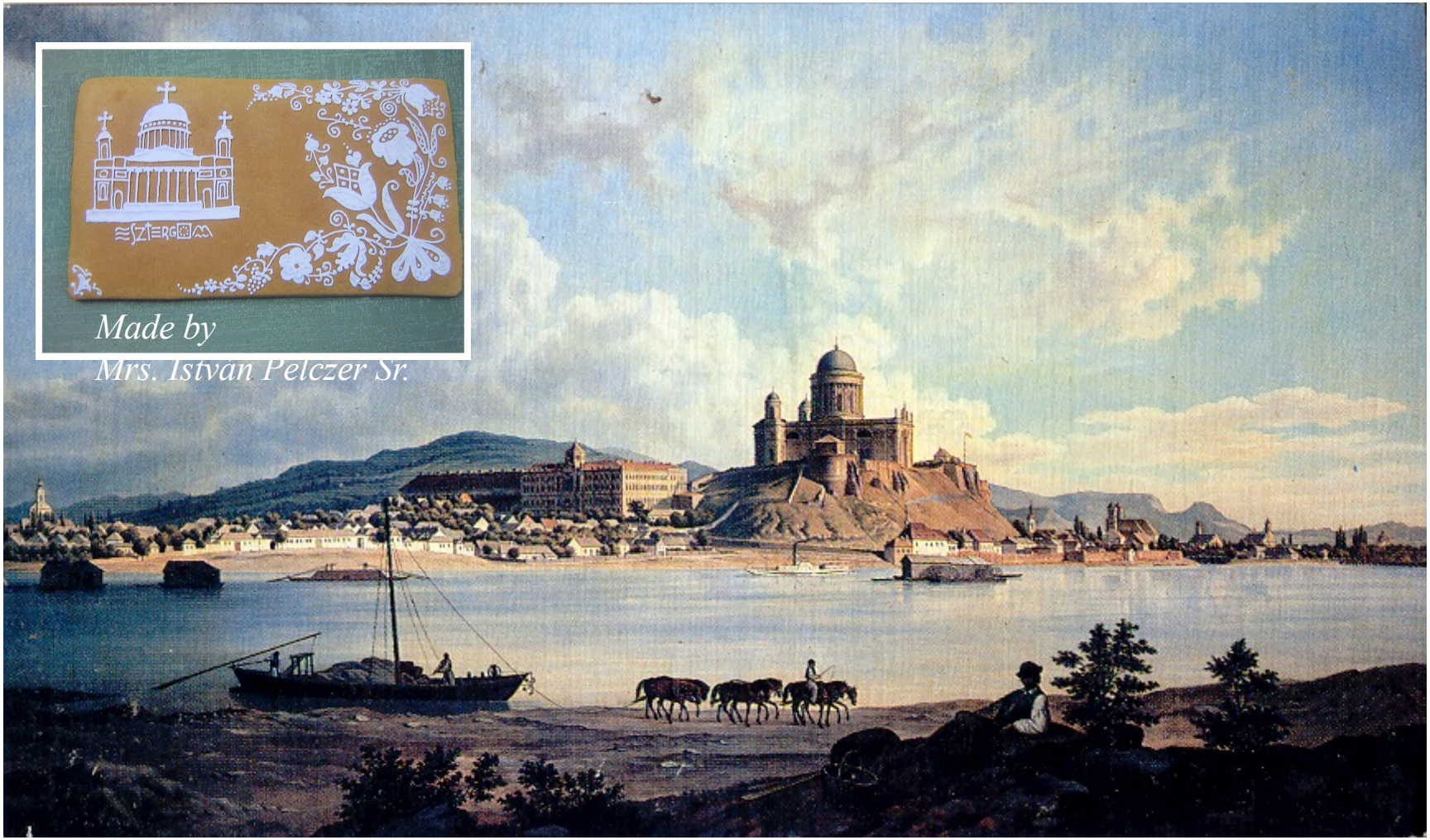
2

Magyar NMR Munkabizottság ülése

Balatonszemes/Princeton
2025. május 8-9.

Esztergom

(my hometown in Hungary…)



Made by
Mrs. István Pelczer Sr.

Hubert Sattler: Esztergom a Dunárol nézve, 1873
(View of Esztergom from the Danube)
Keresztény Múzeum, Esztergom, HU

Akiknem az NMR-t köszönhetem...

The NMR Facility in the Frick Chemistry Laboratory

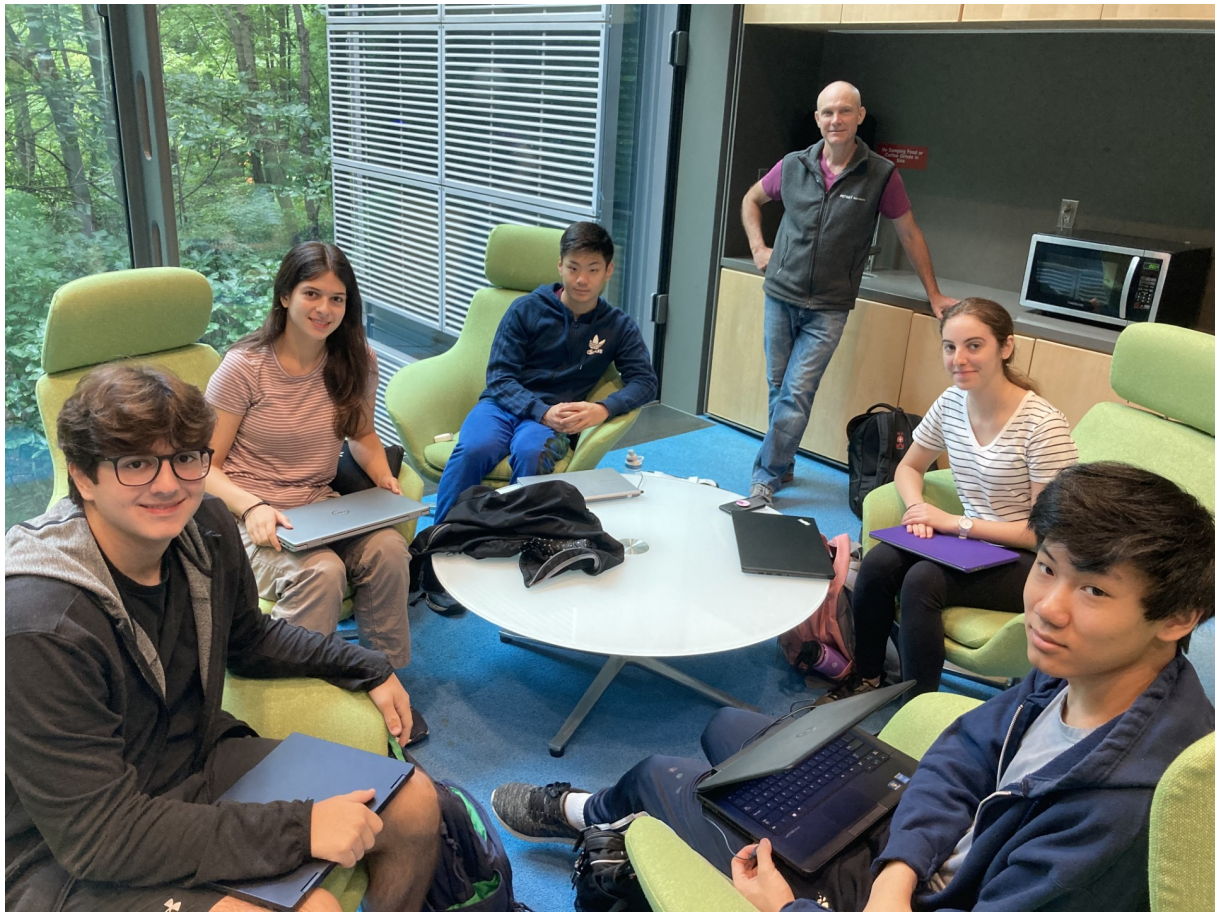
Co-workers

Ken Conover
(NMR Specialist)

