CRAFT
Complete Reduction to Amplitude-Frequency Table

Spectrum to Spreadsheet:
Automated Extraction
of NMR Data

April 2013

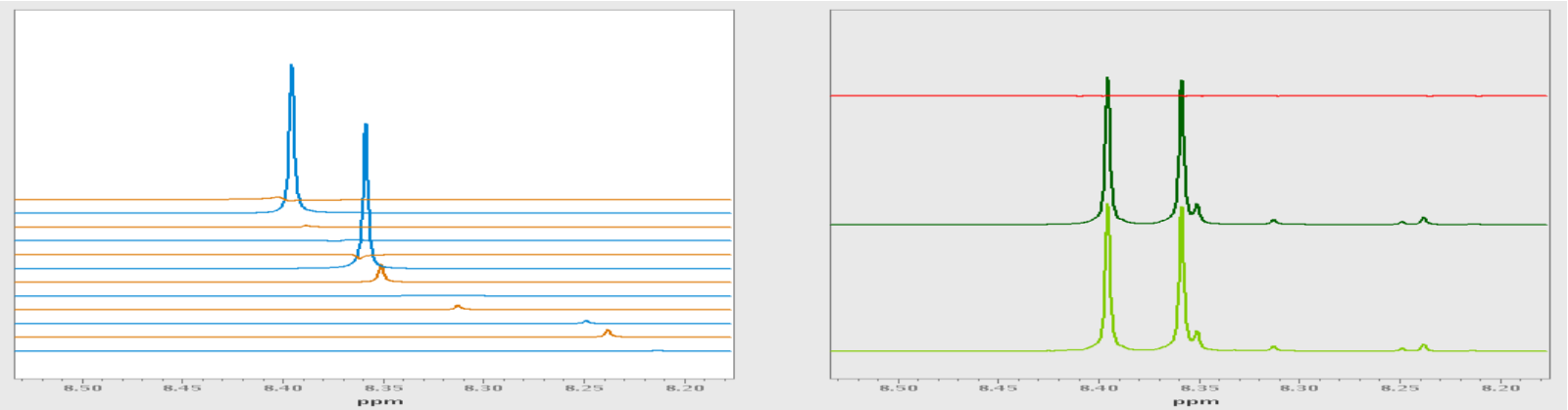
Agilent Technologies

CRAFT

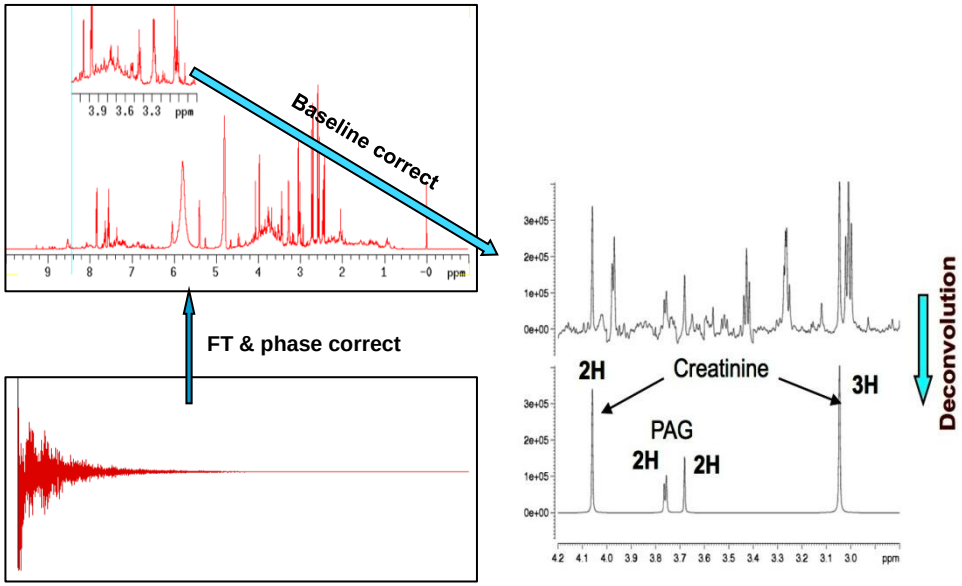
Complete Reduction to Amplitude-Frequency Table

Converting an NMR spectrum into a useable, tabular format has traditionally been a challenge for researchers who want to use NMR to investigate large numbers of samples.

CRAFT is a new utility built into VnmrJ 4 Software that automatically extracts the information inherent in an NMR data set and converts the data into a simple spreadsheet. The result is a simple, rapid, and automated method for interpreting NMR data with high reproducibility and fidelity.



Current NMR Analysis Workflow

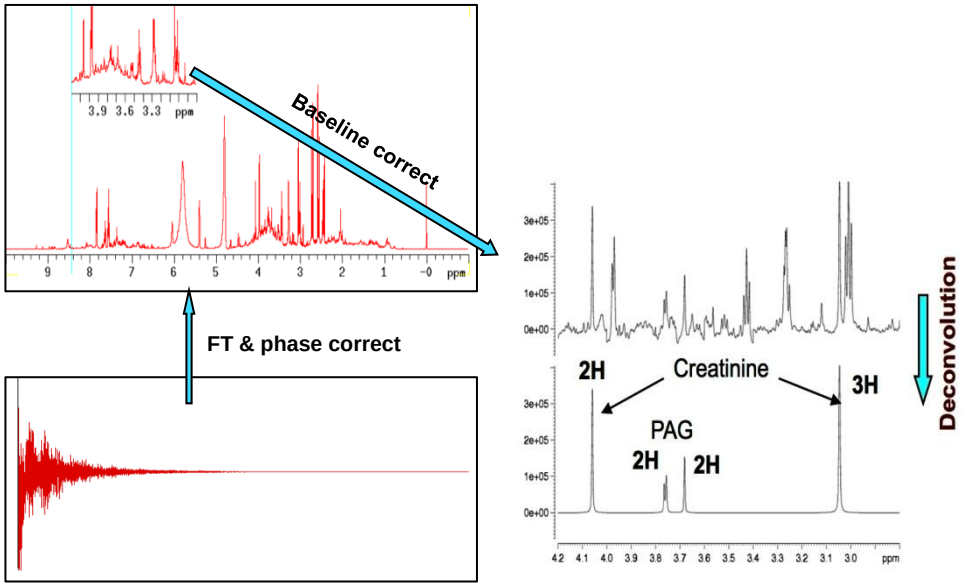


The NMR workflow for data analysis is primarily based on manual processing and interpretation of each individual spectrum.

This is a slow, tedious, and error-prone process.

Manual processing. Manual data reduction. Manual everything!

Current NMR Analysis Workflow

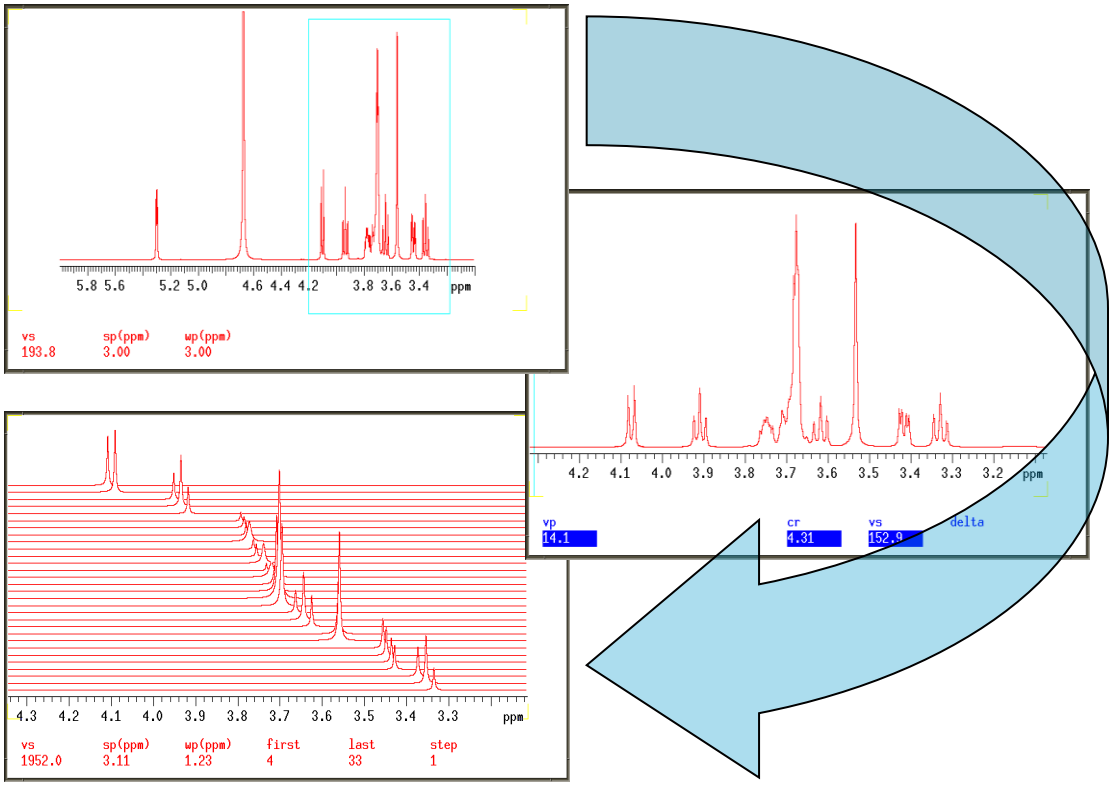


There are various drawbacks to interpretation based on a processed spectrum: significant issues can be introduced by the Fourier Transform, including baseline issues and phase distortions.

Manual processing. Manual data reduction. Manual *everything*!

Spectral Deconvolution

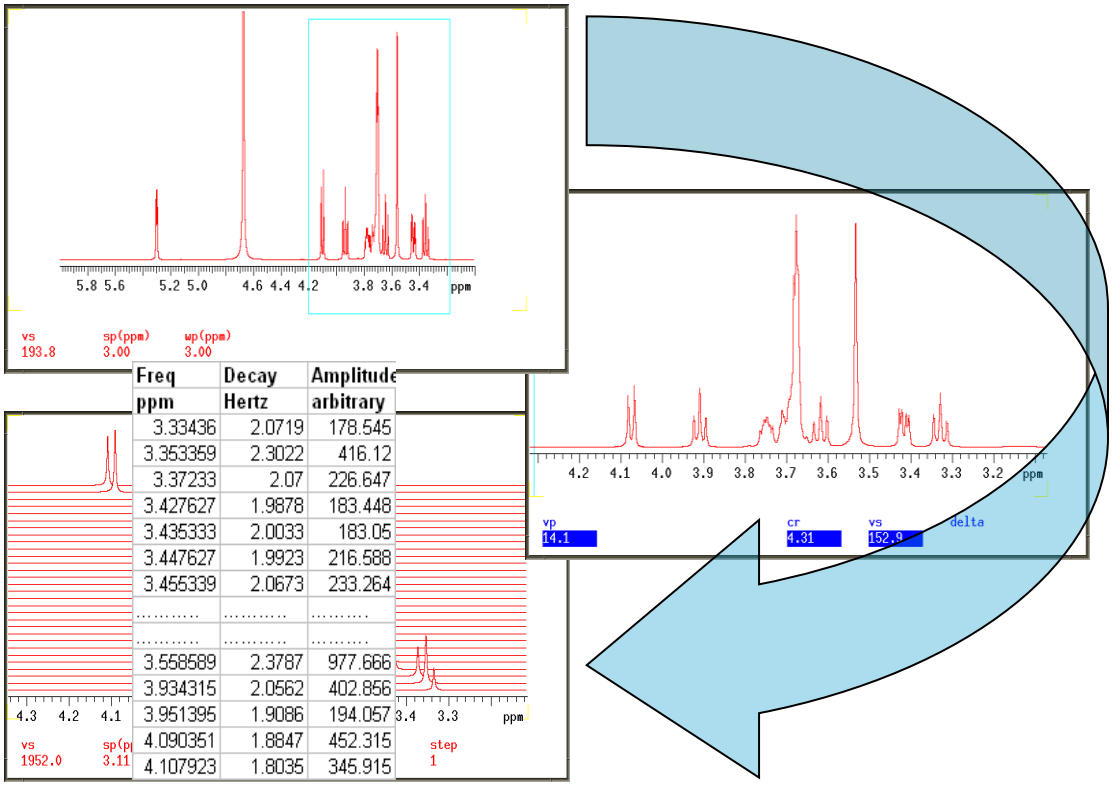
What do we mean by deconvolution?



