Advanced NMR: Metabolite ID by NMR

UAB Metabolomics Training Course
July 17-21, 2016

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NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

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Outline

- Information that NMR Spectroscopy data can provide
  - Chemical shift, J-coupling, chemical structure
- Available NMR methods
  - 1D NMR: ^{1}H, ^{13}C, ^{15}N, ^{31}P
  - 2D NMR: COSY, TOCSY, HSQC, HMBC, NOESY, INADEQUATE
  - Selective 1D: 1D TOCSY, 1D HSQC
- Spectral editing methods
  - CPMG, Diffusion, JRES, DEPT (DEPT 45, DEPT 90, DEPT 135)
- NMR Libraries, software, and databases
  - AMIX, BBREFCODE (Bruker), BATMAN, Chenomx, COLMAR, HMDB, BMRB, Birmingham Metabolite Library, NMR Shift DB
- Other complementary methods
  - Eg. STOCSY, STORM, RANSY
  - MUMA Package (R Based) for STOCSY and STORM
- Tagging, Spike-in of metabolites, Predicting Spectra

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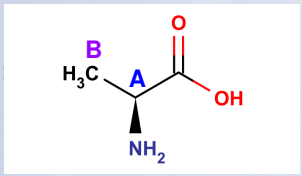
NMR Chemical Shift



Alanine

- Peak position is called "chemical shift"
- It depends on the chemical environment
- Splitting of peak is dependent on neighboring ^1H atom(s)
- Area under peak proportional to the number of ^1H atoms underlying it

Chemical shift of C**H**
4 lines (quadrat)

Chemical shift of CH_3
2 lines (doublet)

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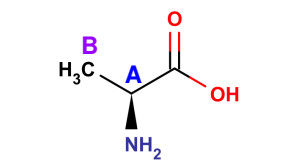
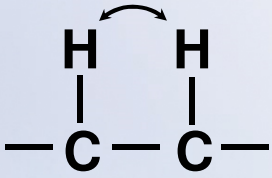
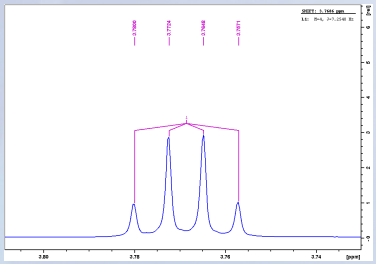
NMR J-Coupling



Alanine



J = 7.2 Hz



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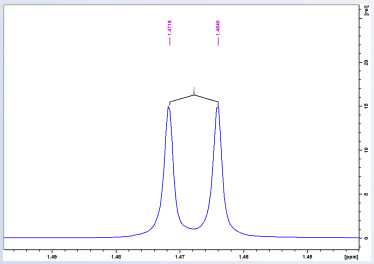
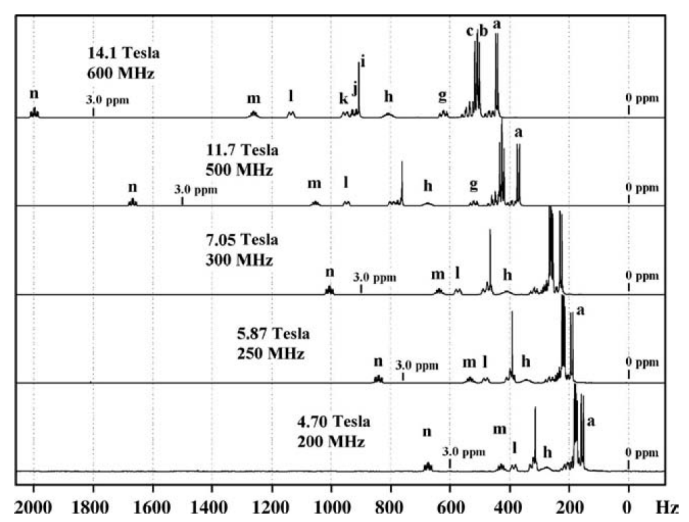