Sarah Ralston
Professor
Emerita (Rutgers U.)
and Fulbright
Scholar

Dr. Merrill Simpson '13
GH Cook Scholar
Rutgers University
Sechelt Animal Hospital, BC

The five + 1 adventurers… - Laboratory Learning Program 2024 summer program, Princeton University



Matheus Guedin

Christina Metaxas

James Chen

Ken Conover

Ayelet Yablon

Joseph Hu

Krish Krishnamurthy

ChemPacker LLC, San Jose, CA 95135
{CRAFT}

Introduction

We have applied the CRAFT (Complete Reduction to Amplitude-Frequency Table) software tool to extract essential inflammatory biomarker signals quantitatively from the full 1D 1H spectrum of horse serum samples. This simple approach is a competitive alternative to a variety of sophisticated experimental methods which aim to suppress the rest of the spectrum and keep only the peaks of interest [DIRE, JEDI]. The CRAFT selective extraction uses the full information content of the spectrum without any modifications or loss of sensitivity.

[DIRE] Lodge S, et al; Anal. Chem., 2021, 93, 3976−3986
[JEDI] Nitschke P, et al.; Anal. Chem., 2022, 94, 1333−1341

Chen J, Yablon A, Metaxas C, Guedin M, Hu J, Conover K, Simpson M, Ralston SL, Krishnamurthy K, Pelczer I;

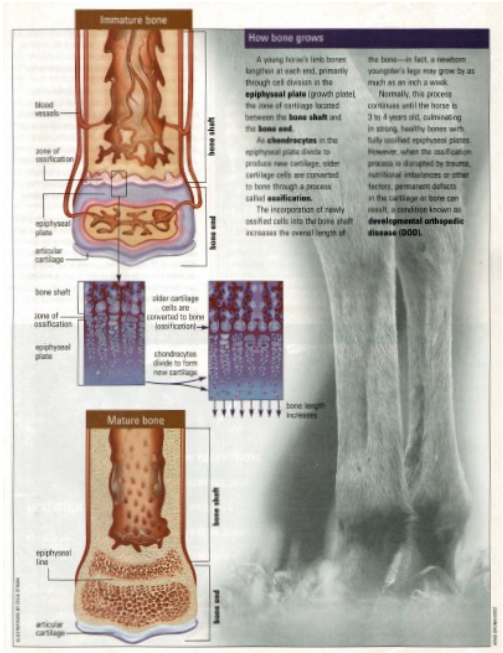
“Suppress or not to suppress? – CRAFT IT! A targeted metabolomics case study extracting essential biomarker signals directly from the 1H-NMR spectra of horse serum samples”

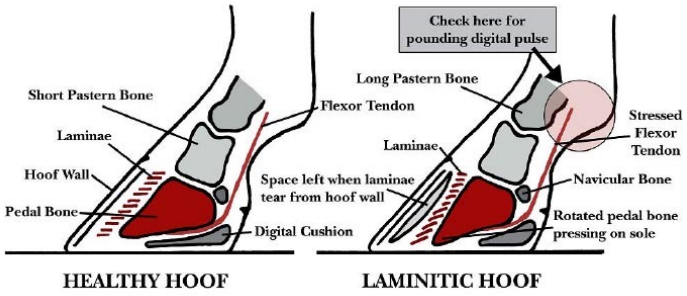
Metabolites (*accepted, in press*)

Osteochondrosis Dessicans, OCD

A growth-related defect, caused by abnormal calcification of cartilage matrices at the growth plates and articular surfaces.

Heritable, correlation between abnormal glucose/insulin metabolism and the presence of lesions reported.

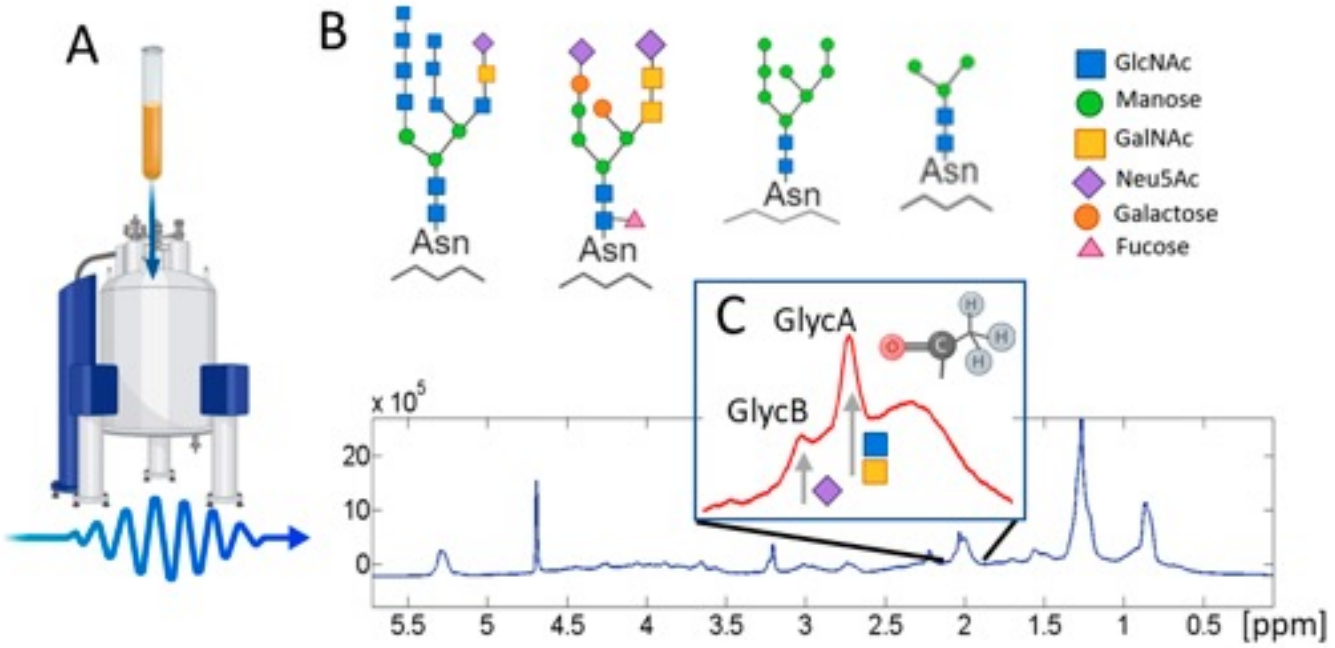




Also:

Obesity, ***Laminitis***

There are a few very specific inflammation markers in blood, namely lipoprotein $N^{+}Me$ signals of protein clusters (GlycA and GlycB)



Fuertes-Martín R, Correig X, Vallvé JC, Amigó N; Life 2021, 11, 1407

In addition, there is a composite resonance of phospholipids (SPC, 3.21 ppm), which is also an essential inflammatory biomarker. The relative integrals of these resonances provide clear indication of inflammatory conditions, often related to serious diseases like cancer or COVID-19 infection.