When we talk about “deconvolution” we mean extracting individual peaks from a complex spectrum.

Spectral Deconvolution

What do we mean by deconvolution?



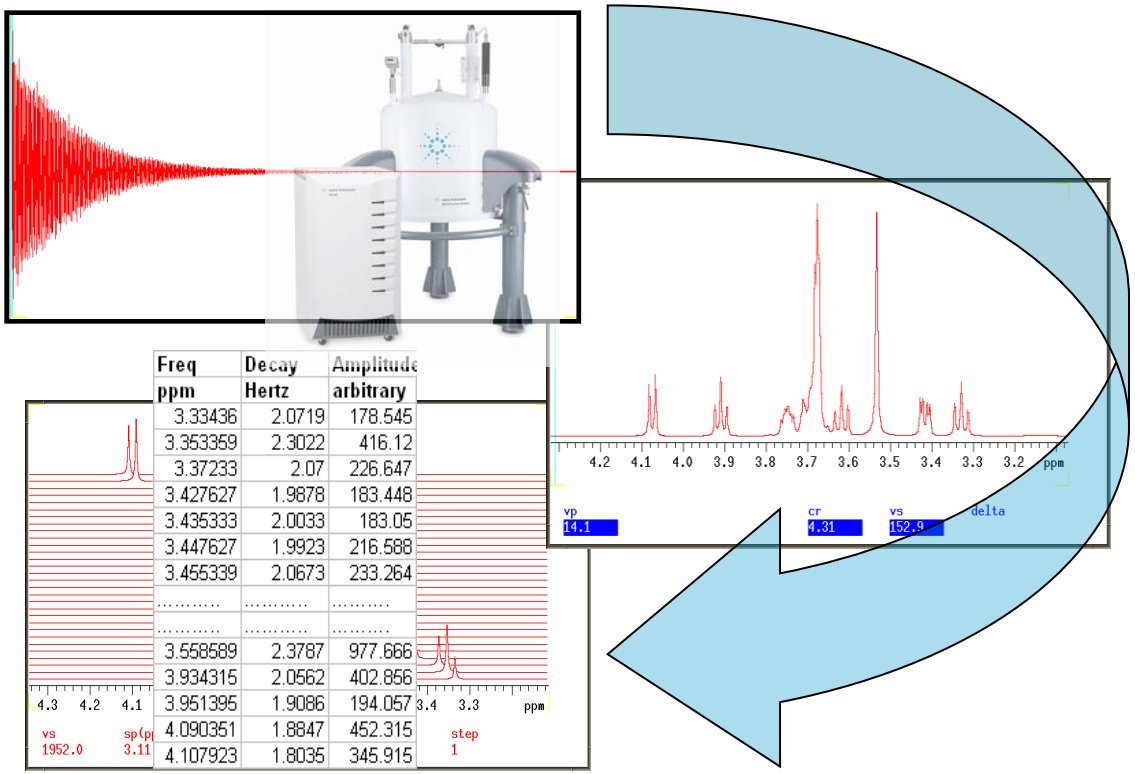
Freq	Decay	Amplitude
ppm	Hertz	arbitrary
3.33436	2.0719	178.545
3.353359	2.3022	416.12
3.37233	2.07	226.647
3.427627	1.9878	183.448
3.435333	2.0033	183.05
3.447627	1.9923	216.588
3.455339	2.0673	233.264
.....	.....	.....
.....	.....	.....
3.558589	2.3787	977.666
3.934315	2.0562	402.856
3.951395	1.9086	194.057
4.090351	1.8847	452.315
4.107923	1.8035	345.915

What we really want is to convert a human-readable NMR spectrum into a data format that can be used for computer-based data manipulation.

This means converting the data into a spreadsheet.

Spectral Deconvolution

What do we “really, really” want from deconvolution?



By analyzing the data directly in the time domain, all the issues induced by using a Fourier Transform are avoided.

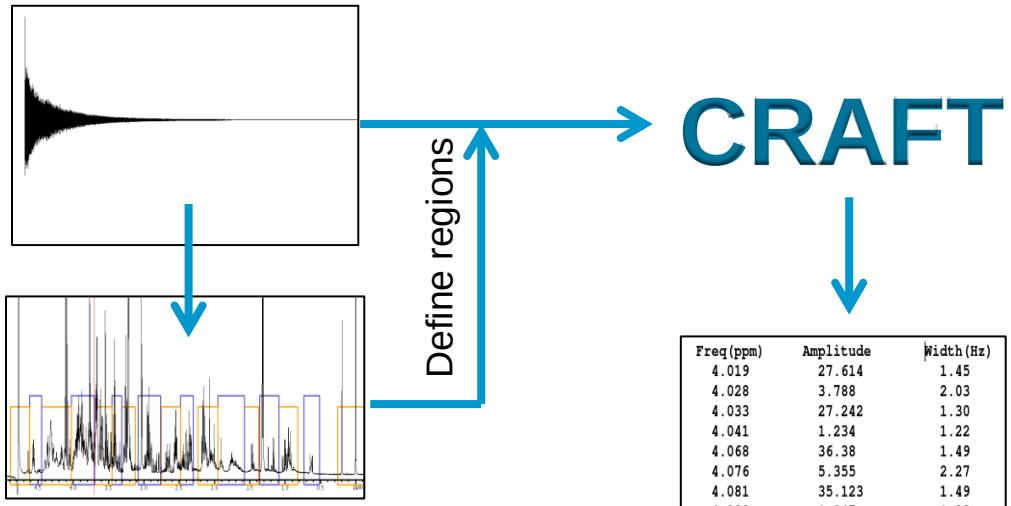
Phase and baseline corrections are no longer relevant, and overlap is based solely on acquisition time and decay constant.

CRAFT

Complete Reduction to Amplitude-Frequency Table

Spectrum to Spreadsheet

CRAFT – Deconstructing a Spectrum

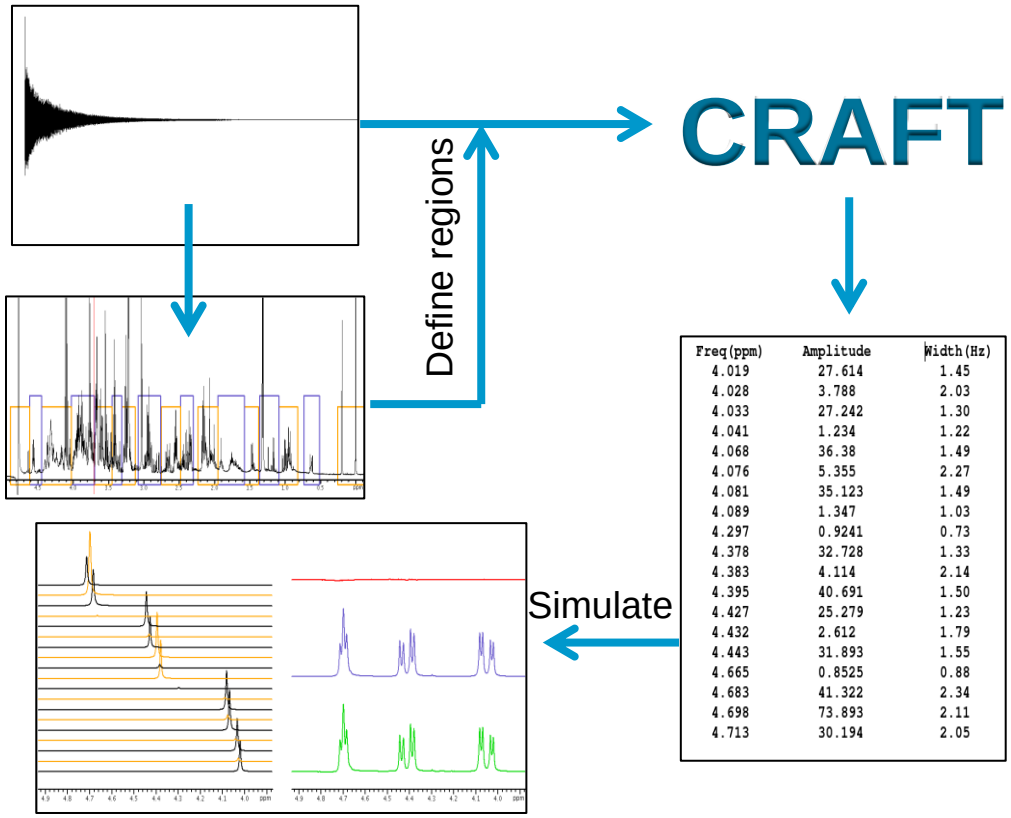


Freq(ppm)	Amplitude	Width(Hz)
4.019	27.614	1.45
4.028	3.788	2.03
4.033	27.242	1.30
4.041	1.234	1.22
4.068	36.38	1.49
4.076	5.355	2.27
4.081	35.123	1.49
4.089	1.347	1.03
4.297	0.9241	0.73
4.378	32.728	1.33
4.383	4.114	2.14
4.395	40.691	1.50
4.427	25.279	1.23
4.432	2.612	1.79
4.443	31.893	1.55
4.665	0.8525	0.88
4.683	41.322	2.34
4.698	73.893	2.11
4.713	30.194	2.05

The CRAFT workflow is simple. Once data are collected, Regions of Interest (ROI) are selected interactively, and then CRAFT does the work.

The result is a spreadsheet containing all the information that was captured in the original experiment.

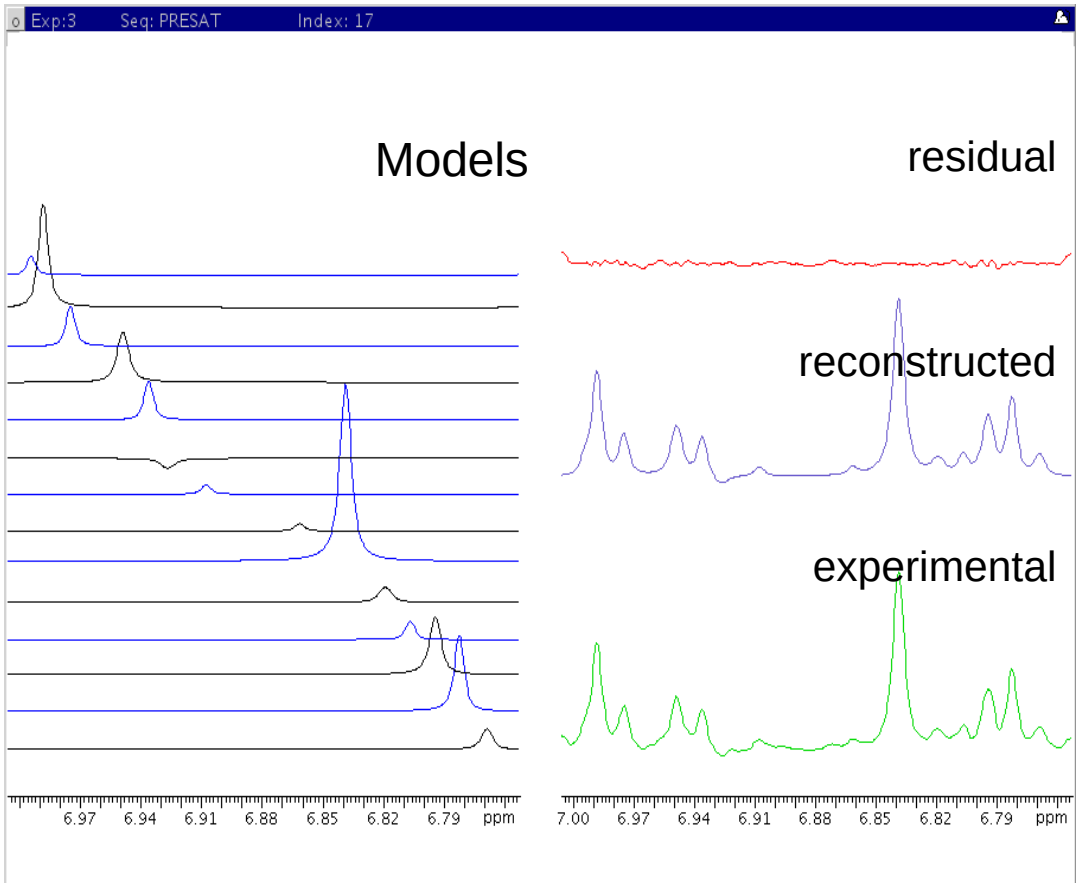
CRAFT – Deconstructing a Spectrum



Once data have been captured in the spreadsheet, analysis is essentially complete.