Dispersion of NMR Signal with Magnetic Field Strength



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1D and 2D NMR Methods

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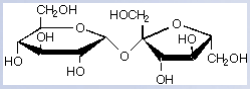
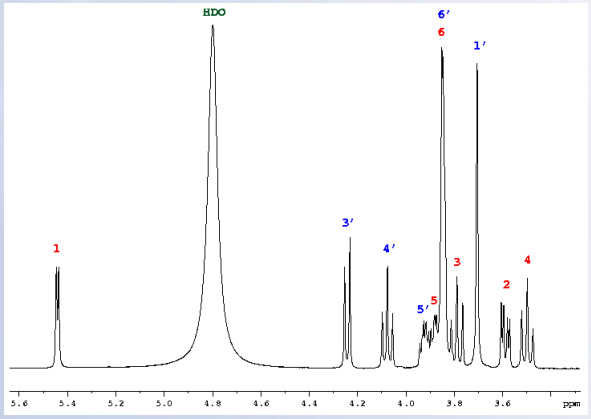
^{1}H NMR: Sucrose





NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

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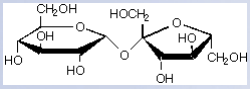
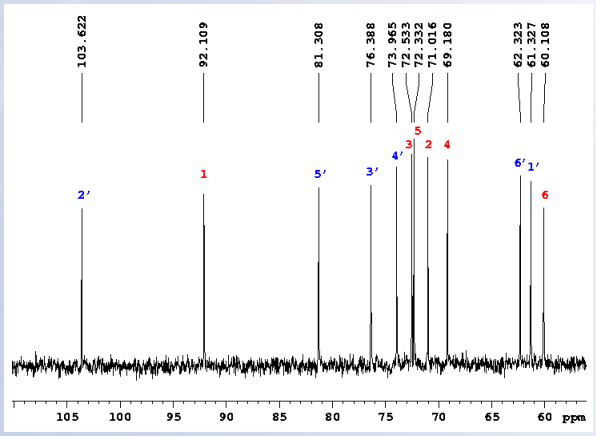
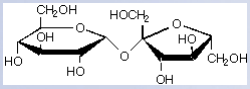
^{13}C NMR: Sucrose

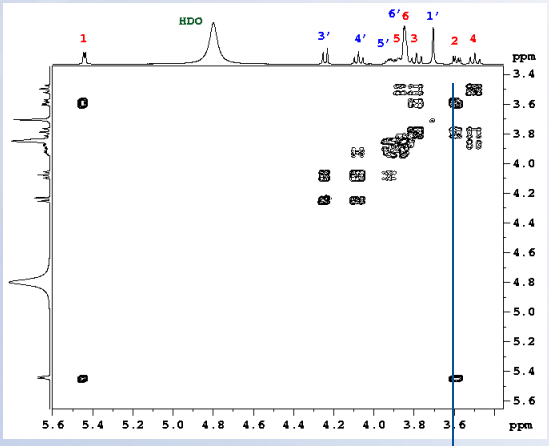




NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin



COSY: Sucrose



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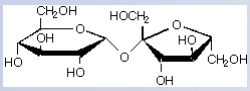
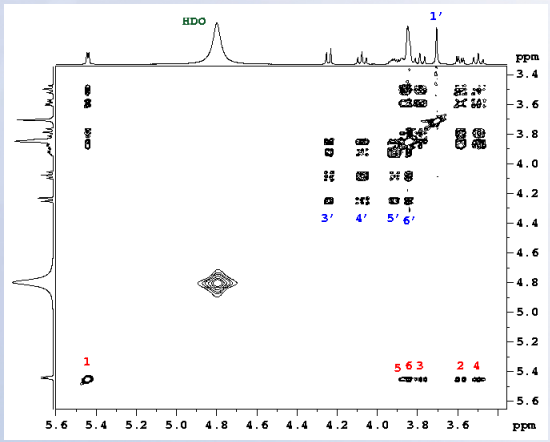


