NMR Training: Metabolite ID

UAB Metabolomics Training Course
July 17-21, 2017

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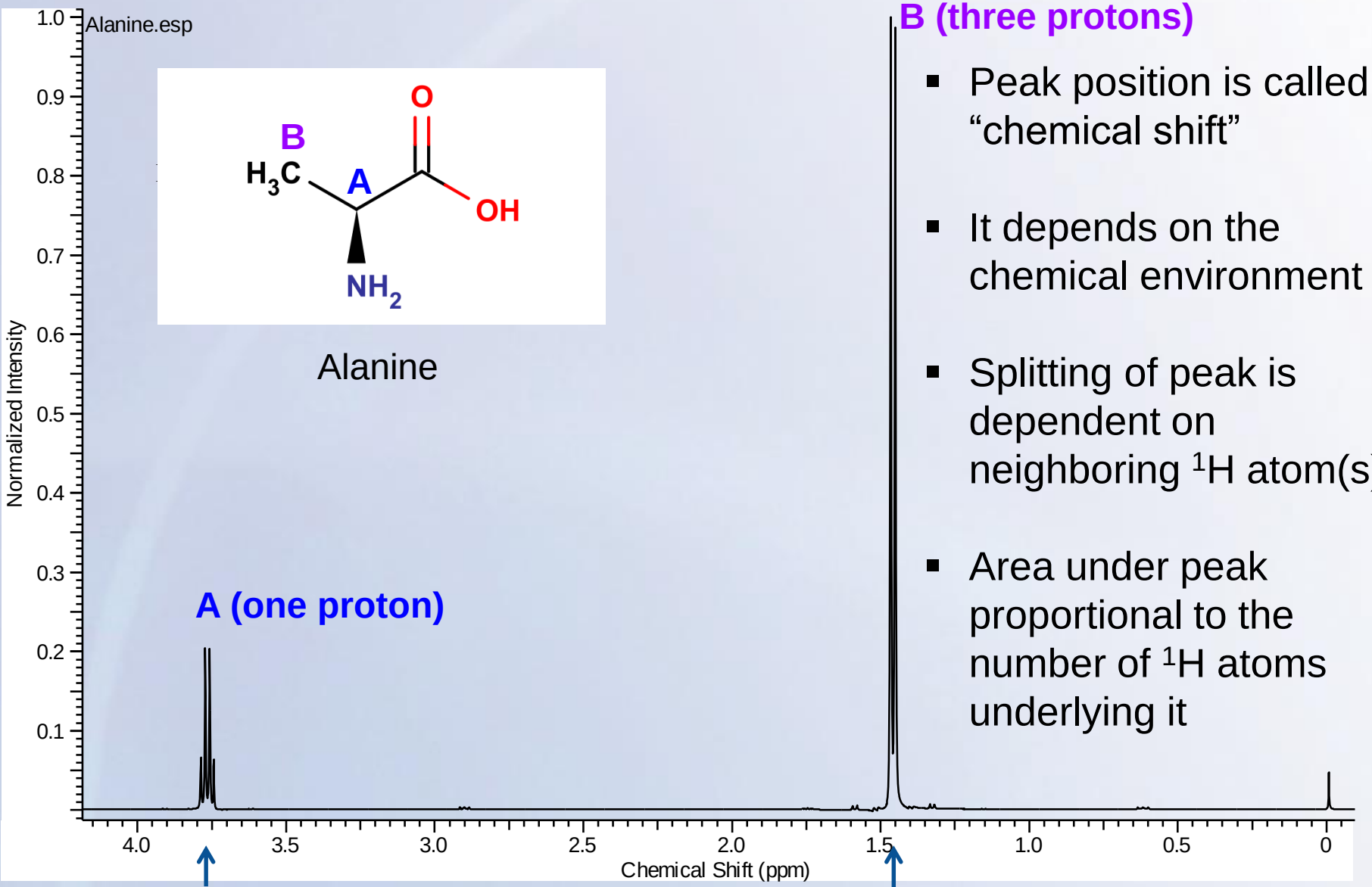
Department of Nutrition – Nutrition Research Institute
University of North Carolina at Chapel Hill