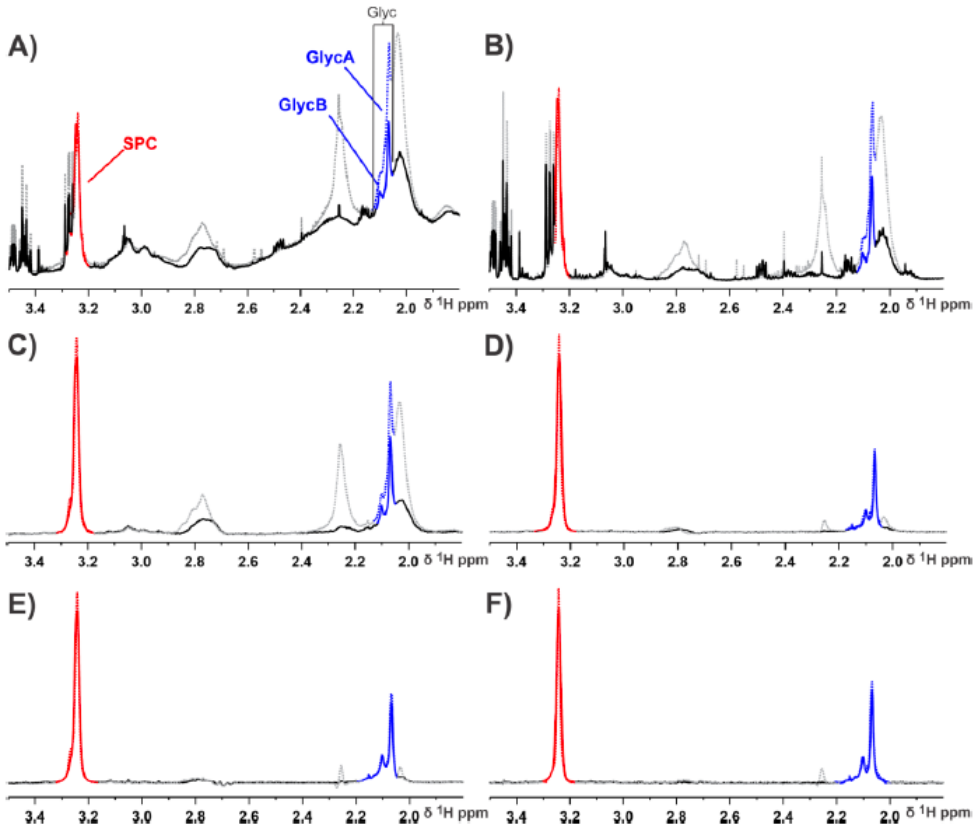
Supporting Information

J-Edited DIffusional (JEDI) Proton Nuclear Magnetic Resonance Spectroscopic Measurement of Glycoprotein and Supramolecular Phospholipid Biomarkers of Inflammation in Human Serum

Philipp Nitschke[1*], Samantha Lodge[1], Torben Kimhofer[1], Reika Masuda[1], Sze-How Bong[1], Drew Hall[1], Hartmut Schäfer[2], Manfred Spraul[2], Nils Pompe[2], Tammo Diercks[3], Ganeko Bernardo-Seisdedos[3], José M Mato[3], Oscar Millet[3], Daniella Susic[4,5], Amanda Henry[4,5], Emad M El-Omar[6], Elaine Holmes[1,7], John C. Lindon[7], Jeremy K. Nicholson[1,8*],and Julien Wist[1,9*]