TOCSY: Sucrose



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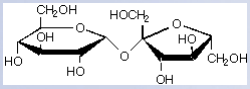
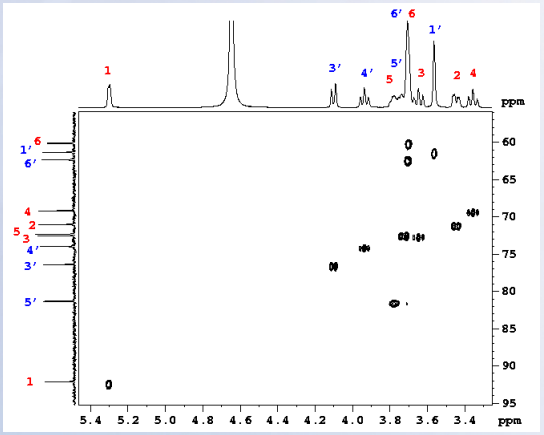
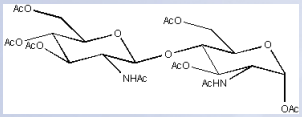
HSQC: Sucrose

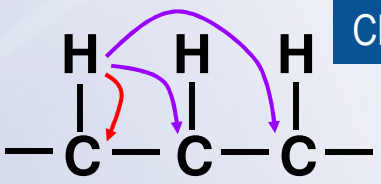


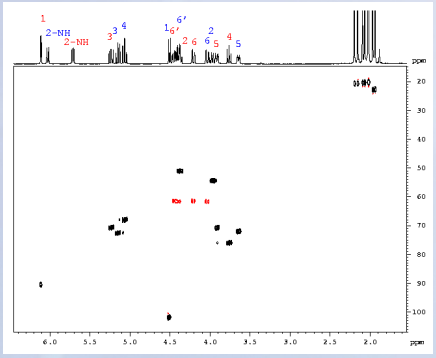


NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

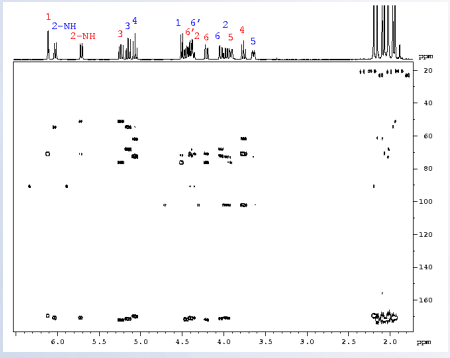
Chentobiose







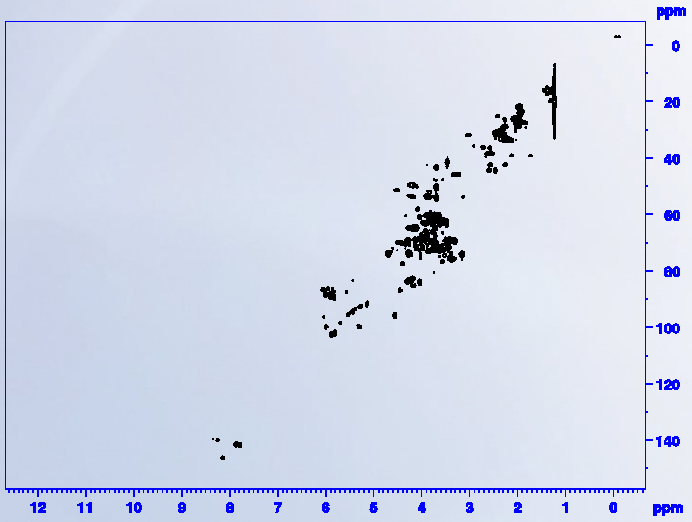
HSQC ($^{1}J_{CH}$)



HMBC ($^{2}J_{CH}$, $^{3}J_{CH}$)