Most users want to see how well the data reduction step worked. CRAFT includes a complete set of tools to allow visual inspection of the results.

CRAFT – Reconstructing Spectra for Comfort

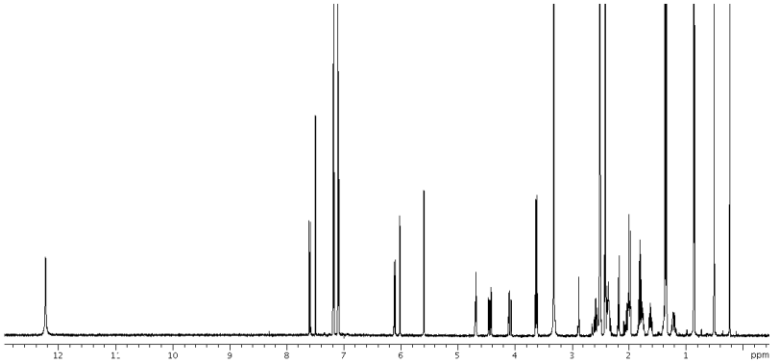


The common method for displaying CRAFT data is to show the experimental NMR spectrum after Fourier Transform, the CRAFT spectrum reconstructed from the final spreadsheet, and the residual signal, or the “difference between the two” spectrum.

This step is for user convenience; it is not required for analysis!

Example Spectrum

CRAFT vs. experimental results

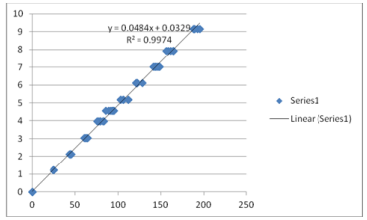


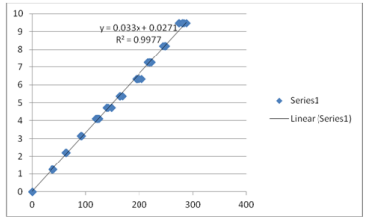
An initial proof-of-concept study used eleven samples containing prednisone and ibuprofen made in a range of concentrations. After data collection (3x of each conc., 2 spectra per sample, 66 spectra total), CRAFT deconstructed the spectra.

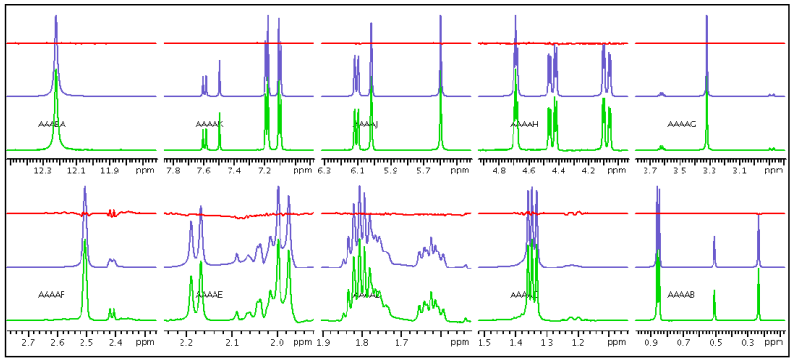
The result table was used to generate concentration values for each component.

Finger print chemical shift table

Prednesone:	7.60283	2
Prednesone:	7.58193	2
Prednesone:	6.11666	2
Prednesone:	6.11336	2
Prednesone:	6.09686	2
Prednesone:	6.09356	2
Prednesone:	6.01436	2
Prednesone:	5.59524	2
Prednesone:	4.6965	2
Prednesone:	4.68329	2
Prednesone:	4.67229	2
Prednesone:	4.46438	2
Prednesone:	4.45118	2
Prednesone:	4.42588	2
Prednesone:	4.41268	2
Prednesone:	4.10137	2
Prednesone:	4.09147	2
Prednesone:	4.06287	2
Prednesone:	4.05187	2
Prednesone:	2.89241	2
Prednesone:	2.86821	2
Prednesone:	2.18948	2
Prednesone:	2.16638	2
Prednesone:	1.99697	2
Prednesone:	1.97277	2
Prednesone:	1.36004	2
Prednesone:	0.505304	2
Ibuprofen:	7.19031	2
Ibuprofen:	7.17381	2
Ibuprofen:	7.10561	2
Ibuprofen:	7.0902	2
Ibuprofen:	3.62615	2
Ibuprofen:	3.61295	2
Ibuprofen:	2.41829	2
Ibuprofen:	2.40399	2
#Ibuprofen:	1.34574	2
#Ibuprofen:	1.33144	2
Ibuprofen:	0.86282	2
Ibuprofen:	0.84962	2

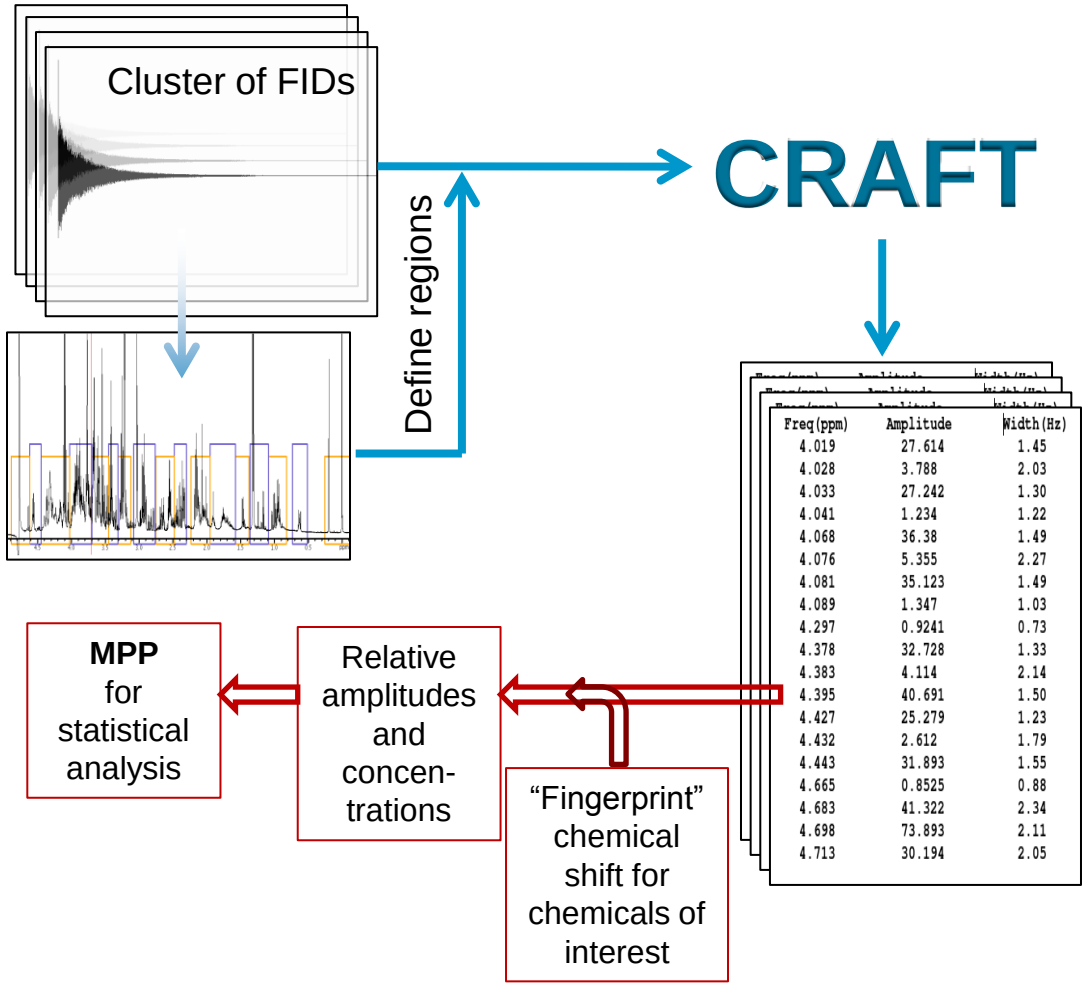






CRAFT results (green=experimental; blue=reconstructed; red=residual). Vertical scaling in each display is independent of one another.

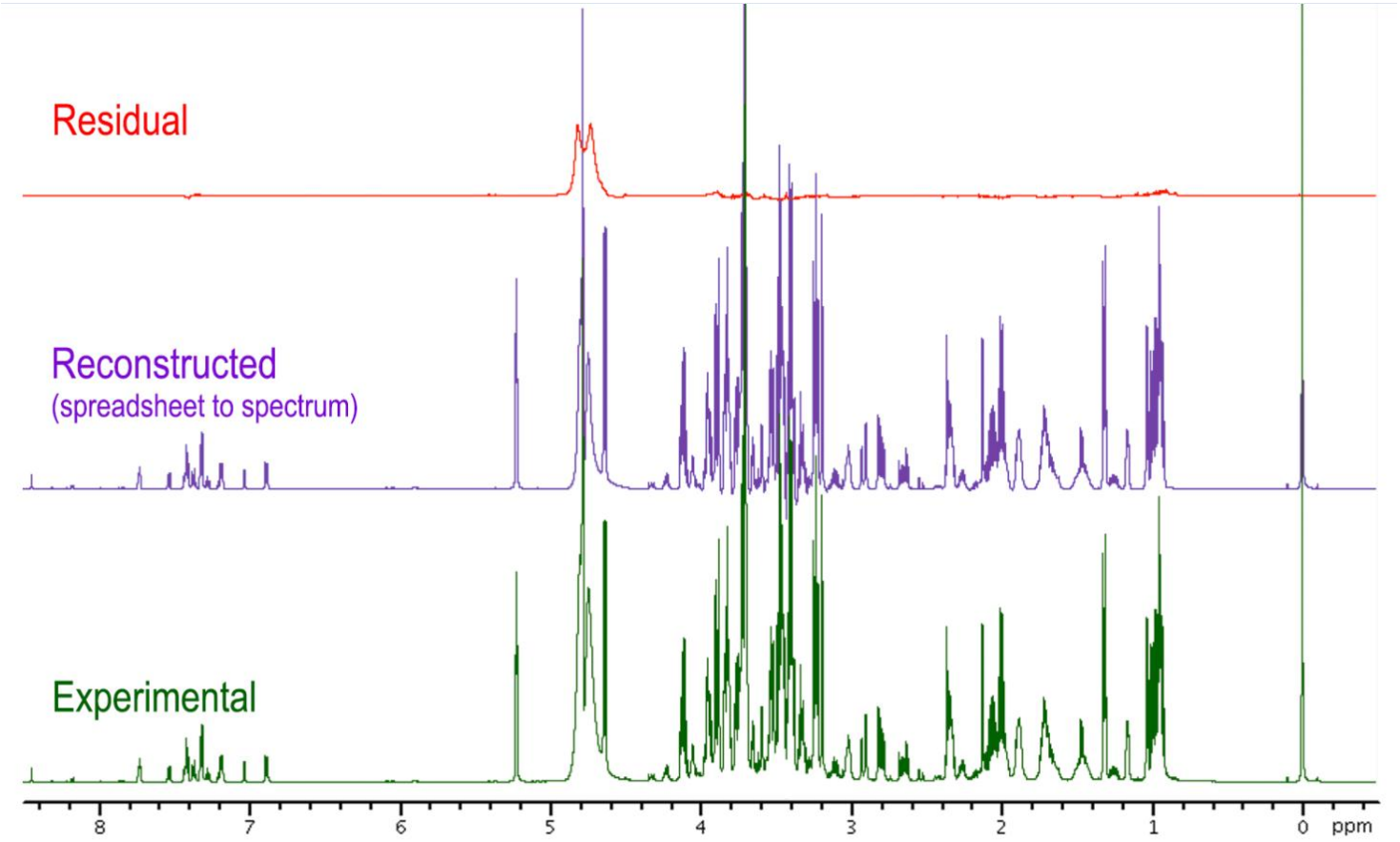
Total CRAFT Study Workflow



The CRAFT workflow for a whole collection of spectra is simple:

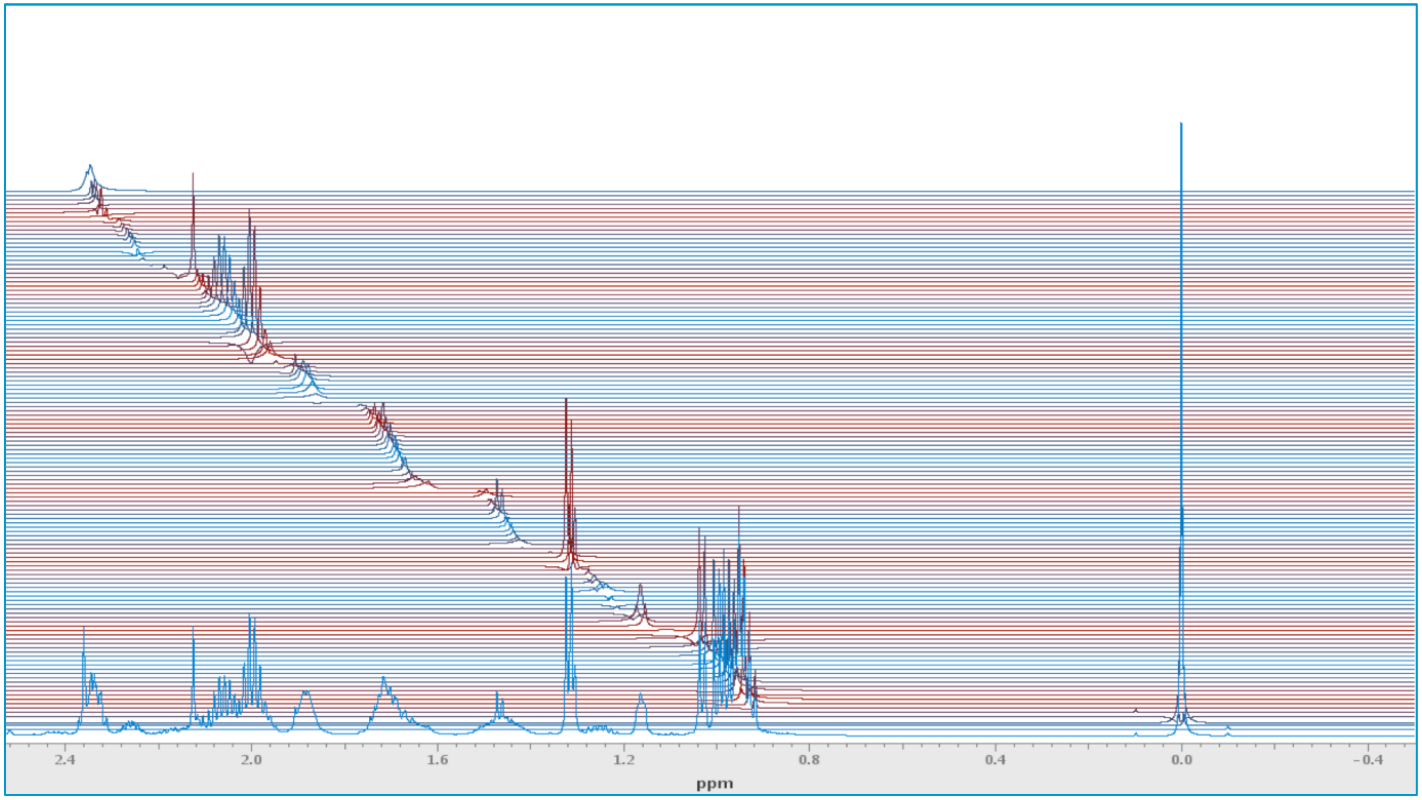
1. Collect the data
2. Create a study cluster
3. Assign cluster ROIs
4. CRAFT
5. Extract library components
6. Generate final results table
7. Export for analysis

CRAFT – Fermentation Broth



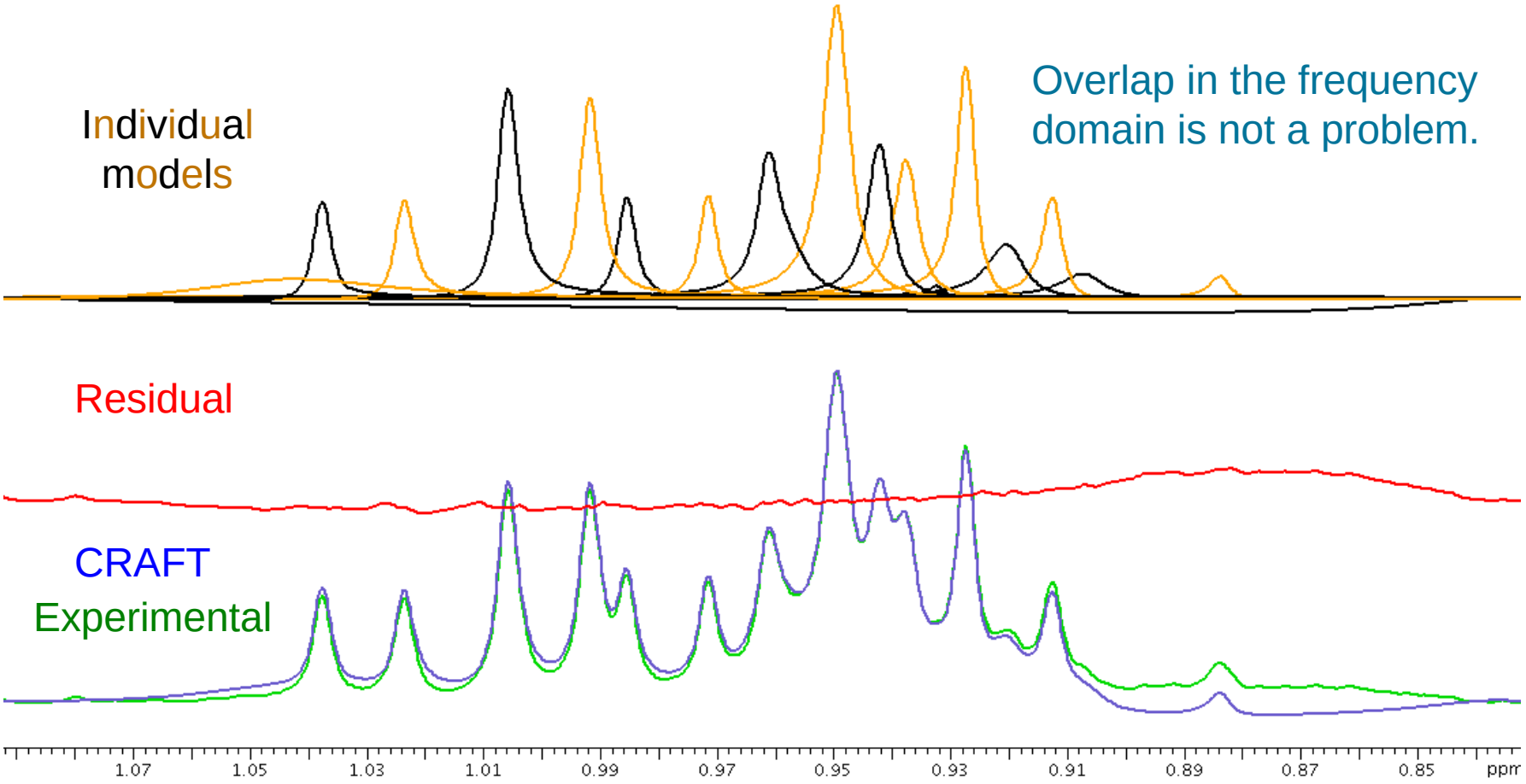
Even complex data sets are easily handled. Broad resonances can be excluded based on user preferences.

CRAFT – Fermentation Broth

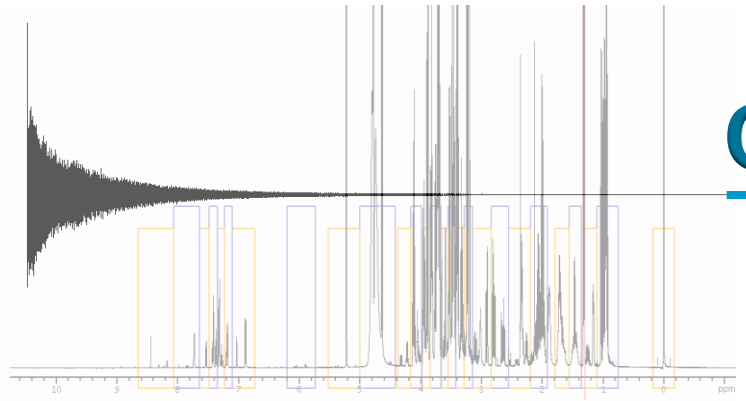


This is another way to visually inspect CRAFT results. A stacked plot was created showing each line extracted from an expansion of the complete data set.

CRAFT ~2.3 Min/Spectrum



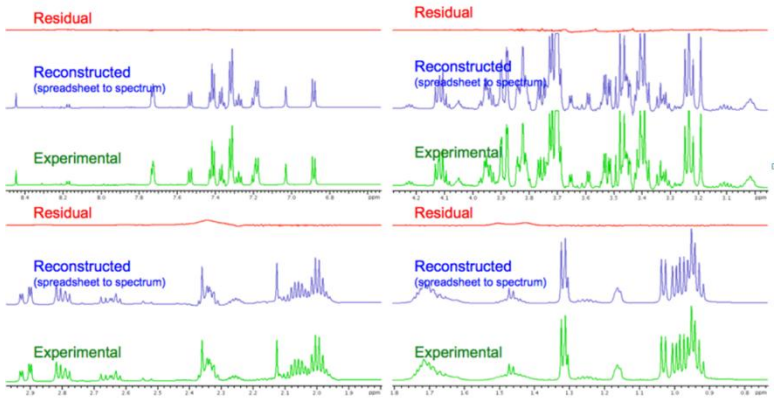
Fermentation Broth



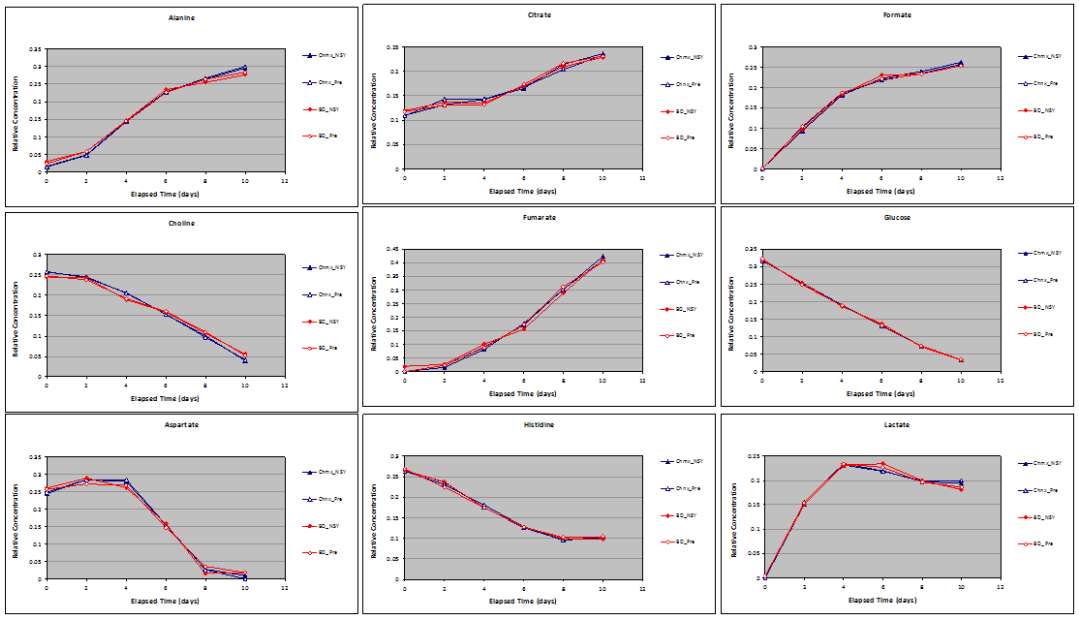
CRAFT

(2.3 min)

CRAFT (bottom line)