Anal. Chem. 2022, 94, 1333−1341

Spectral comparison for 1D ^{1}H, CPMG, DIRE and JEDI



A : full spectrum

B : CPMG diffusion filtered

C : DIRE filtered

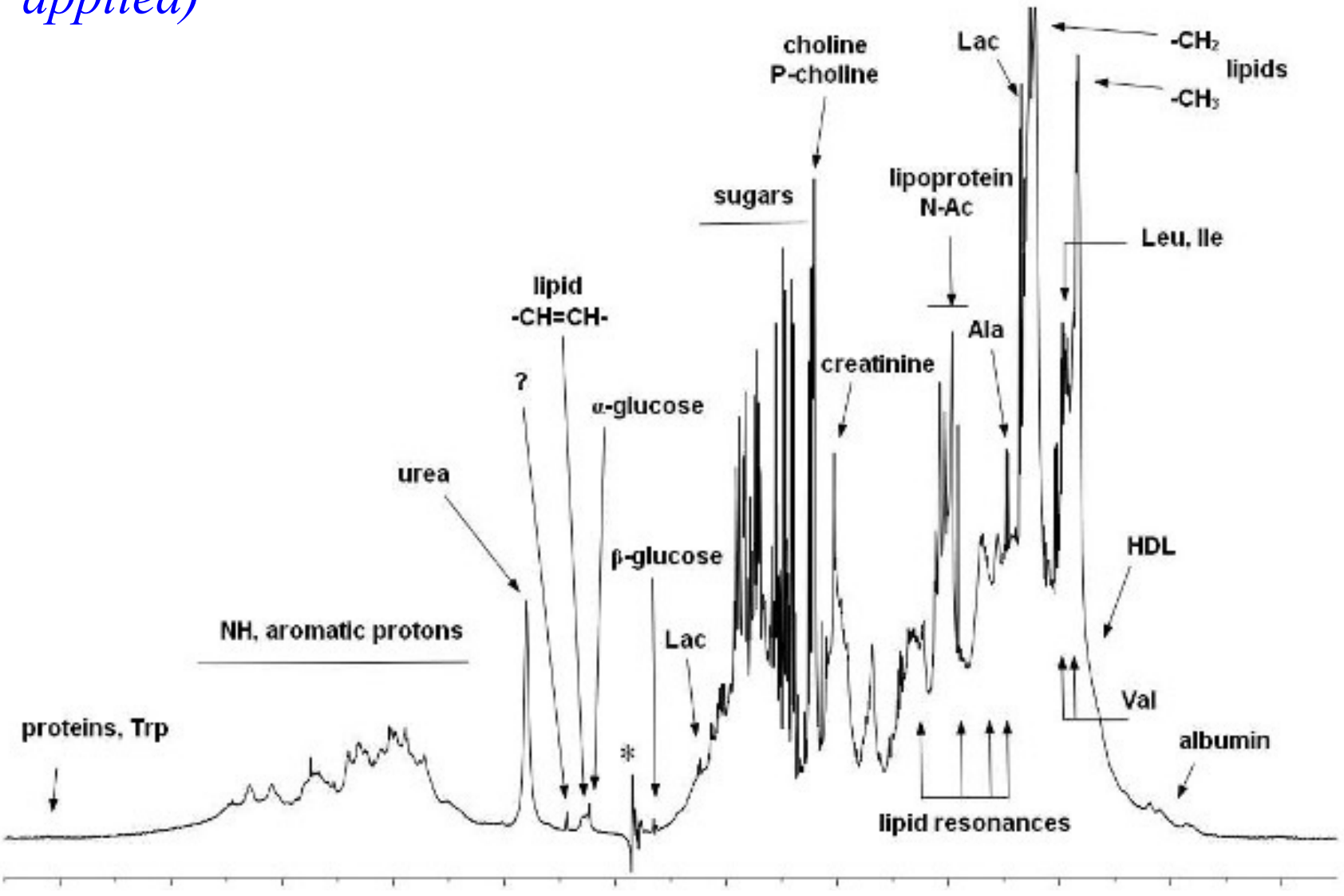
D : JEDI-PGPE

E : JEDI-PGSE

F : JEDI-PGDE

A representative ^{1}H-NMR spectrum of horse blood serum

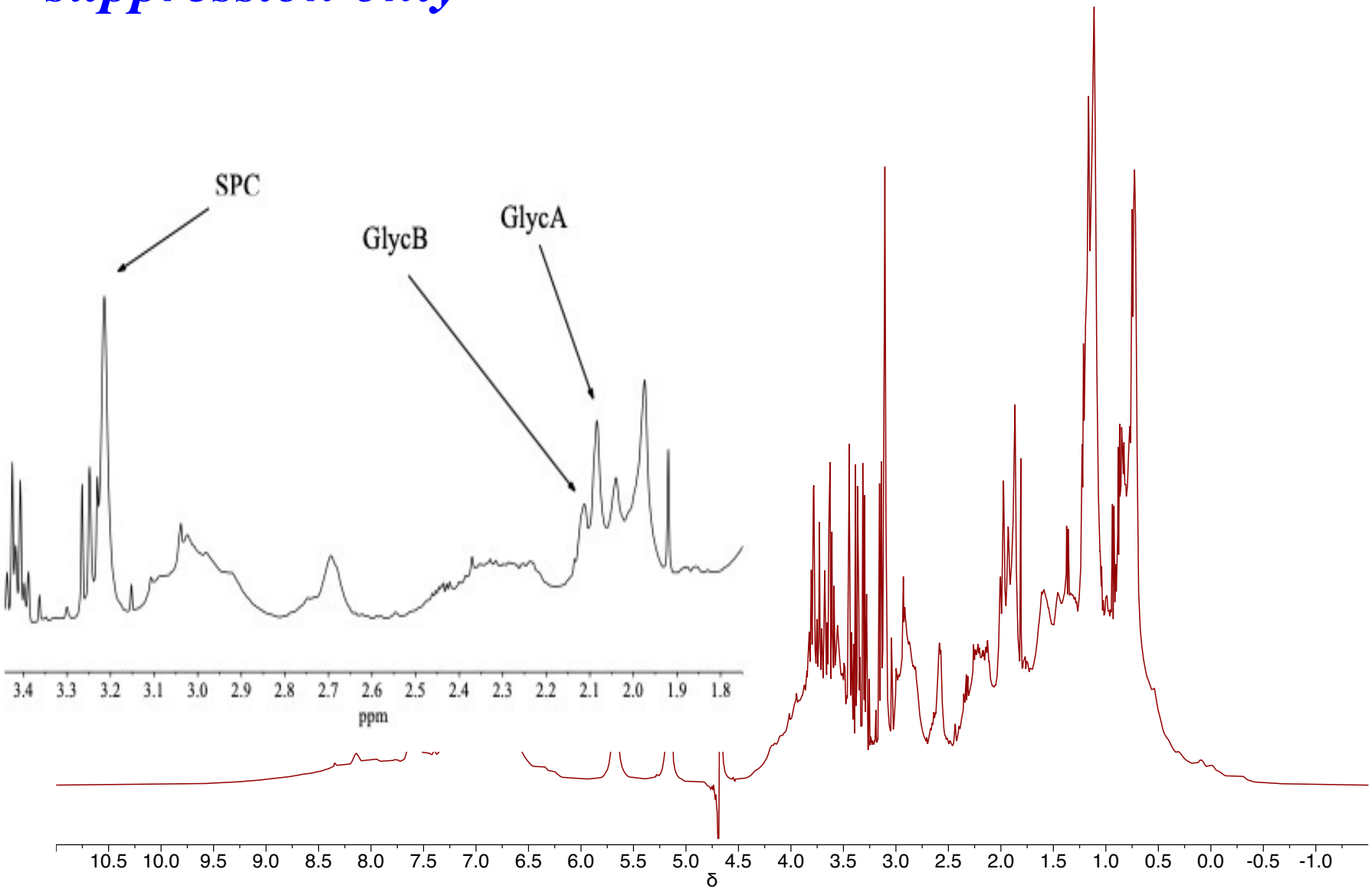
(only water suppression applied)



Experimental

For this case study we used a cohort of 20 + 20 horse samples, healthy and OCD diagnosed, respectively, as described and discussed in the Cook Thesis of Merrill Simpson [George H. Cook Scholars Program Thesis, 2013, School of Environmental and Biological Sciences, Rutgers University]. The CRAFT analysis can be done retrospectively on many datasets acquired earlier as the water suppressed full spectrum is naturally part of the acquisition package.

The routine protocol – full spectrum with water suppression only



And now…

Krish Krishnamurthy

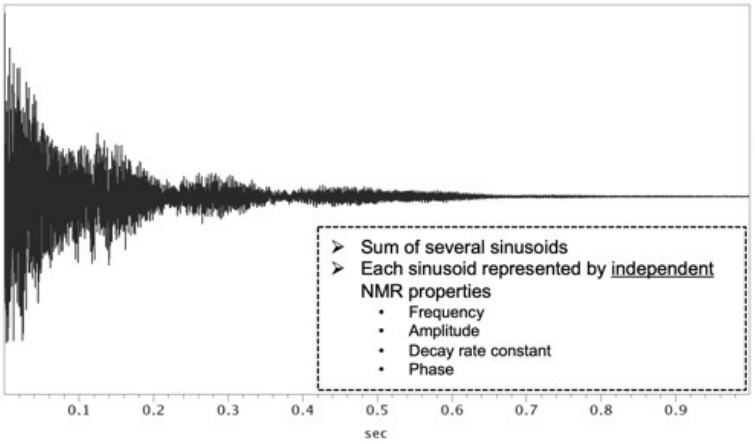
the magician of

CRAFT !

A schematic representation of CRAFT analysis workflow

(from MRC 2013, 51, 821–829)

CRAFT FID => Table => Spectrum



Extract information

- No precondition
- Iterative process
- Stop: Residual is random (i.e., no probability of exponential decay model)

Offset	Amplitude	decay rate	Phase
-3381.84	10.395	0.22727	95.0
-3071.89	200.091	0.21883	95.2
-2816.81	205.187	0.18838	90.9
-2685.30	44.507	0.10155	93.8
-2630.59	17.423	0.08195	96.5
-2498.65	110.320	0.13337	96.2
-2245.51	695.838	0.18692	94.7
.....			
-1698.98	20.776	0.14821	95.0
.....			
-1292.25	49.424	0.13773	93.5
-1162.14	46.537	0.13112	95.0
.....			
290.63	39.522	0.21040	89.4
2606.89	142.060	0.03783	90.1

Each row defined b a single exponentia decay function

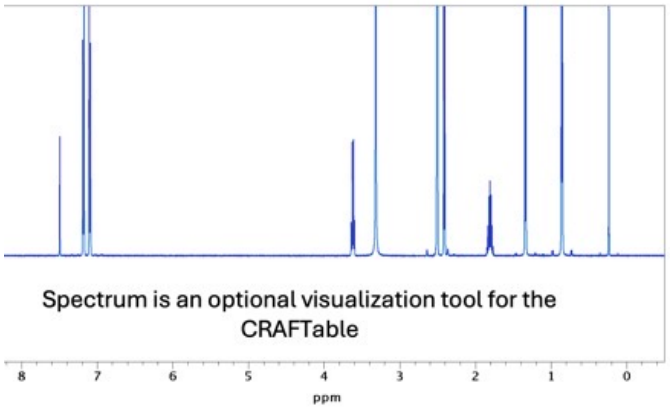
Sort
Combine
Filter
Search
Group
etc.