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

^{1}H-^{13}C HSQC Spectrum of Cell Extract



Spectral Editing

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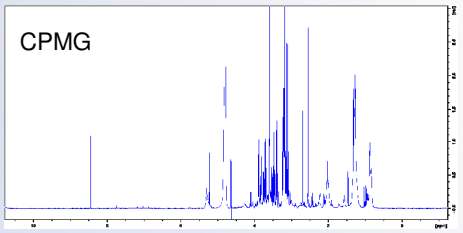
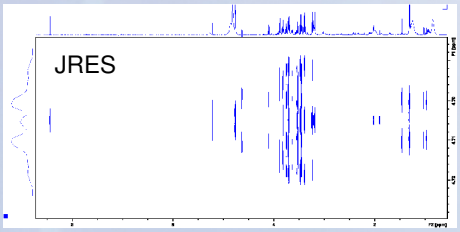
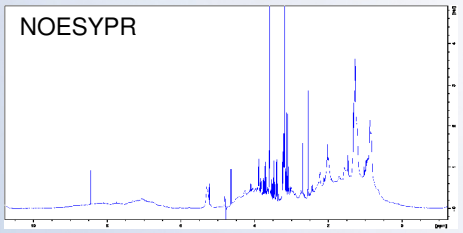
Spectral Editing

- Relaxation editing
  - CPMG Pulse sequence
- J- Modulated
  - J-RES

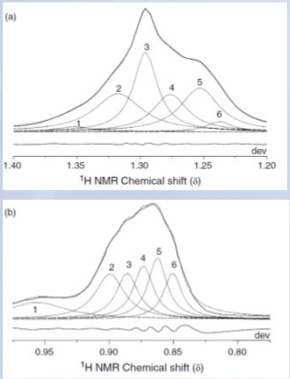


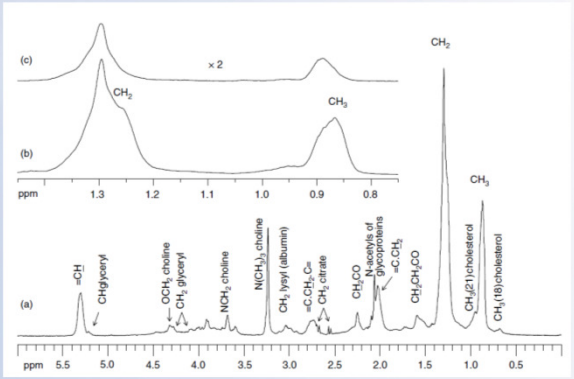




Analysis of Lipoproteins

- Lipoproteins are classified based on their size and density (VLDL, LDL, HDL)
- Lipoproteins can be assigned to these sub fractions by deconvoluting the CH_3 and $(CH_2)_n$ in diffusion edited spectra