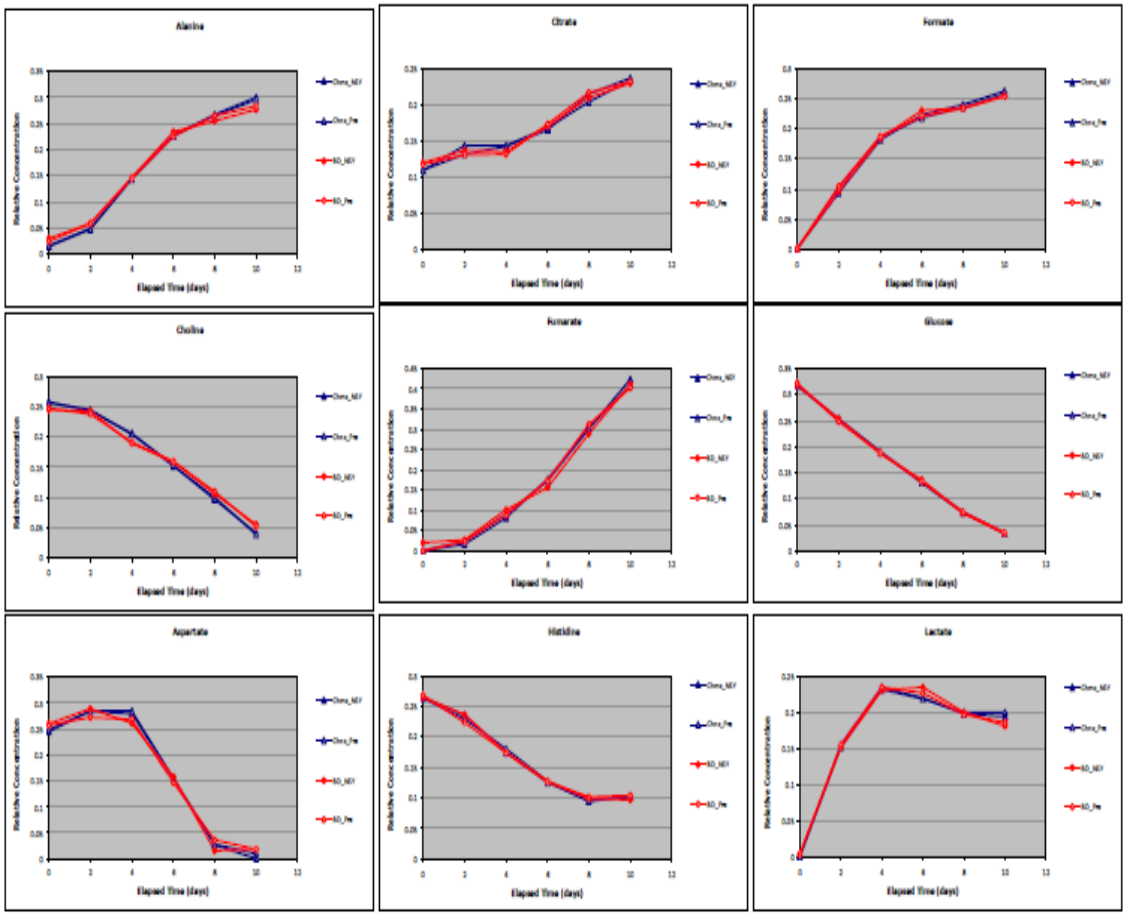


Spectrum to spreadsheet



A major pharmaceutical customer collaborated with us to test CRAFT against a skilled human analyst.

CRAFT Analysis vs. The Human Expert



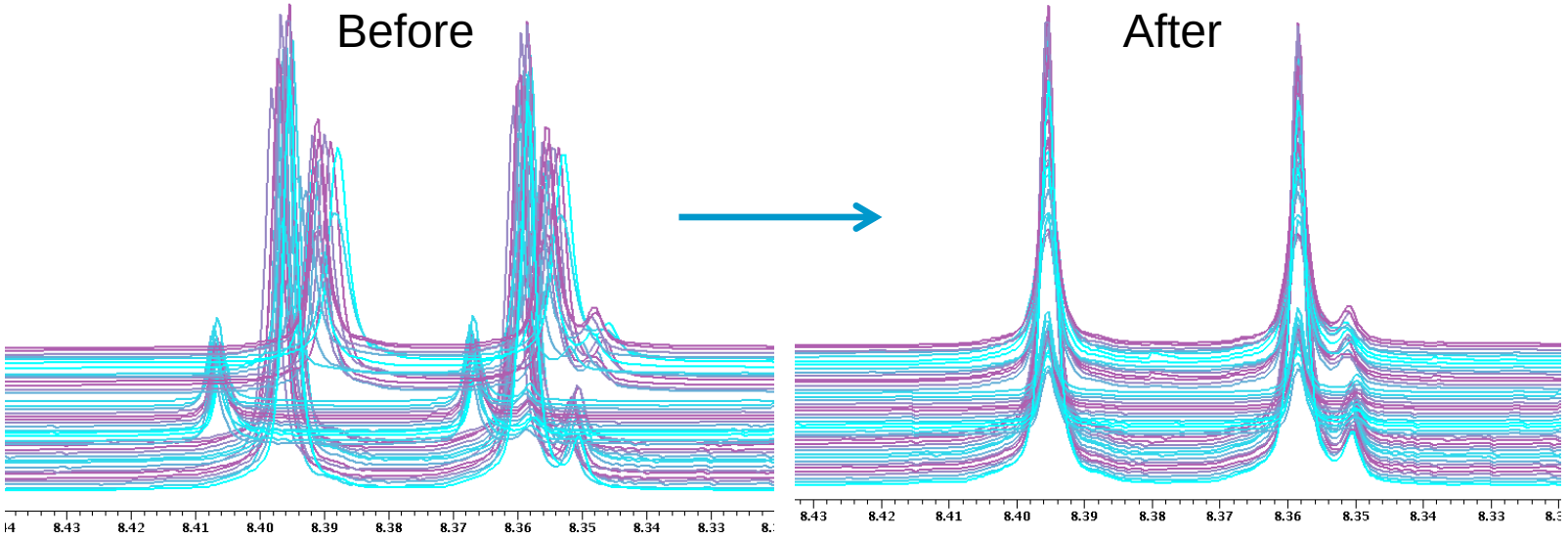
Results were performed on each spectrum by the analyst and plotted against those recovered by CRAFT's fully automated workflow.

It took 10 hours of human interpretation to get the same results that CRAFT provided automatically in minutes.

CRAFT – Spectral Alignment Tool

For many applications, spectral alignment of NMR resonance is required before meaningful analysis can be accomplished. This is due to the sensitivity of some NMR signals to the local chemical environment.

CRAFT provides a robust and intuitive alignment tool.



CRAFT and Metabolomics

CRAFT is a widely applicable utility that can be used by many different types of NMR users on many different kinds of analysis problems.

The nature of Metabolomic and Food Science studies implies spectra collected on a large number of nominally equivalent samples.

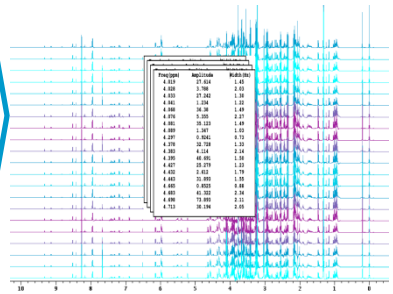
Agilent Technologies is a market leader in these areas, and CRAFT interfaces seamlessly with Agilent's downstream data analysis solution, Mass Profiler Professional.

NMR Metabolomics Workflow

Acquisition

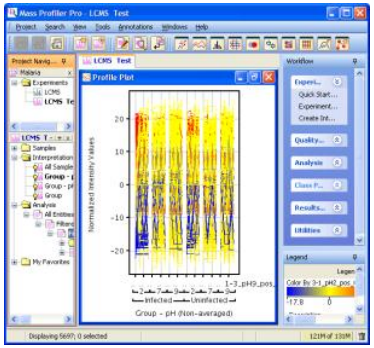
NMR

Identify & Quantify



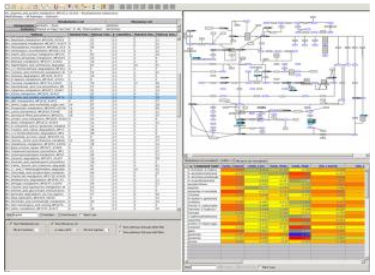
CRAFT
VnmrJ 4

Alignment & Statistics



Mass Profiler Professional

Enrich & Visualize



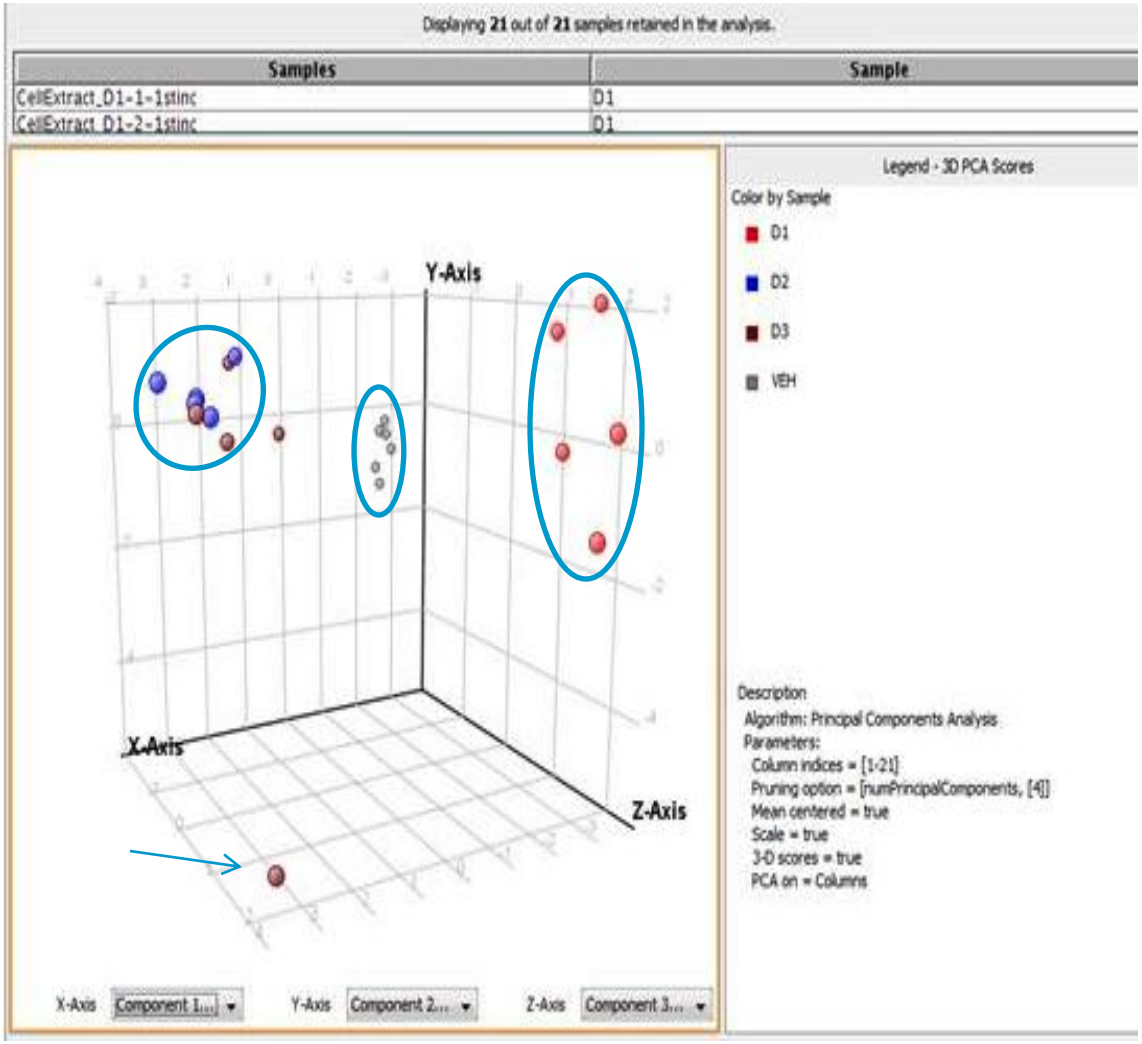
Pathway Architect

CRAFT – MPP Compatible Output

One of the default methods for exporting a CRAFT analysis is an MPP-compatible .cef file format.