NMR rules (User definable)

ppm	Amplitude	width	Phase
0.2286	10.395	1.40	95.0
0.8492	200.091	1.45	95.2
1.3600	205.187	1.69	90.9
1.6233	44.507	3.13	93.8
1.7329	17.423	3.88	96.5
1.9971	110.320	2.39	96.2
2.5040	695.838	1.70	94.7
.....			
3.5983	20.776	2.15	95.0
.....			
4.4127	49.424	2.31	93.5
4.6733	46.537	2.43	95.0
.....			
7.5823	39.522	1.51	89.4
12.2203	142.060	8.41	90.1

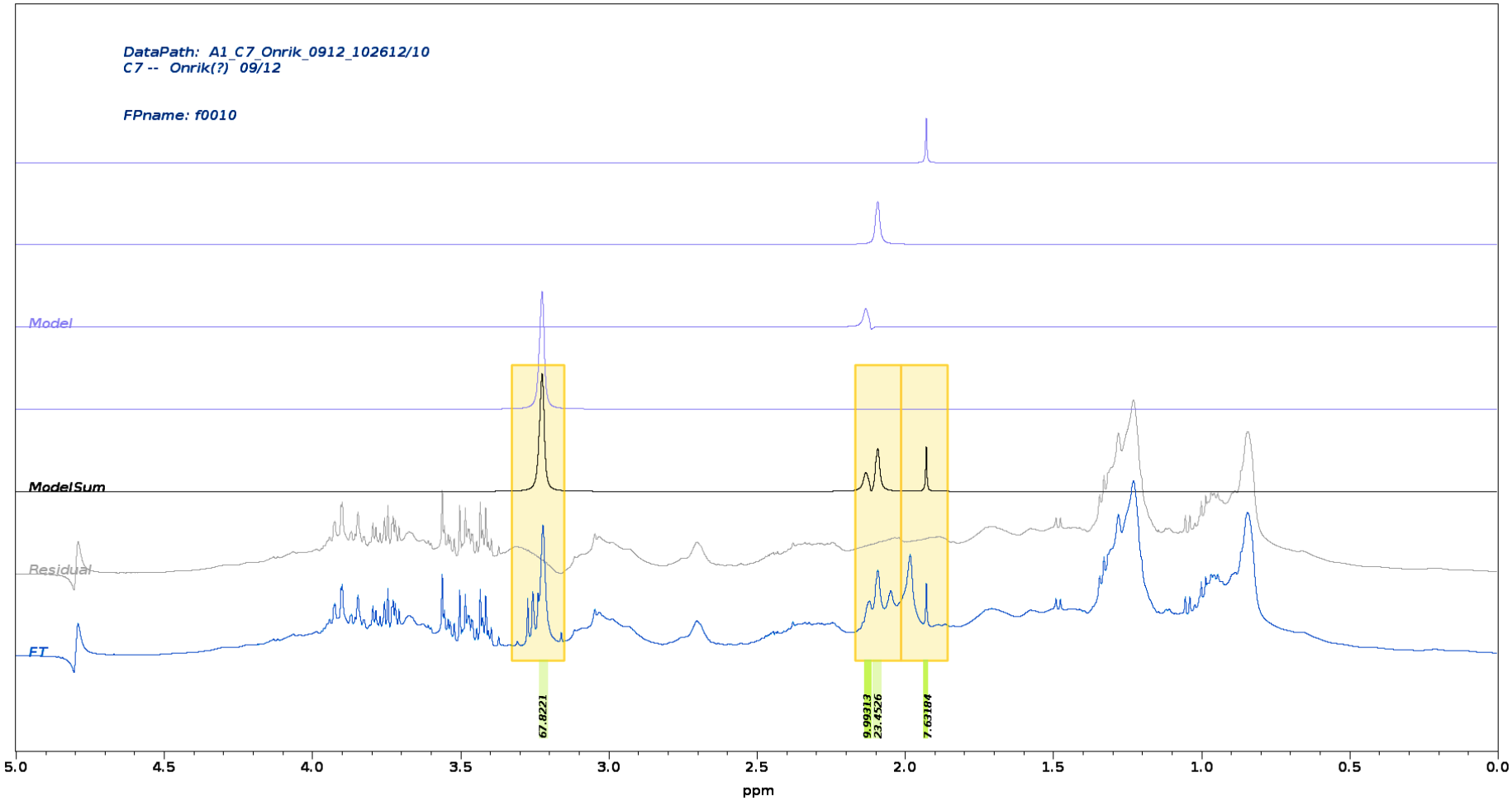
Interpretation

Each row defined by one or more exponential decay functions

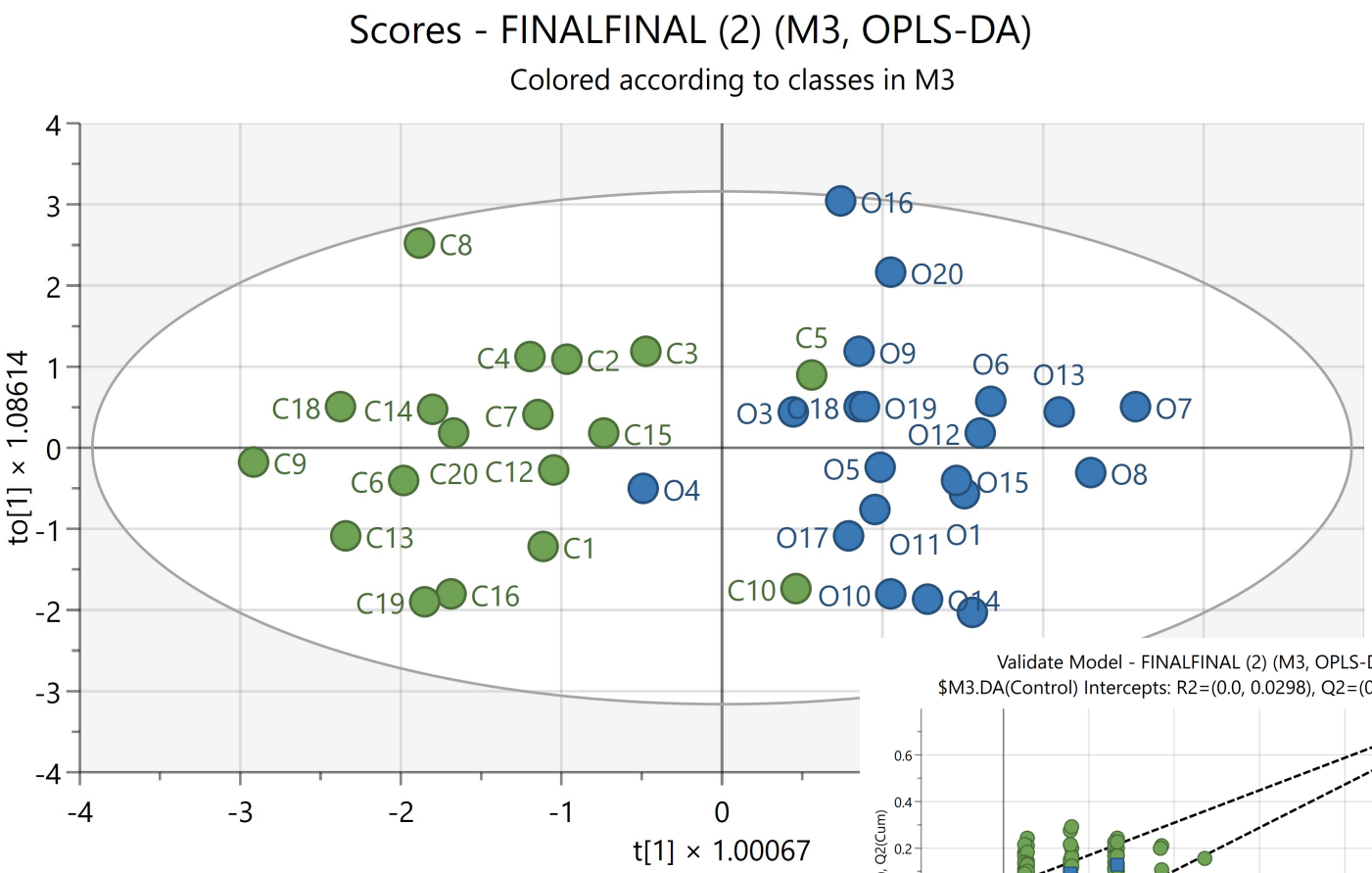


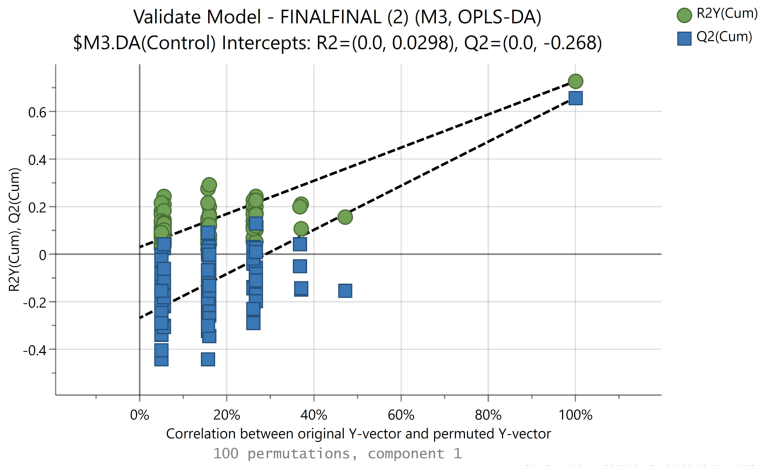
Spectrum is an optional visualization tool for the CRAFTable

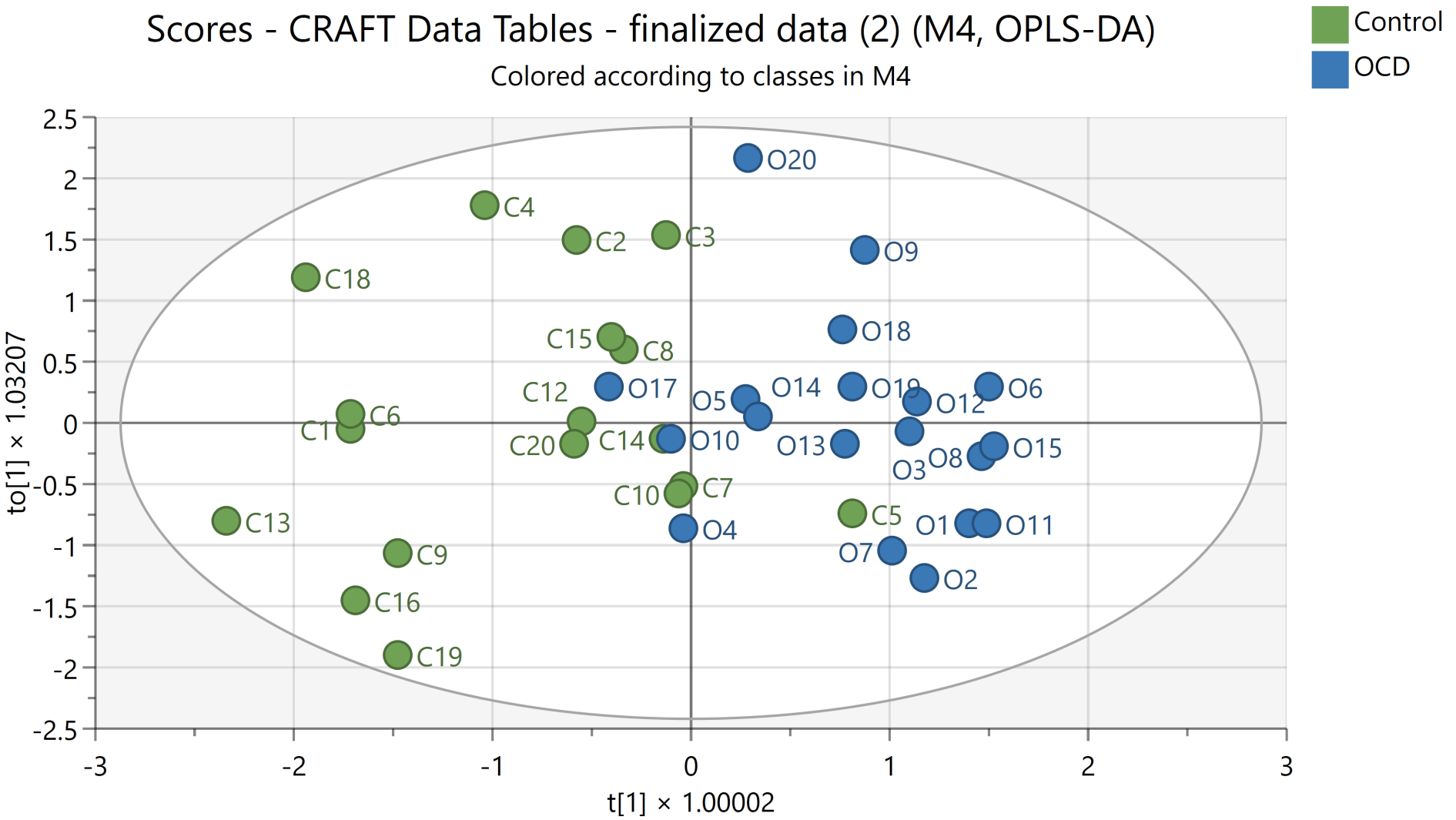
A representative CRAFT model on a randomly selected spectrum from the set of 20+20 cohort