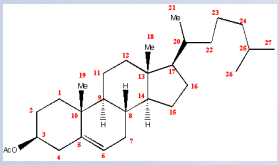


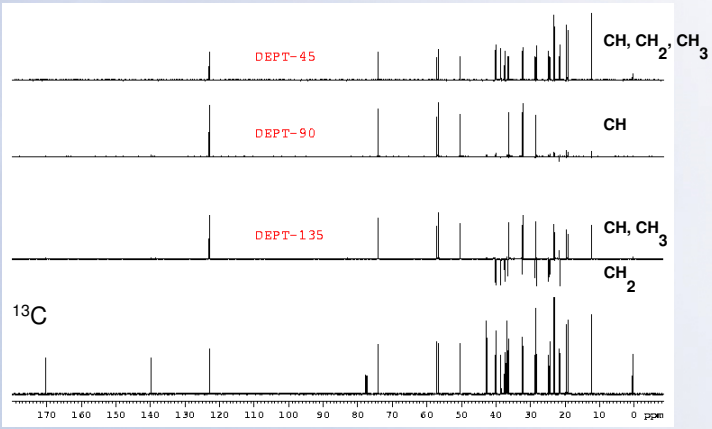


Diffusion Edited NMR Spectra

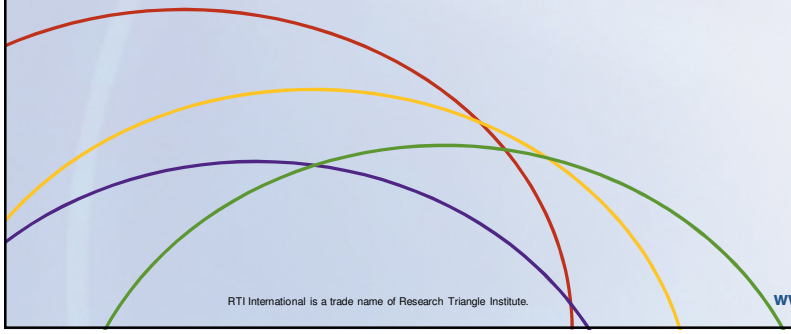
Peak	δ (ppm)	Width (Hz)	D ($cm^2\ s^{-1}$ $\times 10^7$)[a]	Area (%)[b]	Assignment
$(CH_2)_n$ 1	1.353	19.7	2.72	2.3	LDL + VLDL
$(CH_2)_n$ 2	1.317	28.0	1.98	26.4	VLDL
$(CH_2)_n$ 3	1.296	14.0	1.85	27.9	VLDL
$(CH_2)_n$ 4	1.276	17.8	3.15	15.5	LDL
$(CH_2)_n$ 5	1.255	20.5	5.19	19.9	HDL(60.6%) + LDL(39.4%)
$(CH_2)_n$ 6	1.240	18.4	5.96	7.9	HDL
CH_3 1	0.956	33.9	3.77	15.8	VLDL + HDL
CH_3 2	0.899	16.4	1.70	20.6	VLDL
CH_3 3	0.886	12.4	1.84	15.9	VLDL
CH_3 4	0.873	11.3	3.07	16.7	LDL
CH_3 5	0.862	10.5	4.51	17.9	HDL(40.8%) + LDL(59.2%)
CH_3 6	0.851	10.2	7.11	13.1	HDL

DEPT: Cholesterol





NMR Libraries, Software, and Databases



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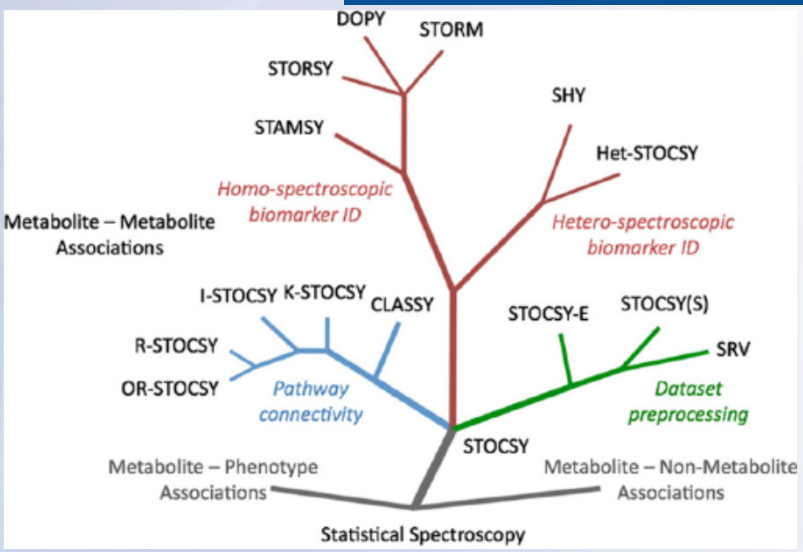
NMR Libraries and Databases

- Continuously emerging
- Databases
  - HMDB (http://www.hmdb.ca/)
  - Birmingham Metabolite Library (http://www.bml-nmr.org/)
  - BMRB (http://www.bmrb.wisc.edu/)
  - NMRShift DB (http://nmrshiftdb.nmr.uni-koeln.de/)
- Online Software
  - COLMAR (http://spin.ccic.ohio-state.edu/)
- Standalone Software
  - Chenomx (http://www.chenomx.com/
  - AMIX/ ASSURE/BBREFCODE
    https://www.bruker.com/products/mr/nmr/nmr-software/software/amix/overview.html
  - BATMAN (http://batman.r-forge.r-project.org/)
  - CCPN Metabolomics (http://www.ccpn.ac.uk/collaborations/metabolomics)
  - rNMR (link)