Profiled Data	RelativeConcentrations								
Export date	Thu May 10 14:38:55 PDT 2012								
Compound N	Mass	Formula	KEGG	CAS ID	CellExtract_D	CellExtract_D	CellExtract_D	CellExtract_D	CellExtr
Acetate	60.052	C2H4O2	C00033	64-19-7	9.00893	6.87555	7.09916	1.28131	2.6
Alanine	89.0932	C3H7NO2	C00041	56-41-7	21.1339	21.1166	20.36346	20.41809	20.2
Choline	104.1708	C5H14NO	C00114	62-49-7	8.92095	8.90574	8.53129	15.7552	18.
Formate	46.0254	CH2O2	C00058	64-18-6	1.45459	0.235959	0.182259	0.304393	0.2
Fumarate	116.0722	C4H4O4	C00122	110-17-8	4.84481	4.57374	4.26157	4.52556	4.5
Glucose	180.1559	C6H12O6	C00031	50-99-7	7.89209	7.78246	7.4931	7.66932	7.9
Histidine	155.1546	C6H9N3O2	C00135	71-00-1			0.365388	0.136593	
Hypoxanthin	136.1115	C5H4N4O	C00262	68-94-0	3.25902	3.52783	3.13117	3.01827	2.7
Lactate	90.0779	C3H6O3	C00186	79-33-4	663.179	669.828	646.987	645.093	648
Leucine	131.1729	C6H13NO2	C00123	61-90-5	31.84432	37.2965	36.145	37.1784	37.
Methionine	149.2113	C5H11NO2S	C00073	63-68-3	2.09859	1.75193	1.90349	2.03501	2.0
myo-Inositol	180.1559	C6H12O6	C00137	87-89-8		3.15985			2.6
Niacinamide	122.1246	C6H6N2O	C00153	98-92-0				0.516886	
Phenylalanin	165.1891	C9H11NO2	C00079	63-91-2	10.830379	11.112116	11.242725	11.746489	12.8
Tryptophan	204.2252	C11H12N2O2	C00078	73-22-3					
Tyrosine	181.1885	C9H11NO3	C00082	60-18-4	7.08139	6.24504	5.8097	5.8144	6.6
Uridine	244.2014	C9H12N2O6	C00299	58-96-8	2.06016				
Valine	117.1463	C5H11NO2	C00183	72-18-4	21.73308	21.05215	20.96291	20.61857	21.2

CRAFT + MPP Analysis

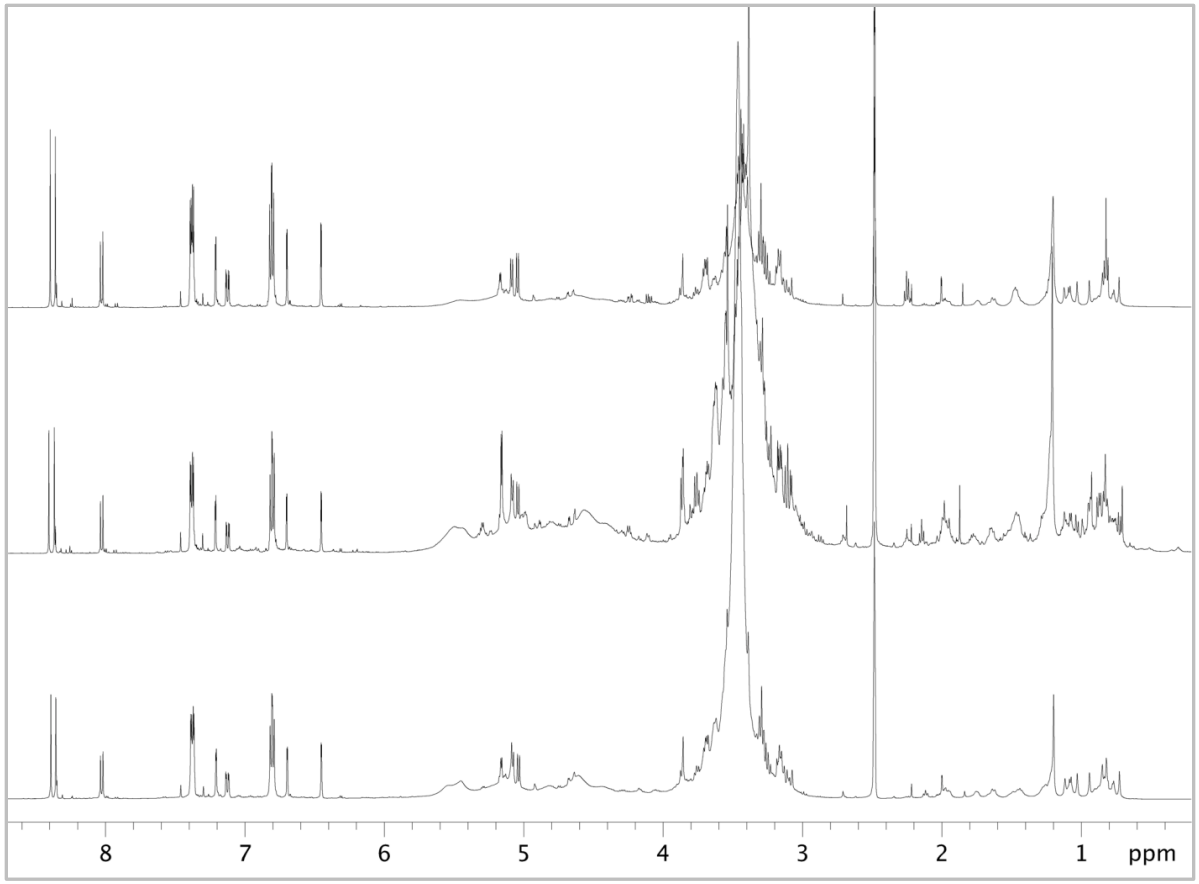


The combination of CRAFT and MPP allows users to exploit the full range of statistical analysis tools available in MPP.

Application to Soy Dietary Supplements

To demonstrate the power of CRAFT, we analyzed three over-the-counter soy dietary supplements.

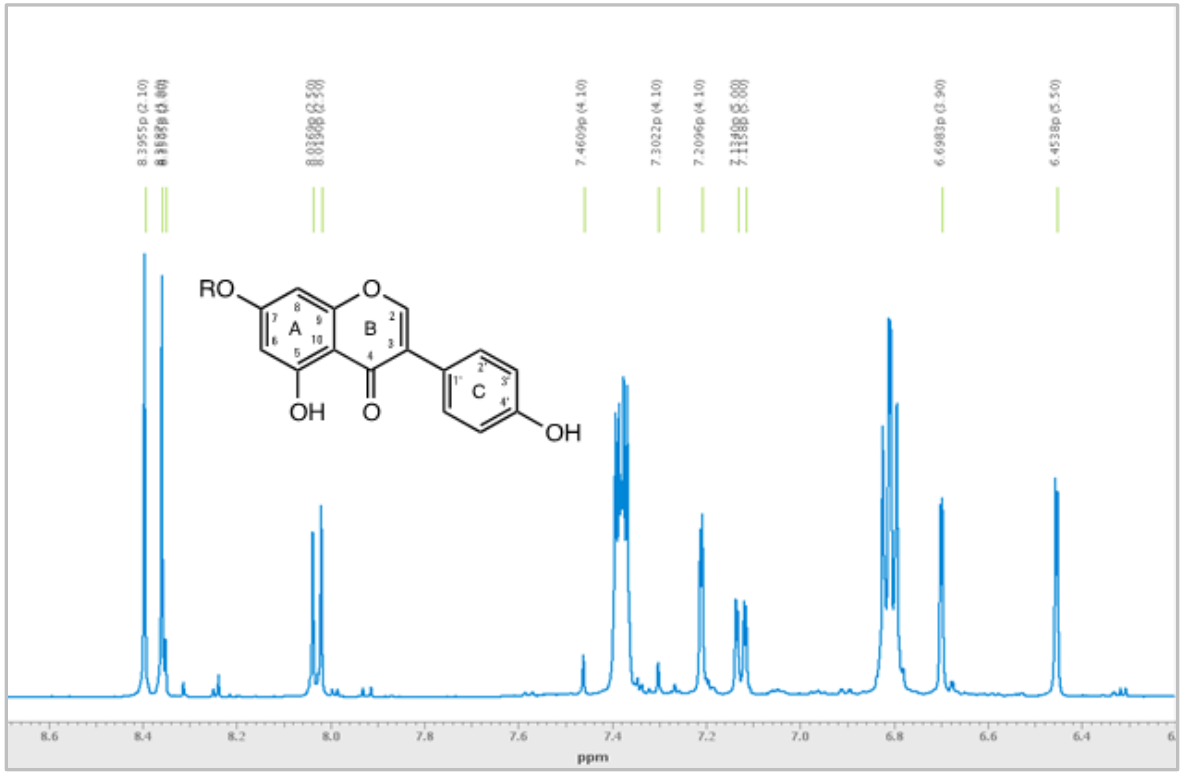
Analysis of Soy



Three different soy supplements were investigated; 15 replicates of each sample were used for the analysis.

These spectra are representative of the results from each sample.

Analysis of Soy – Targeted for Isoflavonoids

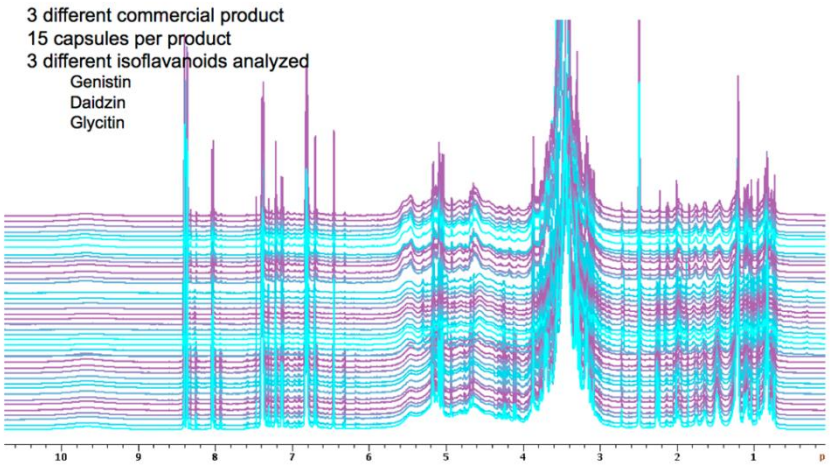


Isoflavonoids are the class of compounds generally associated with the dietary benefits of soy.

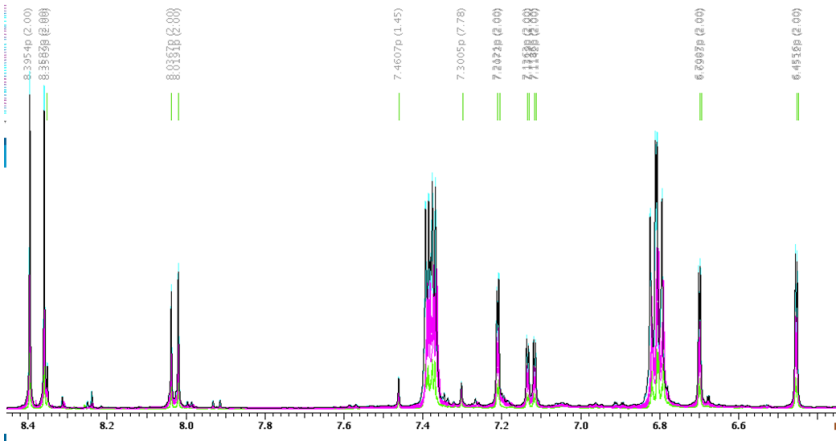
Those aromatic resonances representing the isoflavonoid compounds in the soy extracts were selected for analysis.