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

NMR Chemical Shift



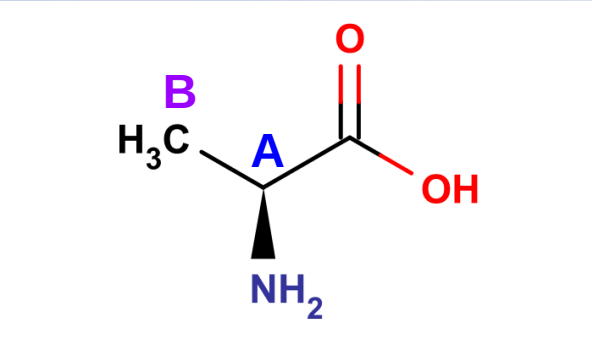
Alanine

- 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

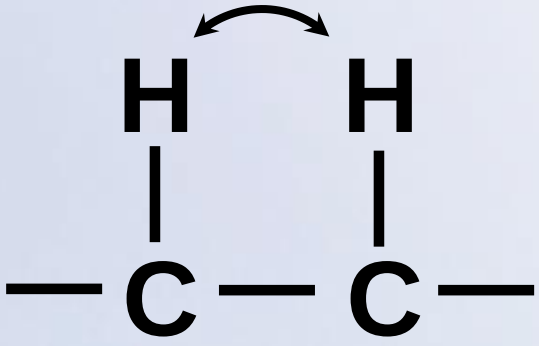
Chemical shift of C**H**
4 lines (quadrat)

Chemical shift of C**H_3**
2 lines (doublet)