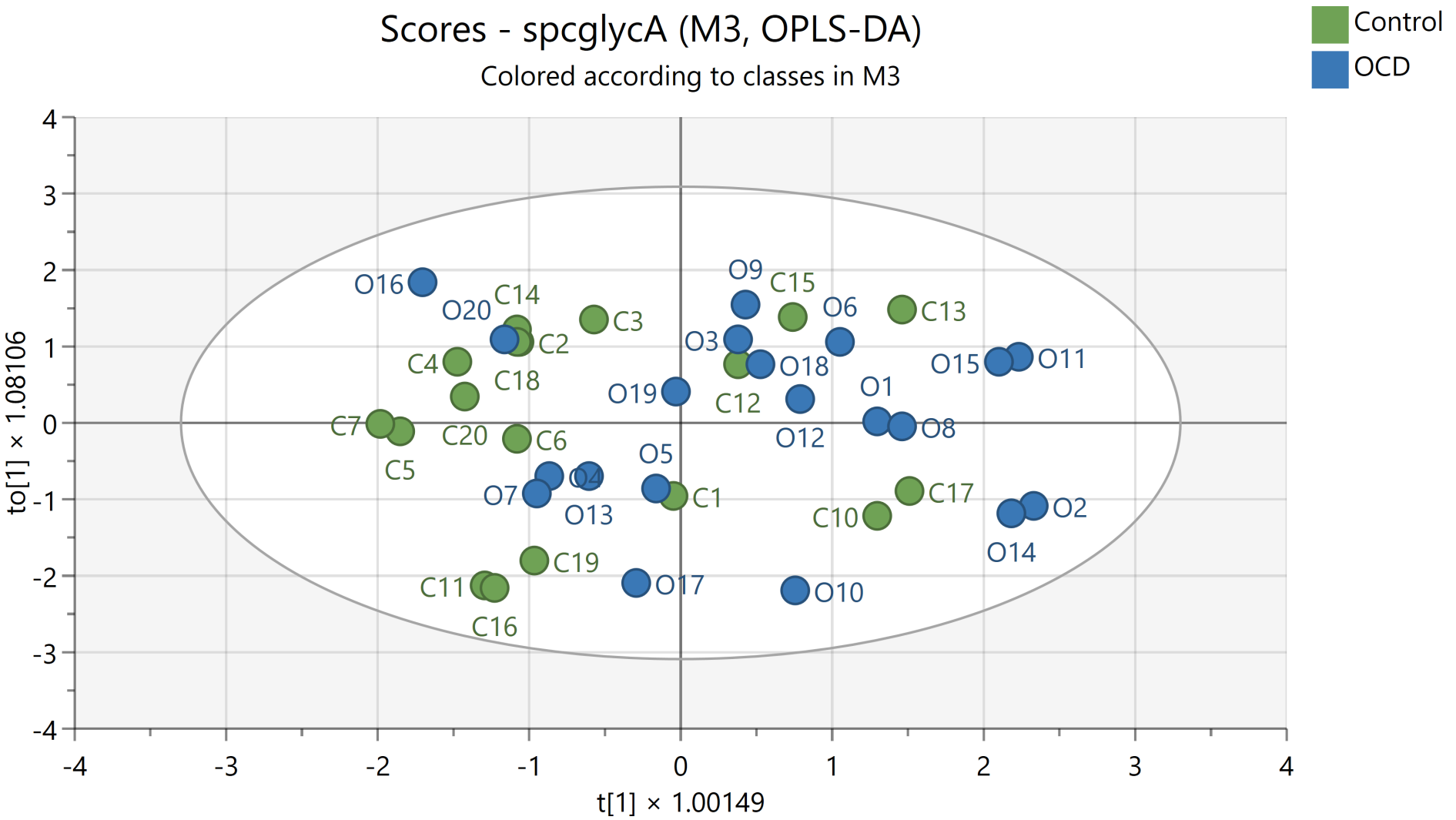
The SIMCA modelling: O-PLS-DA analysis of GlycB/SPC CRAFT amplitude ratios