Other Complementary methods

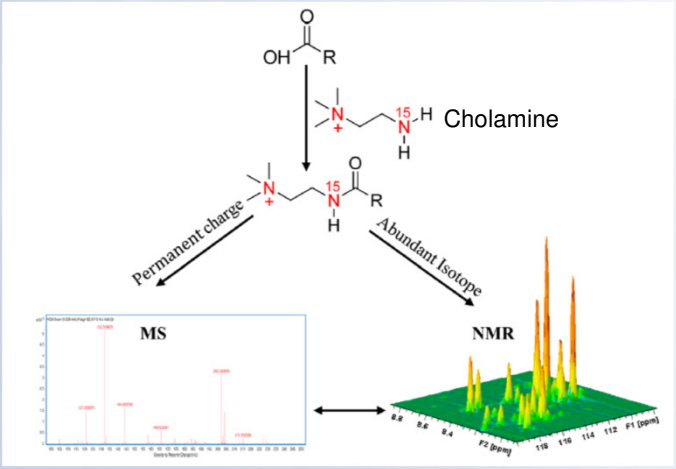
Statistical Spectroscopic Tools



Robinette, S. L., J. C. Lindon and J. K. Nicholson (2013). "Statistical spectroscopic tools for biomarker discovery and systems medicine." Anal Chem 85(11): 5297-5303.

Tagging

Smart Tagging

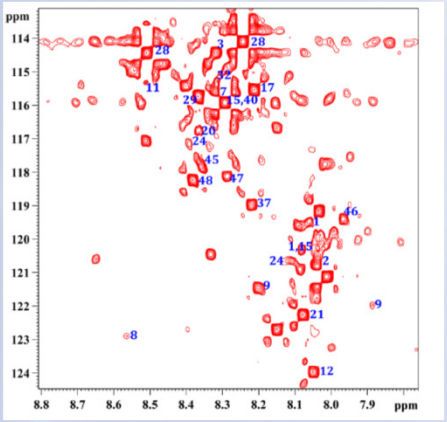


Tayyari, F., G. A. Gowda, H. Gu and D. Raftery (2013). "15N-cholamine--a smart isotope tag for combining NMR- and MS-based metabolite profiling." Anal Chem **85**(18): 8715-8721

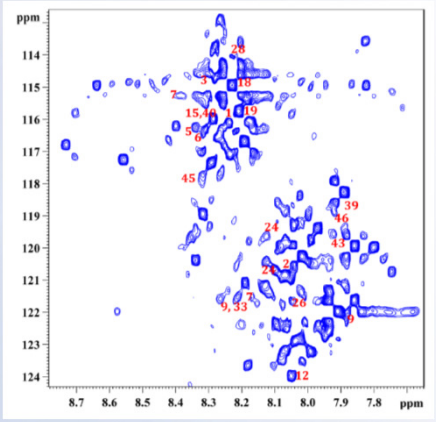
Smart Tagging

^{1}H-^{15}N HSQC

Human Serum



Human Urine



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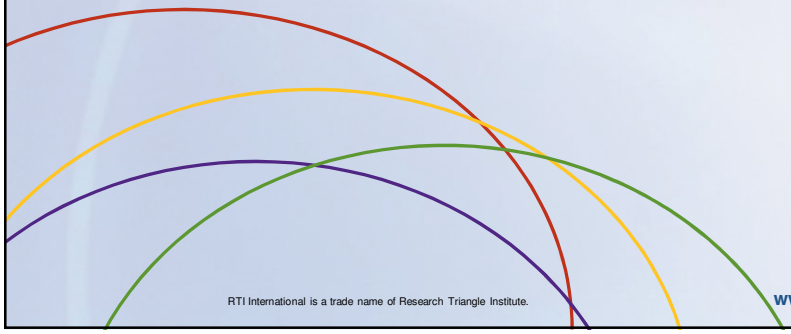
References