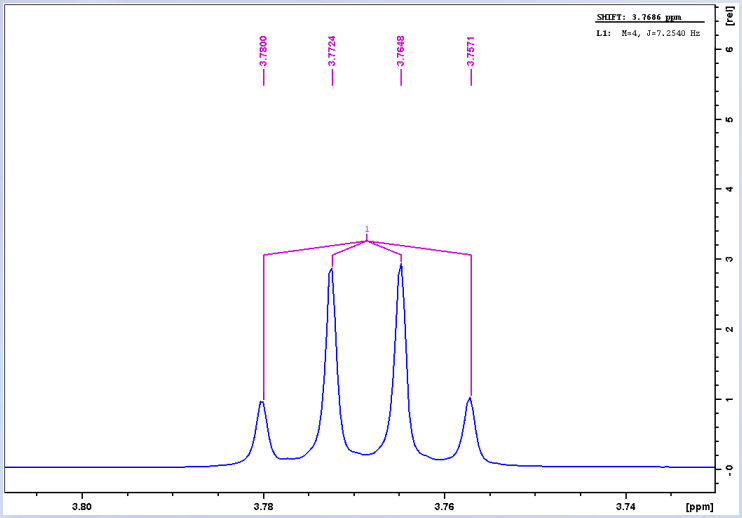
NMR J-Coupling



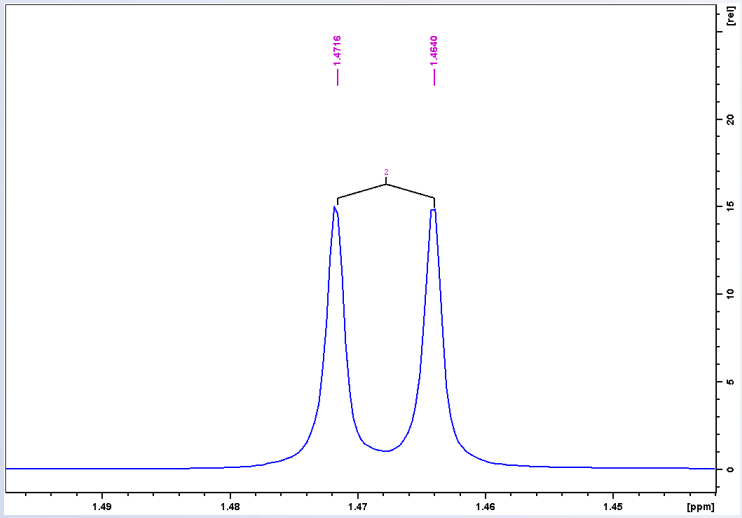
Alanine



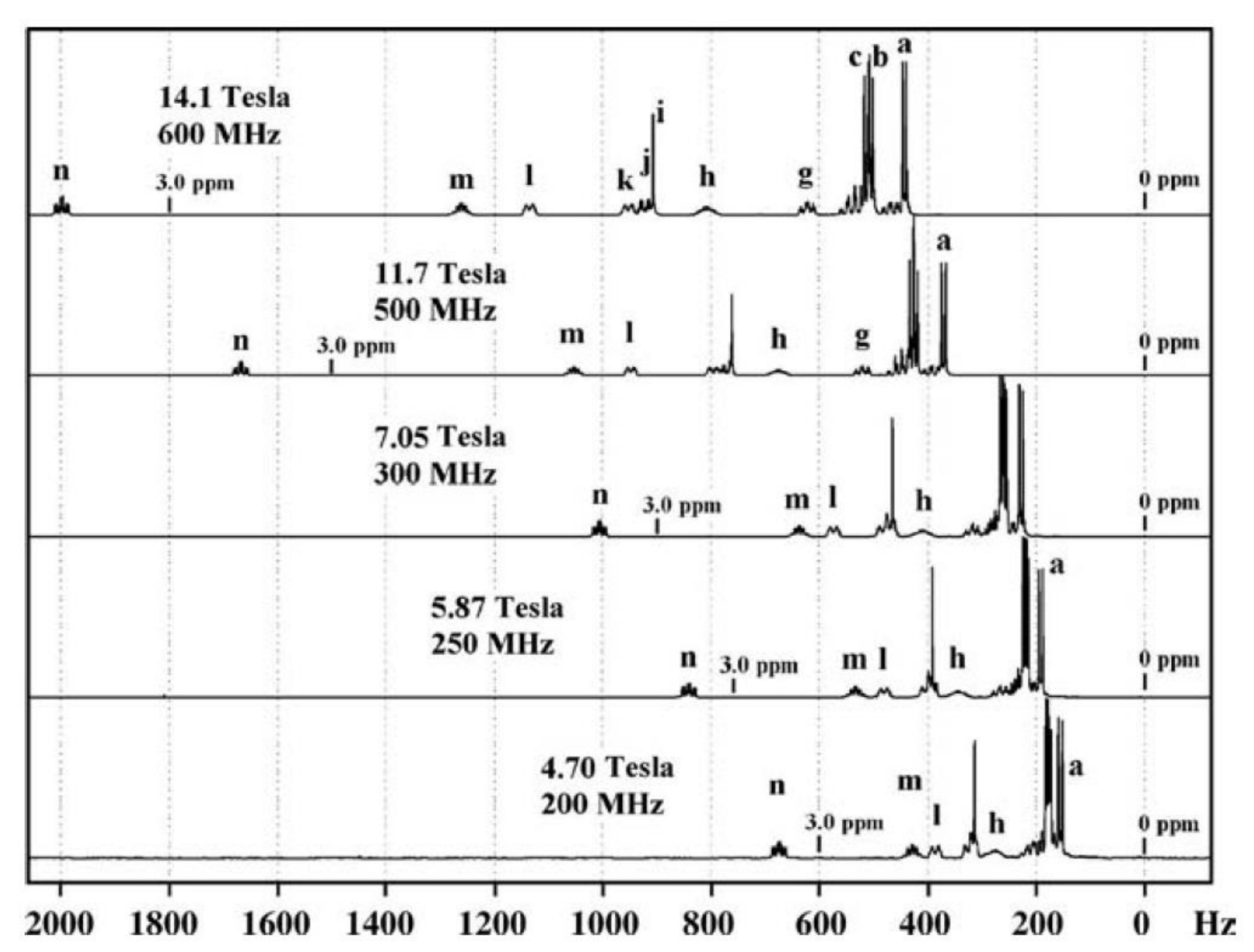
J = 7.2 Hz