Targeted Analysis

Soy dietary supplement extract



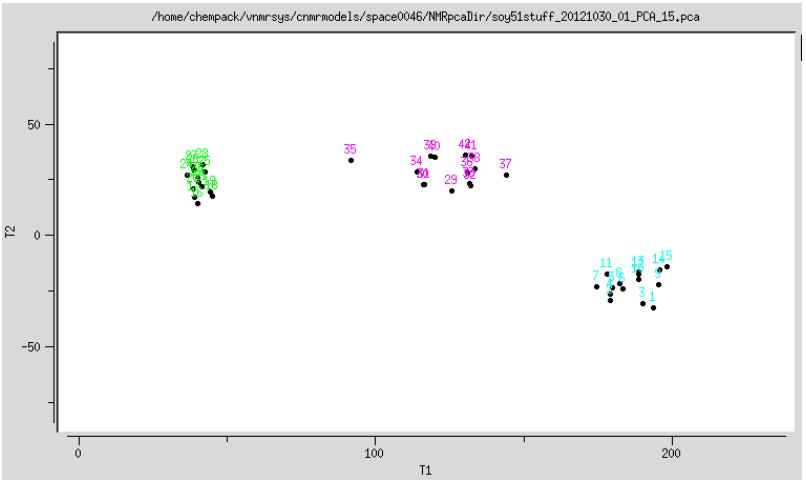
Soy dietary supplement extract + CRAFT
(Avg. CRAFT'ing time: ~1.7 min/spectrum)



CRAFT analysis of the targeted resonances was straightforward and results were exported.

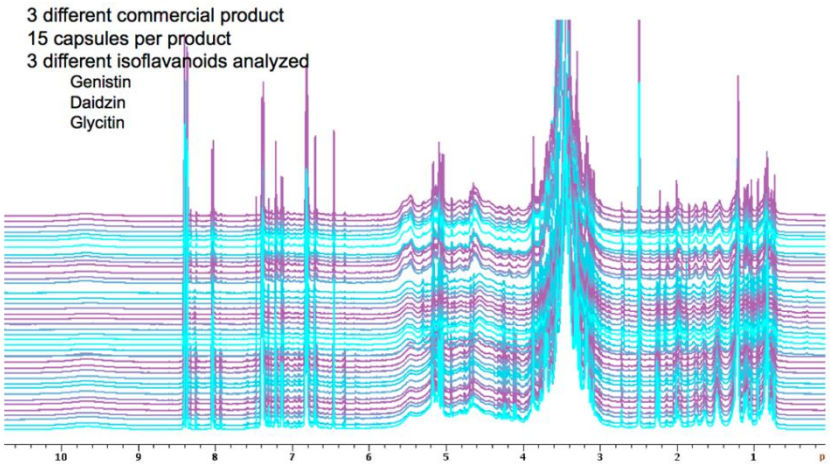
Principal Component Analysis (PCA) readily discriminated between the three different supplements.

Soy supplement CRAFT + MPP (PCA Analysis)

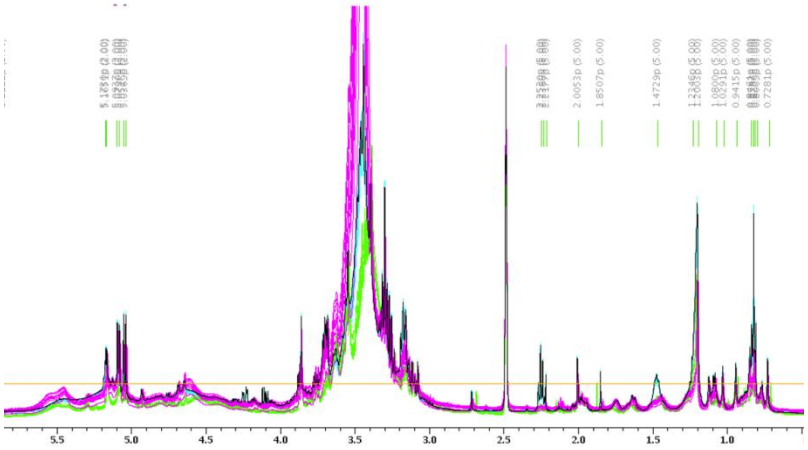


Untargeted Analysis

Soy dietary supplement extract



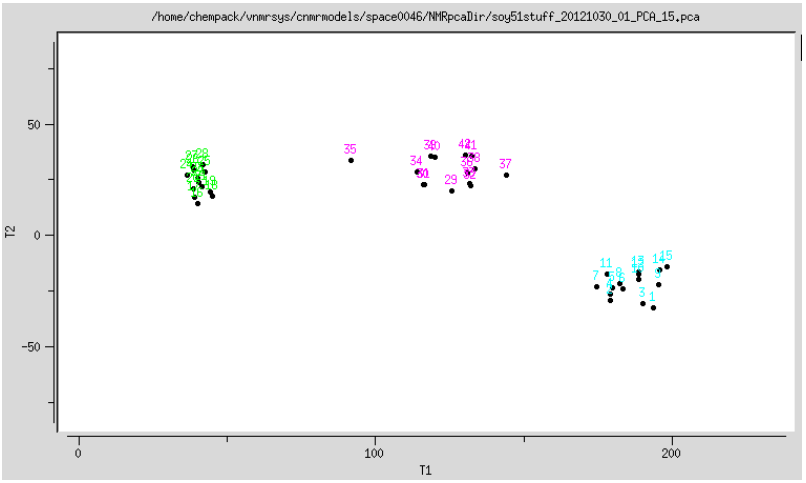
Soy dietary supplement extract + CRAFT
(Avg. CRAFT'ing time: ~1.7 min/spectrum)



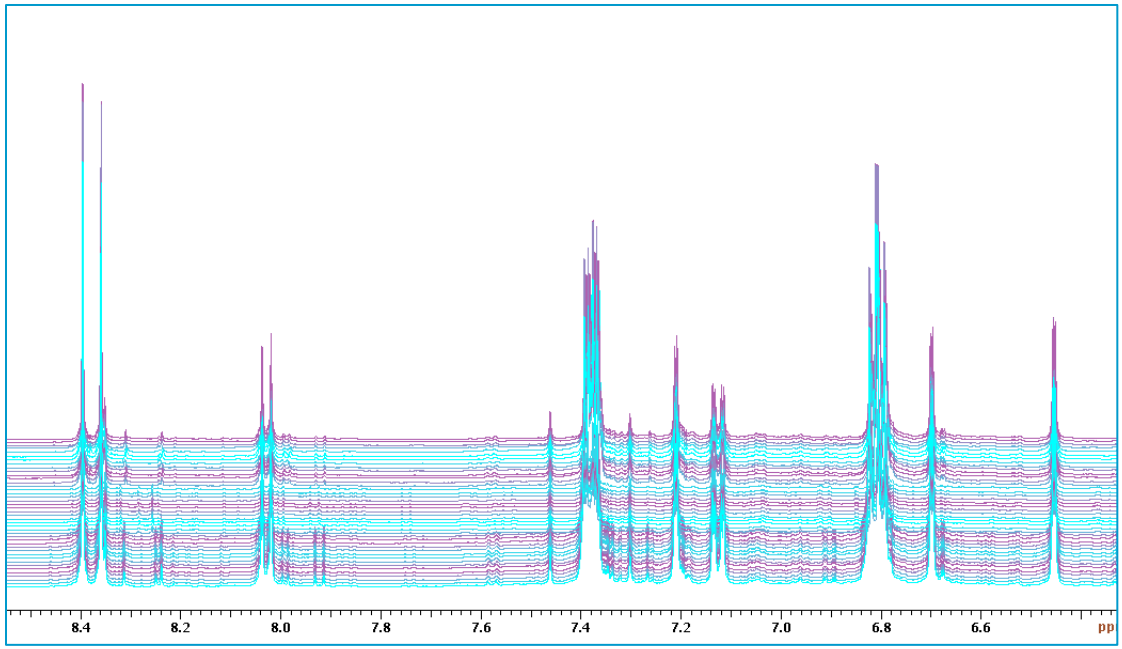
CRAFT analysis was also performed on the non-aromatic resonances, primarily representing lipids, sugars and amino acids. No attempt was made to identify the individual components. Results were exported for analysis.

PCA readily discriminated between the three different supplements.

Soy supplement CRAFT + MPP (PCA Analysis)