- Beckonert, O., H. C. Keun, T. M. Ebbels, J. Bundy, E. Holmes, J. C. Lindon and J. K. Nicholson (2007). "Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts." Nat Protoc 2(11): 2692-2703.
- Cloarec, O., M. E. Dumas, J. Trygg, A. Craig, R. H. Barton, J. C. Lindon, J. K. Nicholson and E. Holmes (2005). "Evaluation of the Orthogonal Projection on Latent Structure Model Limitations Caused by Chemical Shift Variability and Improved Visualization of Biomarker Changes in1H NMR Spectroscopic Metabonomic Studies." Analytical Chemistry **77**(2): 517-526.
- Lindon, J. C. and J. K. Nicholson (2008). "Spectroscopic and Statistical Techniques for Information Recovery in Metabonomics and Metabolomics." Annual Review of Analytical Chemistry 1(1): 45-69.
- Posma, J. M., I. Garcia-Perez, M. De Iorio, J. C. Lindon, P. Elliott, E. Holmes, T. M. Ebbels and J. K. Nicholson (2012). "Subset optimization by reference matching (STORM): an optimized statistical approach for recovery of metabolic biomarker structural information from 1H NMR spectra of biofluids." Anal Chem 84(24): 10694-10701.
- Robinette, S. L., J. C. Lindon and J. K. Nicholson (2013). "Statistical spectroscopic tools for biomarker discovery and systems medicine." Anal Chem 85(11): 5297-5303.
- Sands, C. J., M. Coen, T. M. Ebbels, E. Holmes, J. C. Lindon and J. K. Nicholson (2011). "Data-driven approach for metabolite relationship recovery in biological 1H NMR data sets using iterative statistical total correlation spectroscopy." Anal Chem 83(6): 2075-2082.
- Wei, S., J. Zhang, L. Liu, T. Ye, G. A. Gowda, F. Tayyari and D. Raftery (2011). "Ratio analysis nuclear magnetic resonance spectroscopy for selective metabolite identification in complex samples." Anal Chem 83(20): 7616-7623.
- Tayyari, F., G. A. Gowda, H. Gu and D. Raftery (2013). "15N-cholamine--a smart isotope tag for combining NMR- and MS-based metabolite profiling." Anal Chem **85**(18): 8715-8721.