J = 7.2 Hz

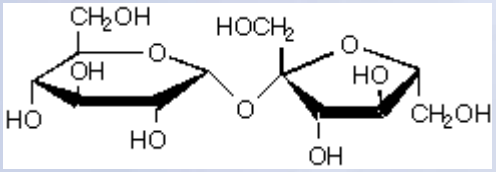


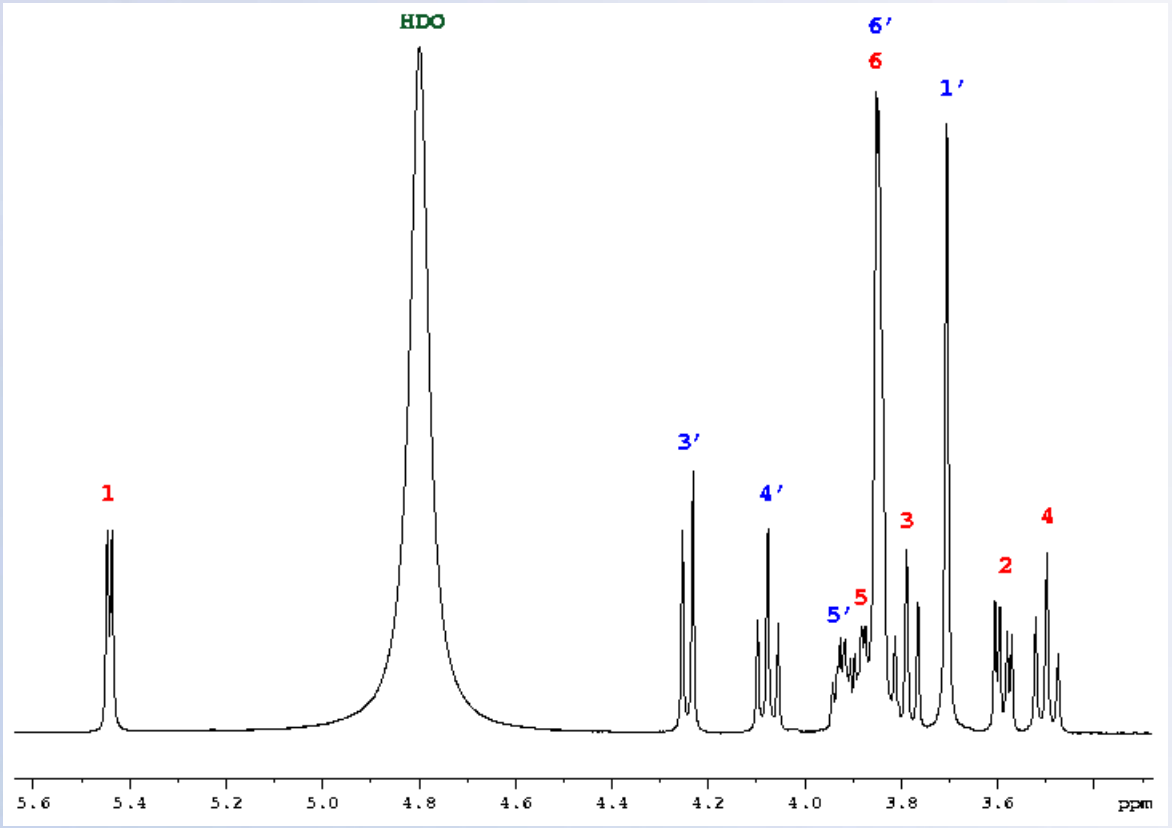
Dispersion of NMR Signal with Magnetic Field Strength