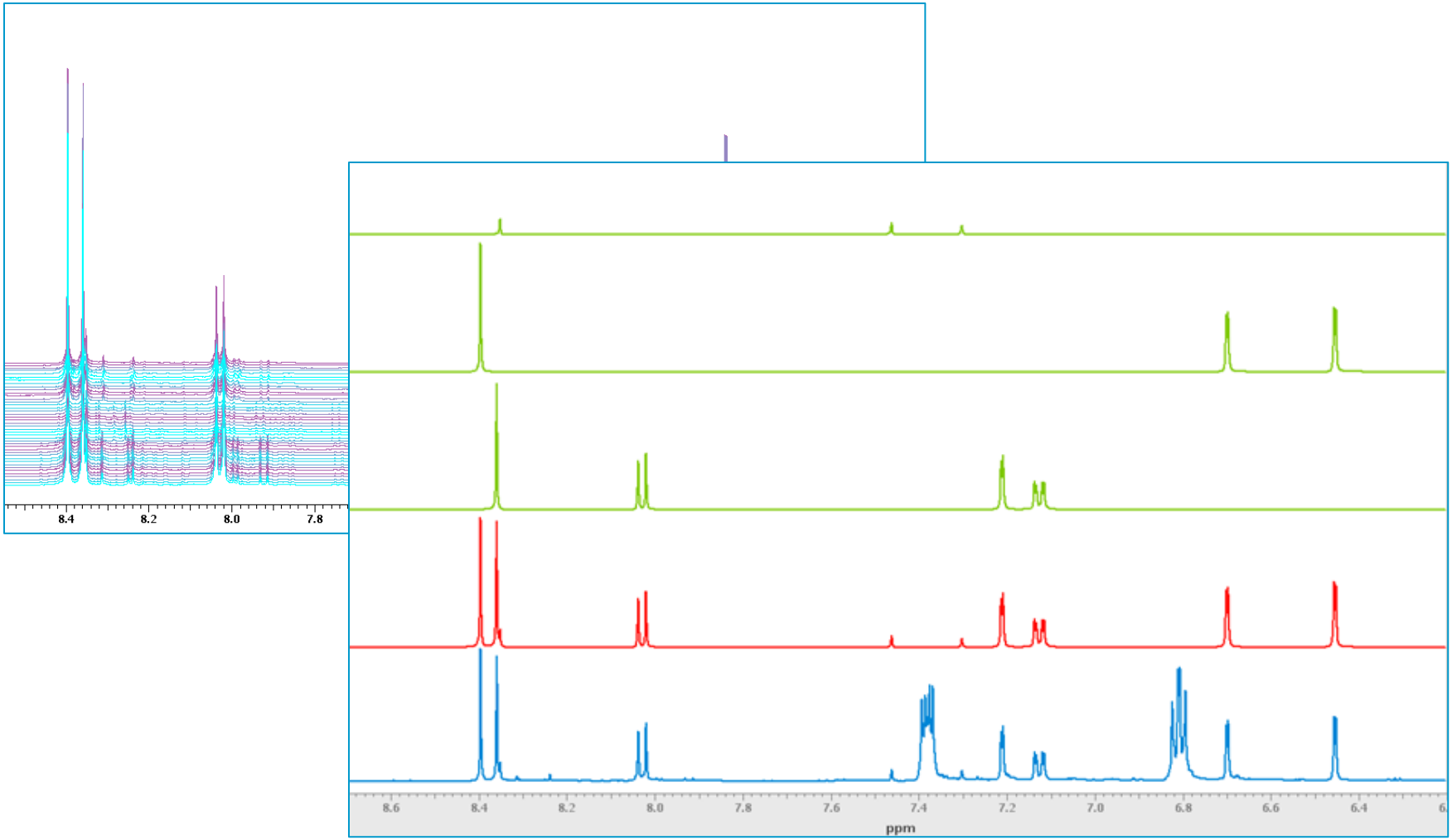
CRAFT – Extract Only the Data You Need



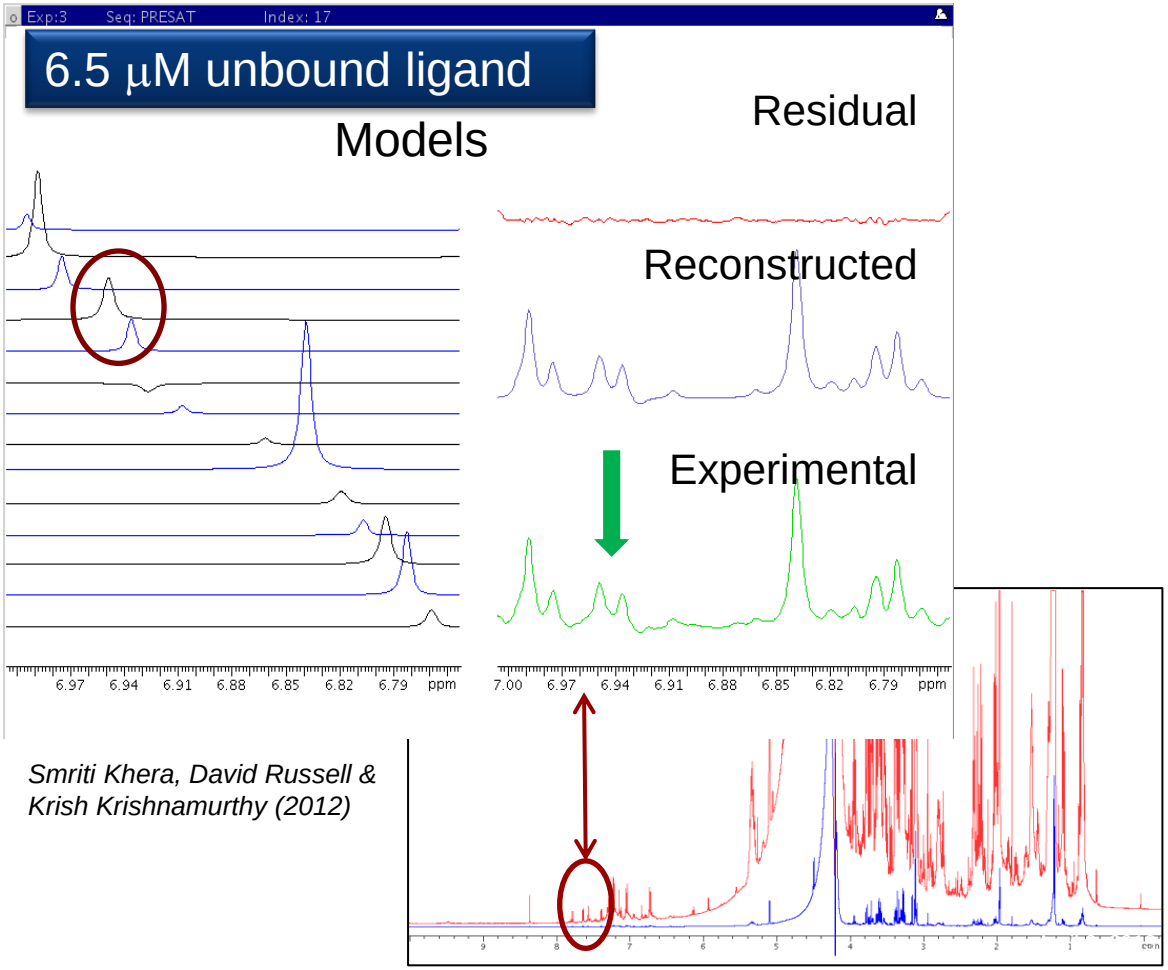
Once a data set has been CRAFT'ed, the CRAFT table can extract useful information from the data set.

For example, those resonances associated with relevant compounds can be used to recreate spectra containing only the peaks of interest.

CRAFT – Extract Only the Data You Need



CRAFT'ing a Needle From a Haystack



Smriti Khera, David Russell & Krish Krishnamurthy (2012)

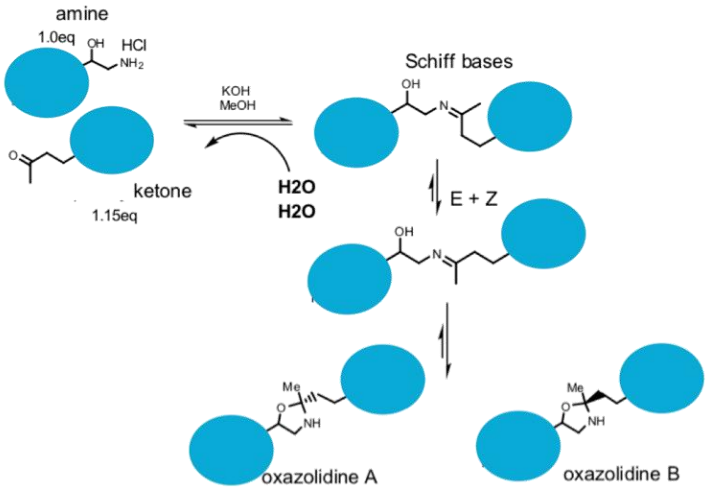
CRAFT can simplify analysis of a very complicated spectrum.

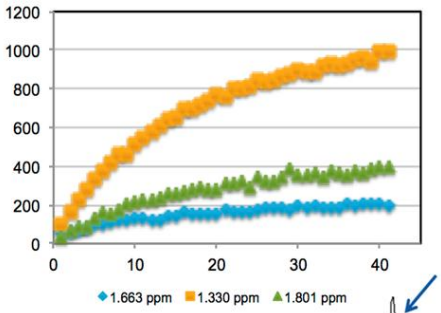
Tetracycline is known to bind to endogenous proteins in plasma. NMR can be used to directly measure the concentration of unbound tetracycline using CRAFT analysis of a fingerprint peak.

Redefining “Overlap”

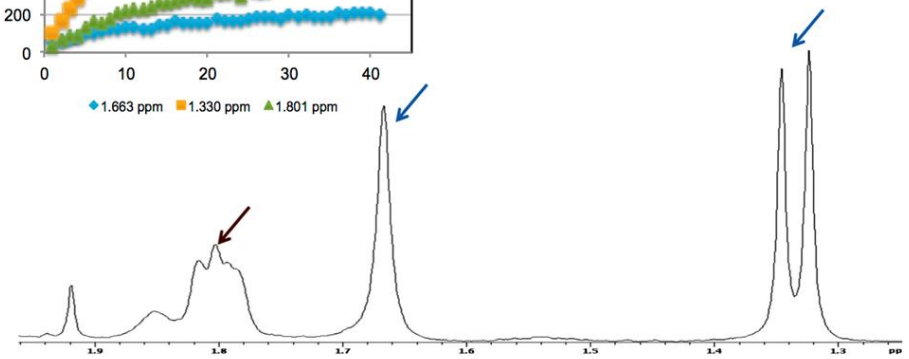
CRAFT analysis is based on time-domain data so peak overlap in the frequency-domain is not an issue. CRAFT was used to extract a peak from a strongly congested region with 100% fidelity.

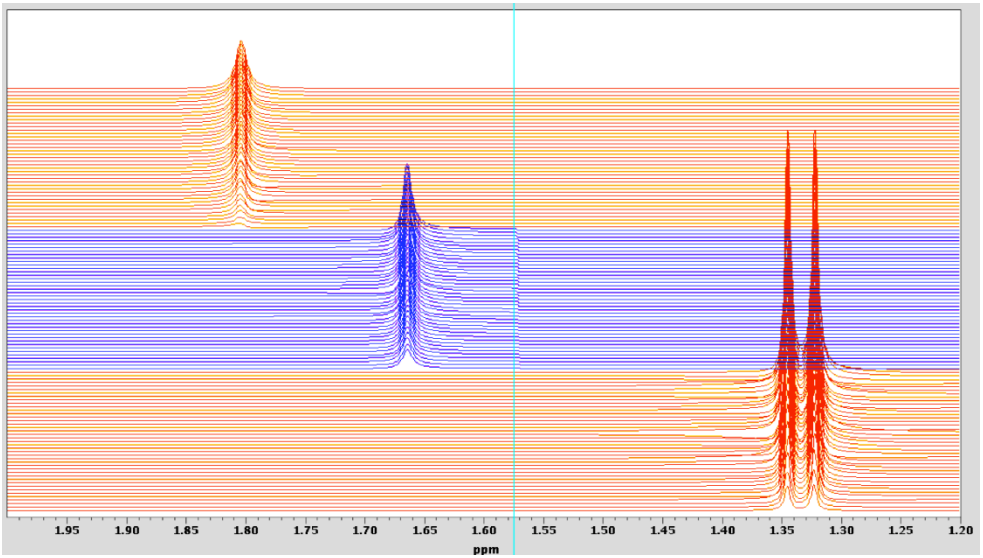
Reaction Monitoring



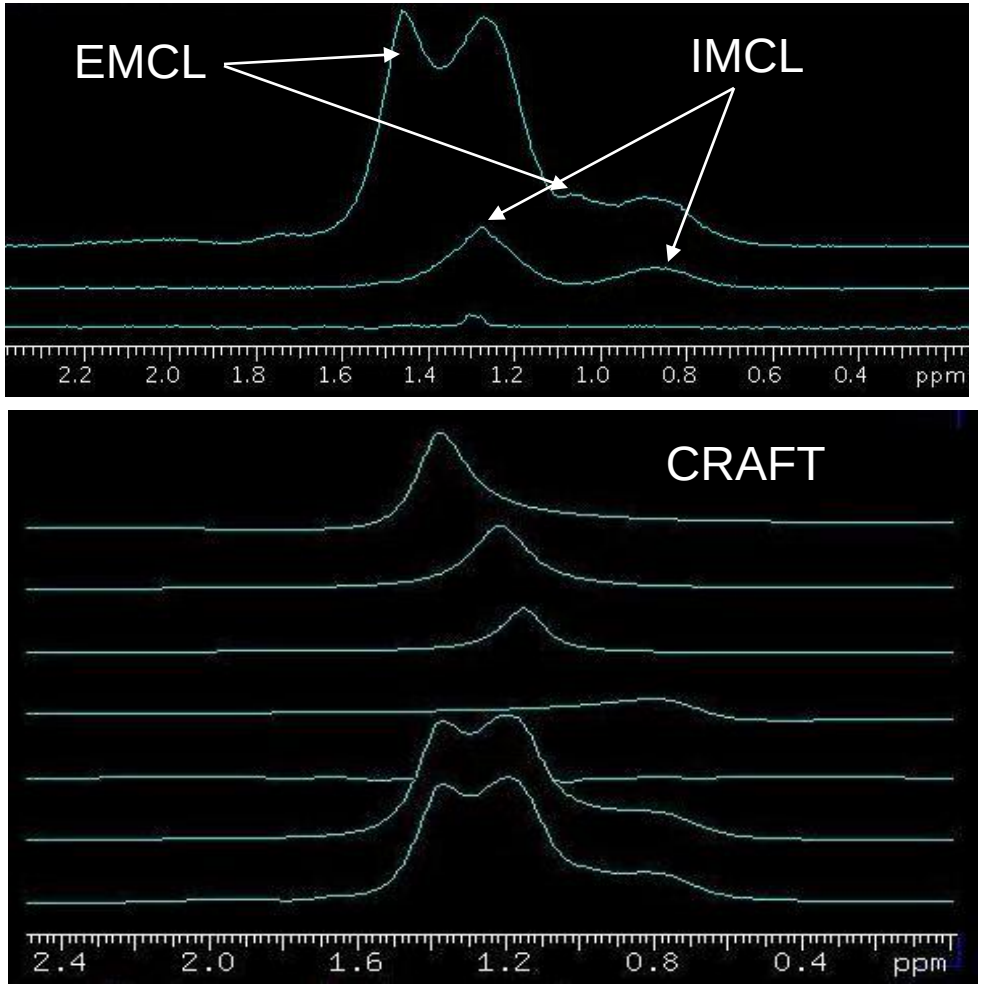


Reaction Monitoring





IMCL vs. EMCL from Lipid Signal



Allen Kline, Krish Krishnamurthy (unpublished)

MRI Chemical Shift Imaging data is often broad, but CRAFT can cleanly extract useful chemical shift and concentration data.

Here, an internal/external mouse muscle cell lipid ratio can be a potential biomarker for muscle atrophy. The ratio would be totally intractable based on standard 1D deconvolution techniques.

Conclusions

- ✓ NMR is a valuable tool for Metabolomics research.
- ✓ CRAFT allows automated reduction of very complex spectra to a spreadsheet result with high fidelity. The requirement for a human to analyze each data set is no longer a research bottleneck.
- ✓ Once converted to a spreadsheet, NMR data can be imported into Agilent's MPP software suite for analysis.
- ✓ The CRAFT technique can provide answers for a wide variety problems.

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