Chenomx Exercise



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Chenomx Library

- Over 320 metabolites
- pH sensitive library of 1H NMR Spectra
- Customizable

1,3-Dihydroxyacetone, 1,3-Dimethylurate, 1,6-Anhydro-β-D-glucose, 1,7-Dimethylxanthine, 1-Methylnicotinamide, 2'-Deoxyadenosine, 2'-Deoxyguanosine, 2'-Deoxyinosine, 2-Aminoadipate, 2-Aminobutyrate, 2-Ethylacrylate, 2-Furoate, 2-Hydroxy-3-methylvalerate, 2-Hydroxybutyrate, 2-Hydroxyglutarate, 2-Hydroxyisobutyrate, 2-Hydroxyisocaproate, 2-Hydroxyisovalerate, 2-Hydroxyphenylacetate, 2-Hydroxyvalerate, 2-Methylglutarate, 2-Octenoate, 2-Oxobutyrate, 2-Oxocaproate, 2-Oxoglutarate, 2-Oxoisovalerate, 2-Phosphoglycerate, 3,4-Dihydroxymandelate, 3,5-Dibromotyrosine, 3-Aminoisobutyrate, 3-Dehydrolysine, 3-Hydroxy-3-methylglutarate, 3-Hydroxybutyrate, 3-Hydroxyisovalerate, 3-Hydroxymandelate, 3-Hydroxyphenylacetate, 3-Indoxylsulfate, 3-Methyl-2-oxovalerate, 3-Methyladipate, 3-Methylxanthine, 3-Phenyllactate, 3-Phenylpropionate, 4-Aminobutyrate, 4-Aminohippurate, 4-Hydroxy-3-methoxymandelate, 4-Hydroxybenzaldehyde, 4-Hydroxybenzoate, 4-Hydroxyhippurate, 4-Hydroxyphenyllactate, 4-Pyridoxate, 5,6-Dihydrothymine, 5,6-Dihydrouracil, 5-Aminolevulinate, 5-Hydroxyindole-3-acetate, 5-Hydroxylysine, 5-Methoxysalicylate, Acetaldehyde, Acetamide, Acetaminophen, Acetate, Acetoacetate, Acetone, Acetylsalicylate, Adenine, Adenosine, Adipate, Alanine, Allantoin, Alloisoleucine, Anserine, Arginine, Argininosuccinate, Asparagine, Aspartate, Benzoate, Betaine, Bilirubin, Caffeine, Caprate, Caprylate, Carnitine, Carnosine, Choline, Cinnamate, Citrate, Citrulline, Creatine, Creatinine, Cysteine, Cystine, Cytidine, Cytosine, DSS (Chemical Shift Indicator), Dimethylamine, Epicatechin, Ethanol, Ethanolamine, Ethylene glycol, Ethylmalonate, Ferulate, Formate, Fructose, Fucose, Fumarate, Galactarate, Galactitol, Galactonate, Galactose, Gentisate, Glucarate, Glucose, Glutamate, Glutamine, Glutarate, Glutaric acid monomethyl ester, Glutathione, Glycerate, Glycerol, Glycine, Glycolate, Glycylproline, Guanidoacetate, Guanine, Hippurate, Histidine, Homocitrulline, Homocystine, Homogentisate, Homoserine, Homovanillate, Hypoxanthine, Ibuprofen, Imidazole, Indole-3-acetate, Inosine, Isobutyrate, Isocaproate, Isocitrate, Isoleucine, Isopropanol, Isovalerate, Kynurenate, Kynurenine, Lactate, Lactose, Leucine, Levulinate, Lysine, Malate, Maleate, Malonate, Mannitol, Mannose, Methanol, Methionine, Methylamine, Methylguanidine, Methylmalonate, Methylsuccinate, N,N-Dimethylformamide, N,N-Dimethylglycine, N-Acetylaspartate, N-Acetylglutamate, N-Acetylglycine, N-Carbamoyl-β-alanine, N-Carbamoylaspartate, N-Isovaleroylglycine, NAD+, Niacinamide, Nicotinate, O-Acetylcarnitine, O-Phosphocholine, O-Phosphoethanolamine, O-Phosphoserine, Ornithine, Oxalacetate, Oxypurinol, Pantothenate, Phenol, Phenylacetate, Phenylacetylglycine, Phenylalanine, Pimelate, Proline, Propionate, Propylene glycol, Protocatechuate, Pyridoxine, Pyroglutamate, Pyruvate, Quinolinate, Riboflavin, Ribose, S-Adenosylhomocysteine, S-Sulfocysteine, Salicylate, Salicylurate, Sarcosine, Serine, Suberate, Succinate, Succinylacetone, Sucrose, Tartrate, Taurine, Theophylline, Threonate, Threonine, Thymine, Thymol, Tiglylglycine, Trigonelline, Trimethylamine, Trimethylamine N-oxide, Tryptophan, Tyramine, Tyrosine, Uracil, Urea, Uridine, Urocanate, Valerate, Valine, Valproate, Vanillate, Xanthine, Xanthosine, Xylose, cis-Aconitate, myo-Inositol, o-Cresol, p-Cresol, trans-4-Hydroxy-L-proline, trans-Aconitate, β-Alanine, π-Methylhistidine, τ-Methylhistidine

Chenomx Exercise

- Save the folder called “Chenomx_Tutorial.zip into your computer
  - Sample files
  - Chenomx NMR Suite Tutorial.pdf
- We will use Processor and Profiler in the exercise
- Processor
  - Sample.fid
- Profiler
  - Basic Start, Basic End
  - Advanced Start, Advanced End
  - Batch fitting

STS Center

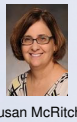

Susan Sumner


Wimal Pathmasiri
NMR & GC-MS


Jim Carlson
LC- and GC-MS


Jessica Gooding
LC-MS


Kelly Mercier
NMR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LC-MS/MS


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Biology and QC


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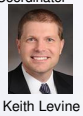
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In vivo and in vitro
Metabolism


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Neurotranmitter
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Systems


Puvi Patel
In vitro metabolsm


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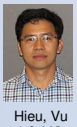
Sue Clark
Administrative
Support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