1D and 2D NMR Methods

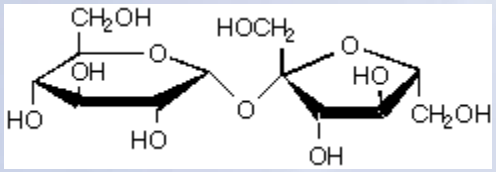
^{1}H NMR: Sucrose

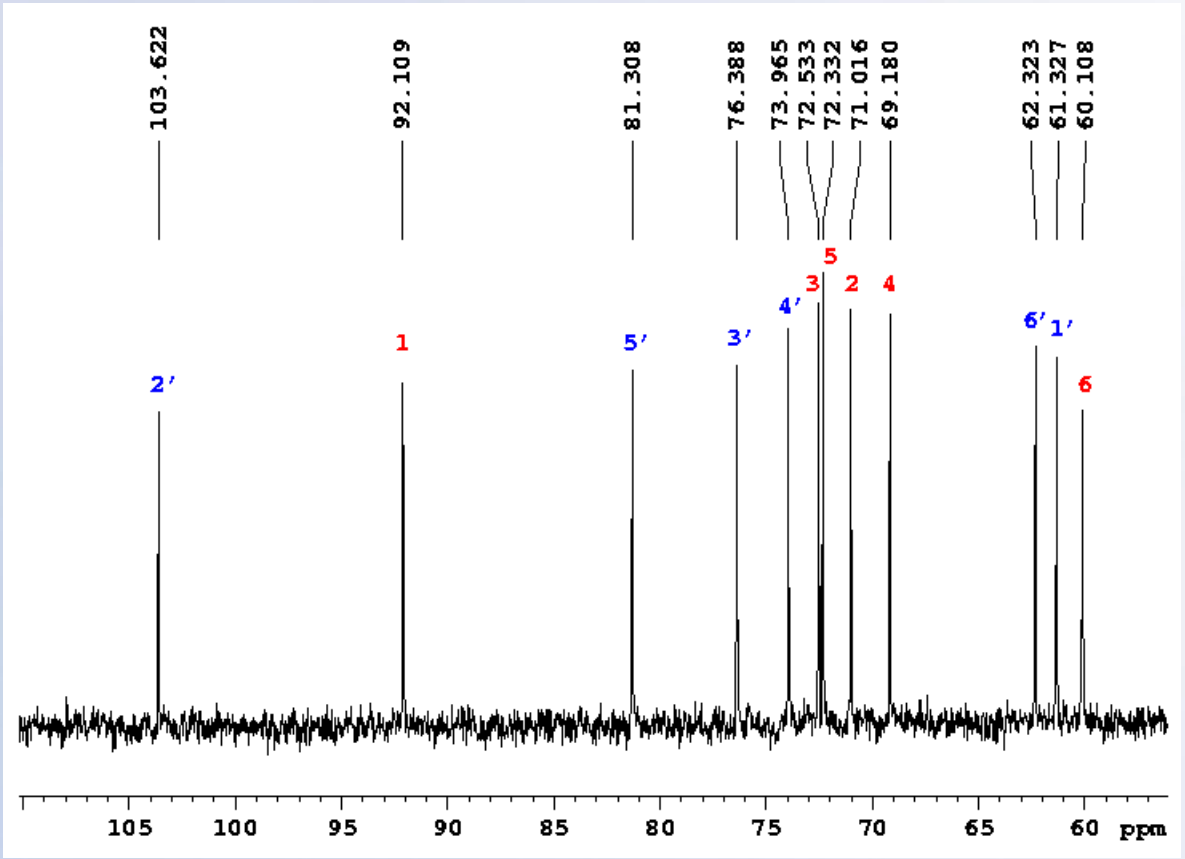




NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

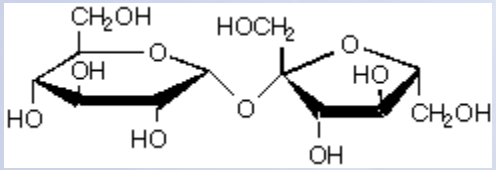
^{13}C NMR: Sucrose

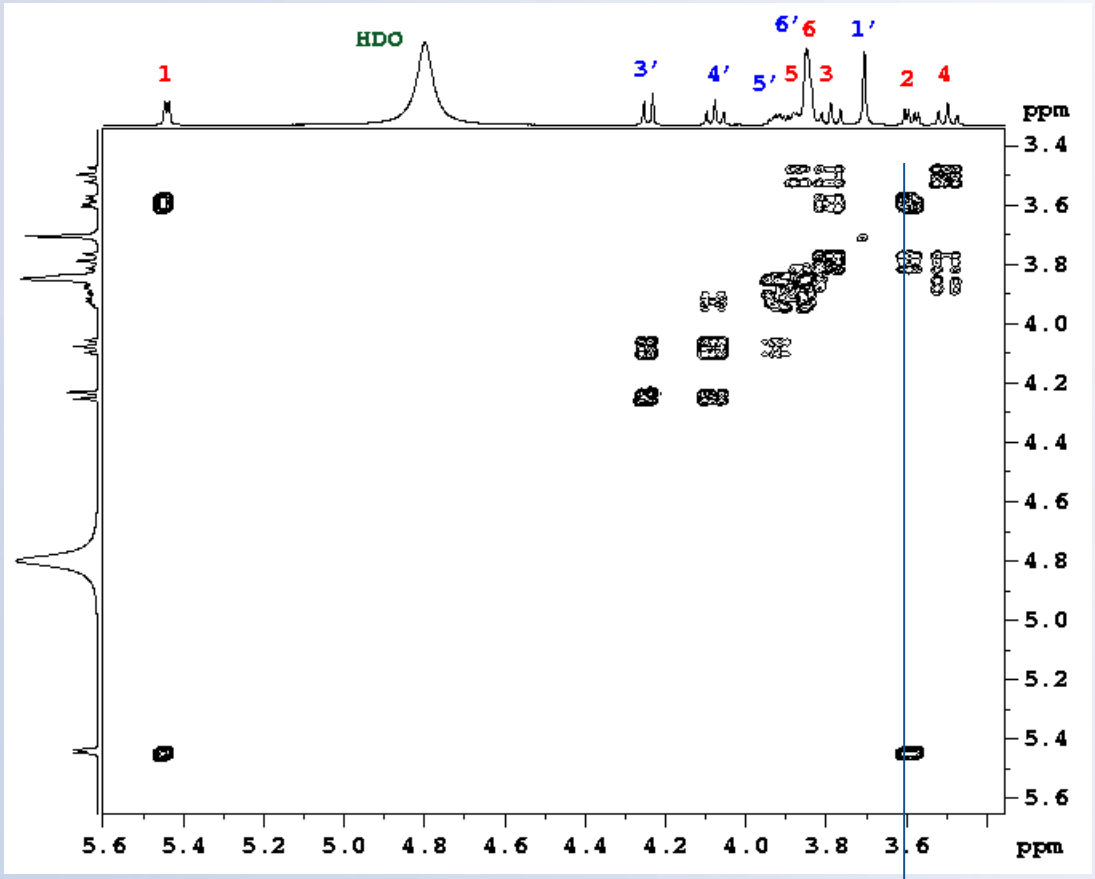




NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

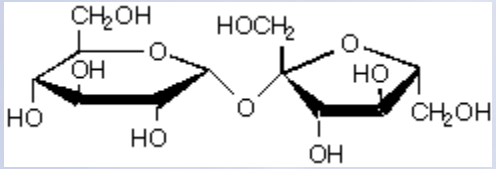
COSY: Sucrose

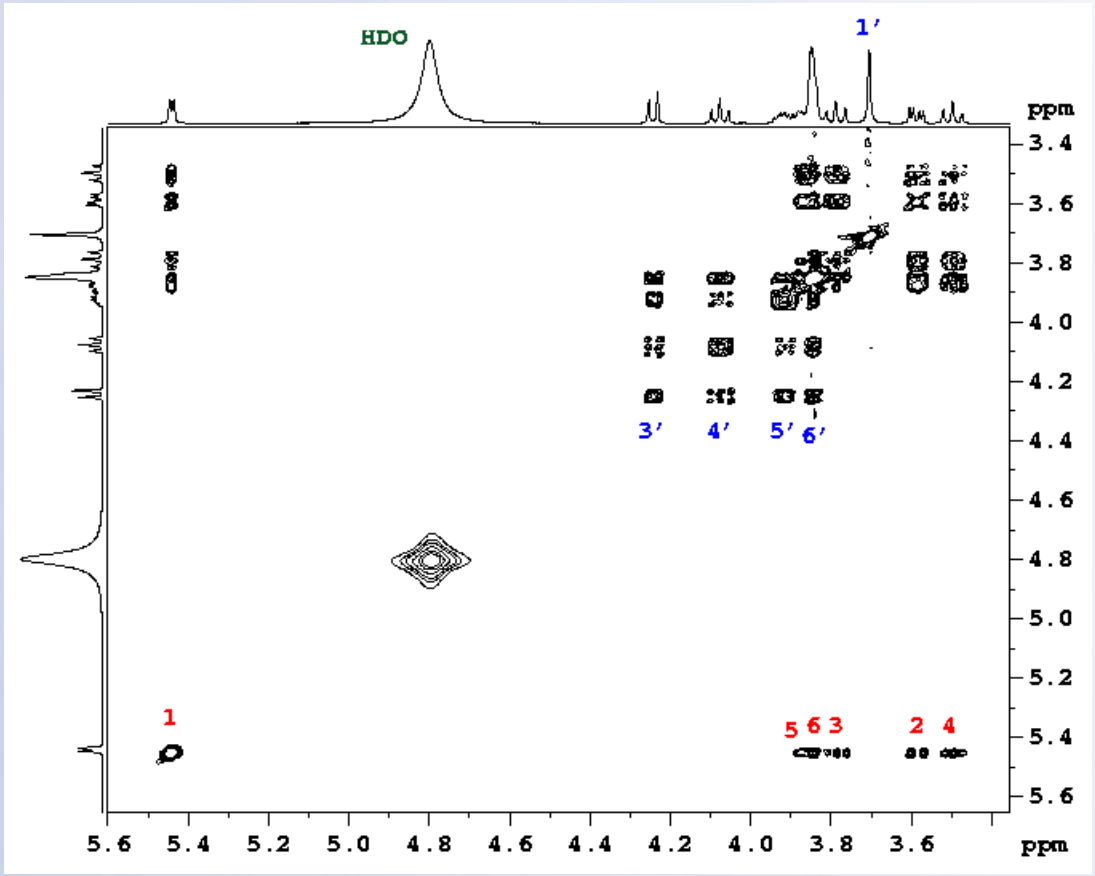




NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

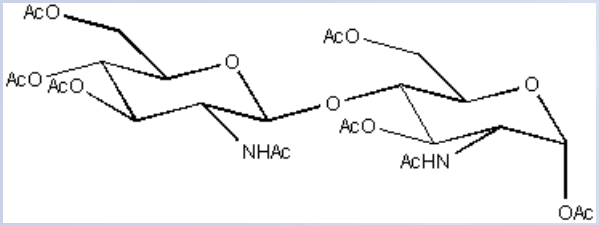
TOCSY: Sucrose

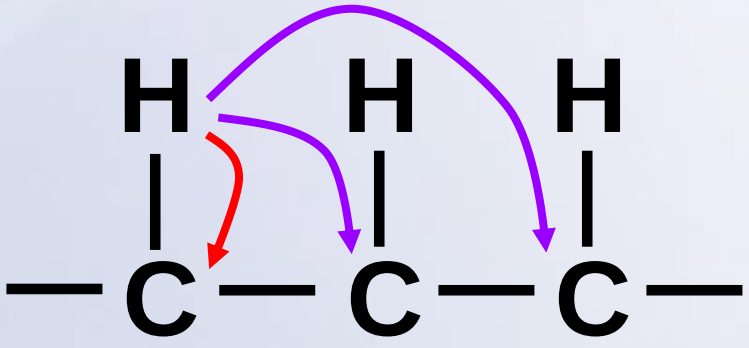