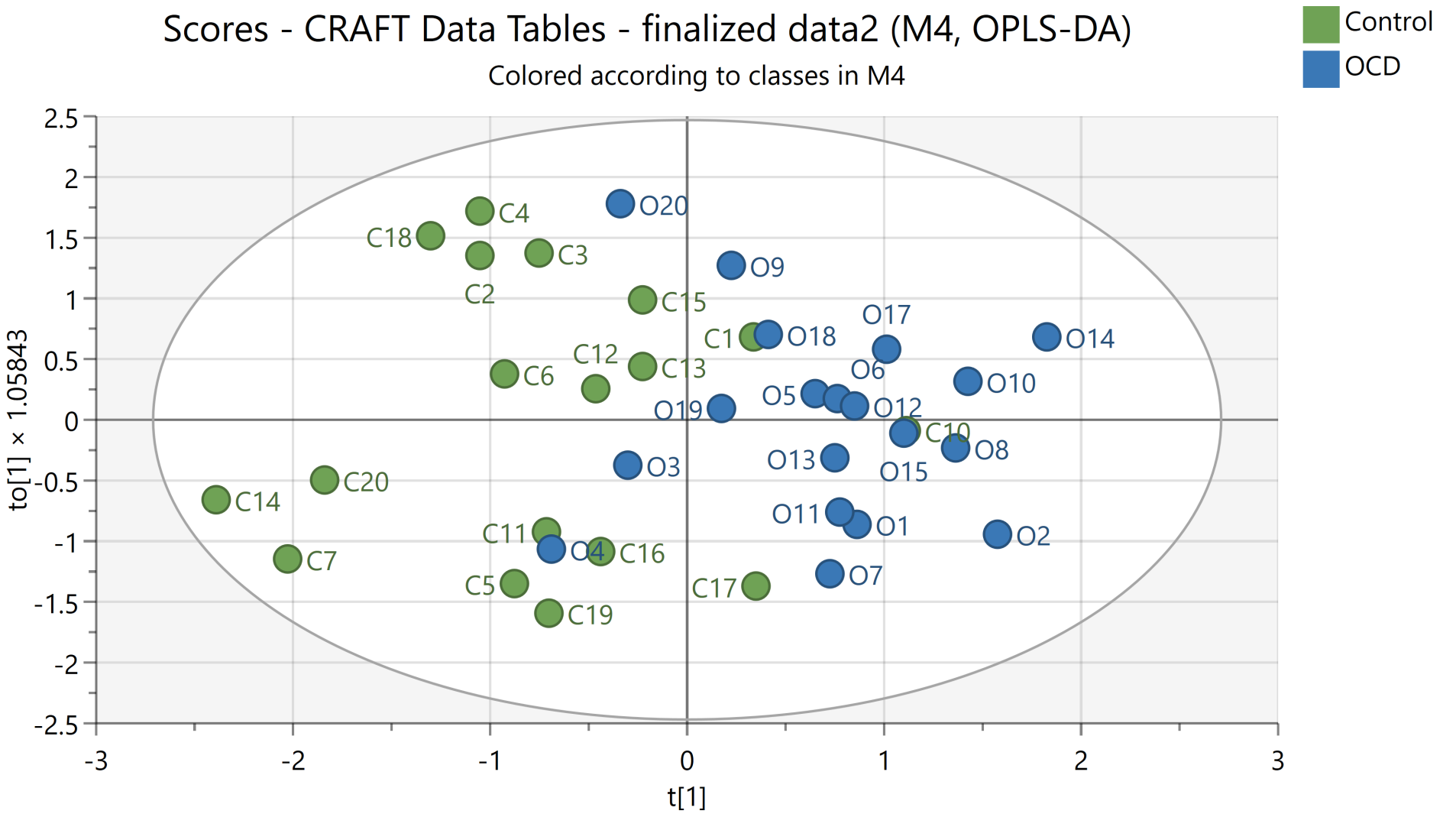



Scores - CRAFT Data Tables - finalized data (2) (M4, OPLS-DA)
Colored according to classes in M4
Control
OCD
to[1] × 1.03207
t[1] × 1.00002
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-2
-1
0
1
2
3
C4
C2
C3
C18
C15
C8
C12
O17
C6
C1
C20
C14
C10
C7
C13
C9
C16
C19
C5
O4
O20
O9
O18
O5
O14
O19
O6
O12
O10
O13
O15
O3
O8
O1
O11
O7
O2
Scaled proportionally to R2X, R2X[1] = 0.409, R2Xo[1] = 0.29,
Ellipse: Hotelling's T2 (95%)
SIMCA 18.0.1 - 2025/04/06 03:36:10 PM (UTC-4)

GlycA/GlycB


Scores - spcglycA (M3, OPLS-DA)
Colored according to classes in M3
Control
OCD
to[1] × 1.08106
t[1] × 1.00149
4
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3
4
O16
O20
C14
C3
C2
C4
C18
O9
C15
O6
C13
O3
O18
O15
O11
O19
C12
O1
C7
C20
C6
O8
O12
C5
O5
O4
O7
C1
C17
O13
C10
O2
O14
C19
C11
O17
O10
C16
Scaled proportionally to R2X, R2X[1] = 0.406, R2Xo[1] = 0.356,
Ellipse: Hotelling's T2 (95%)
SIMCA 18.0.1 - 8/6/2024 10:41:33 AM (UTC-4)

SPC/GlycA



SPC/(GlycA+GlycB)

Summary, conclusions, future work

We proved that ***CRAFT is a viable and highly simplified tool*** for diagnostics from a single and simple spectrum. This work is focusing on the three major inflammatory biomarker signals (GlycA, GlycB and SPC), but ***the concept is valid to all identifiable biomarker signals*** or patterns of those.

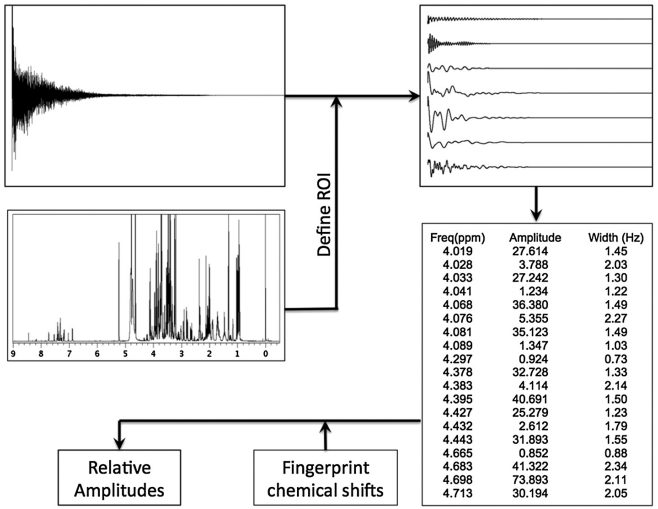
GlycA, GlycB and SPC can be quantitatively extracted from complex spectral environments, including overlap with other, irrelevant signals and broad background resonances.

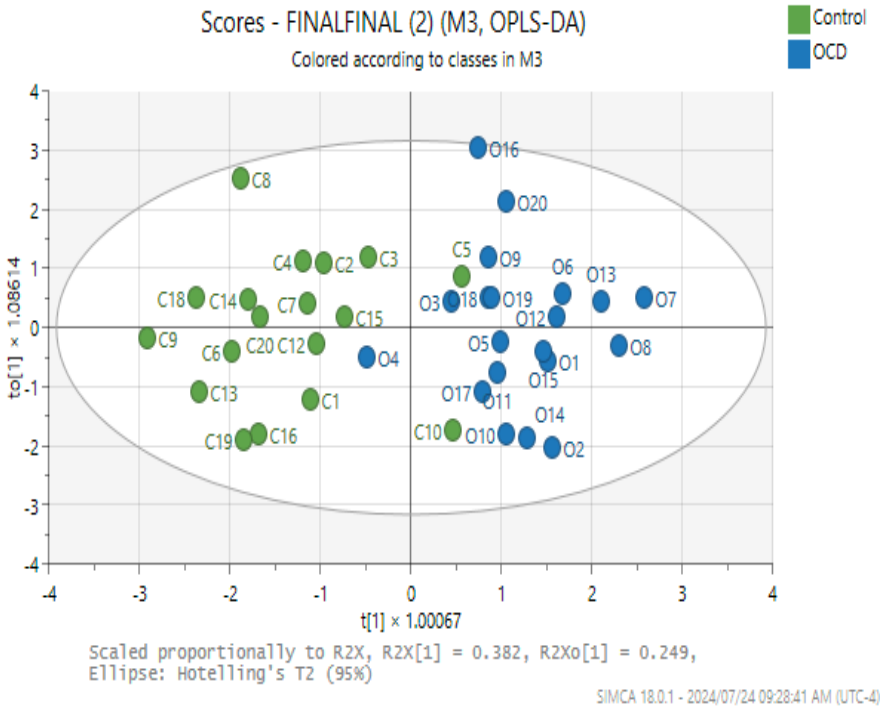
This work proves that ***OCD is an inflammatory condition indeed***, as the ratio of the GlycB and SPC amplitudes clearly separate the healthy and diseased cohorts using multivariate analysis, as shown by the O-PLS-DA results with very good validation. Other combinations of the selected signal amplitudes show limited or no good clustering.

CRAFT can be applied ***RETROSPECTIVELY*** !

We are in the process to extend this application to many other datasets collected earlier, including those on laminitis, age related studies, comparing metabolic signature of mustangs to that of domestic horses, falcon aspergillosis, etc.

Thank you!...


Define ROI
Freq(ppm) Amplitude Width (Hz)
4.019 27.614 1.45
4.028 3.788 2.03
4.033 27.242 1.30
4.041 1.234 1.22
4.068 36.380 1.49
4.076 5.355 2.27
4.081 35.123 1.49
4.089 1.347 1.03
4.297 0.924 0.73
4.378 32.728 1.33
4.383 4.114 2.14
4.395 40.691 1.50
4.427 25.279 1.23
4.432 2.612 1.79
4.443 31.893 1.55
4.665 0.852 0.88
4.683 41.322 2.34
4.698 73.893 2.11
4.713 30.194 2.05
Relative Amplitudes
Fingerprint chemical shifts


Scores - FINALFINAL (2) (M3, OPLS-DA)
Colored according to classes in M3
Control
OCD
to[1] × 1.08614
t[1] × 1.00067
Scaled proportionally to R2X, R2X[1] = 0.382, R2Xo[1] = 0.249,
Ellipse: Hotelling's T2 (95%)
SIMCA 18.0.1 - 2024/07/24 09:28:41 AM (UTC-4)

Check out our new ssNMR/DNP!...