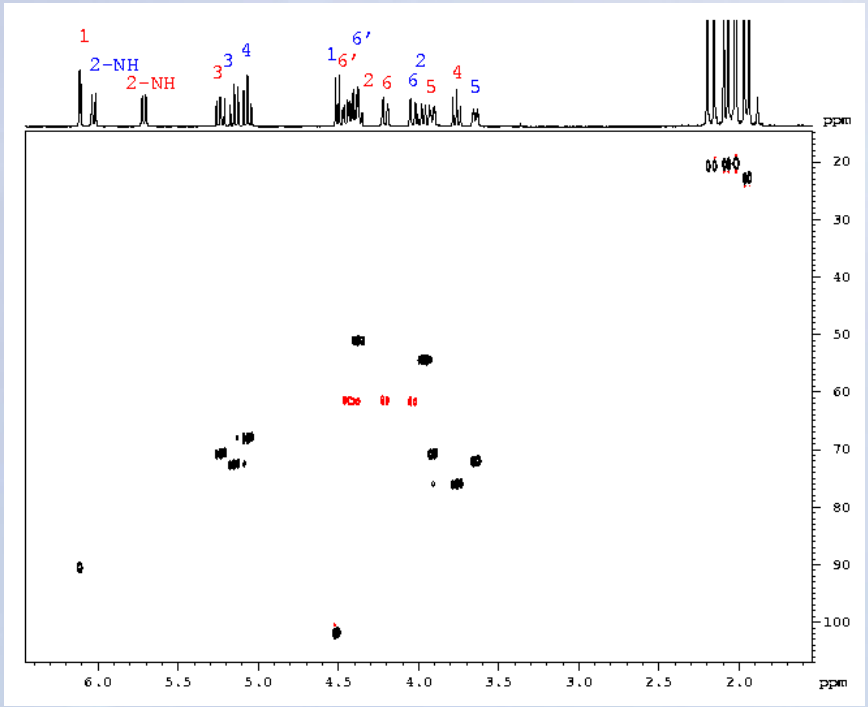


NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

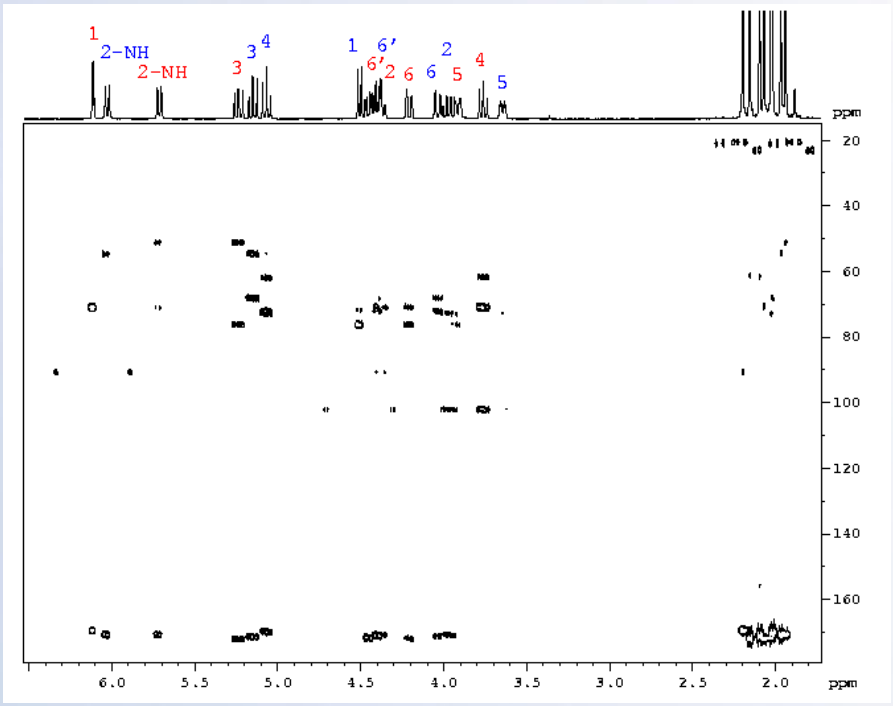
Chentobiose







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



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

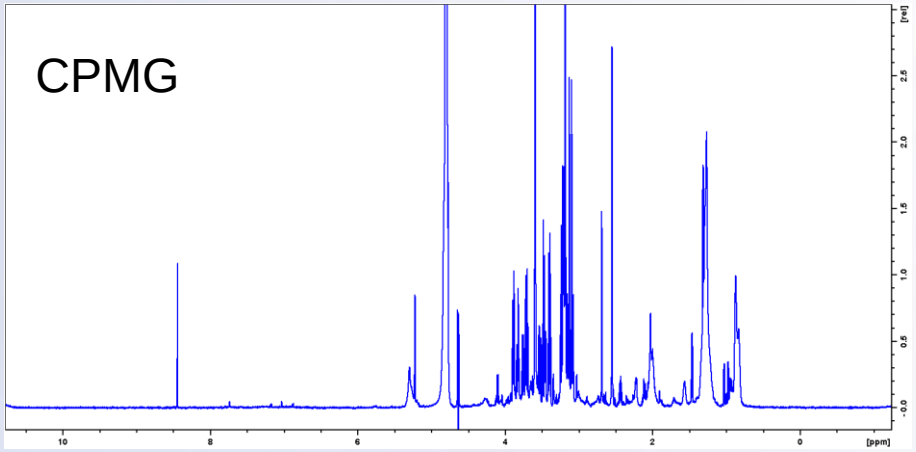
NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

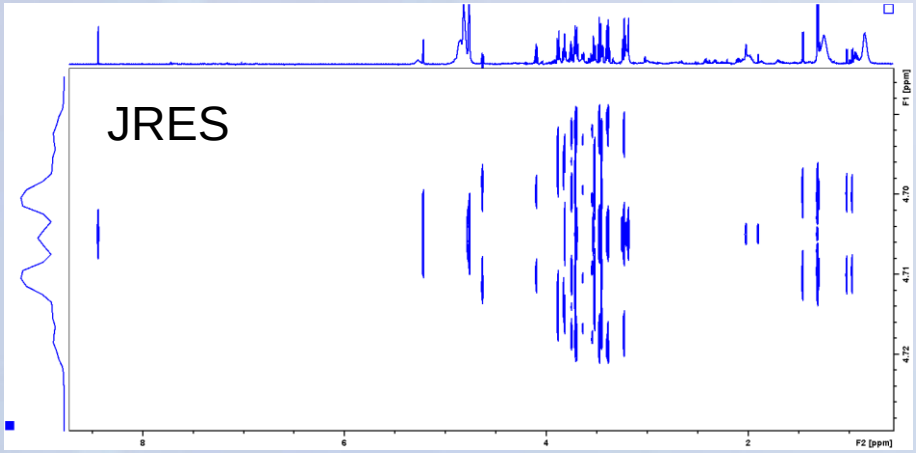
^{1}H-^{13}C HSQC Spectrum of Cell Extract

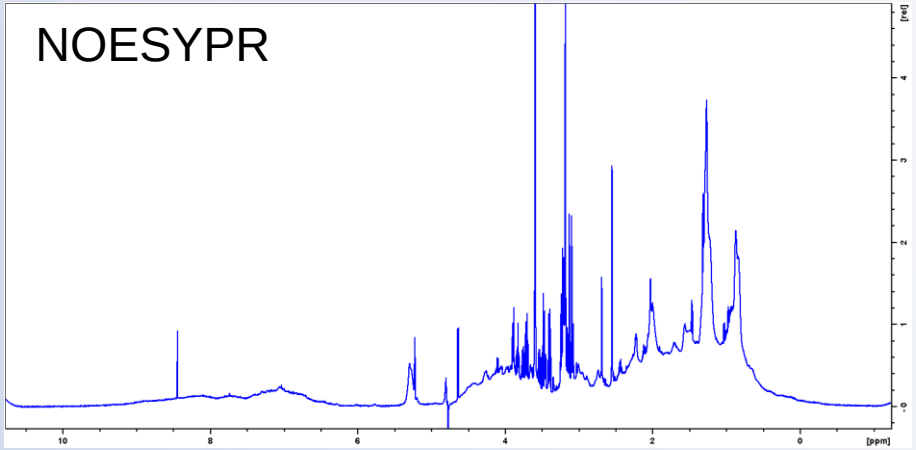
Spectral Editing

Spectral Editing

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

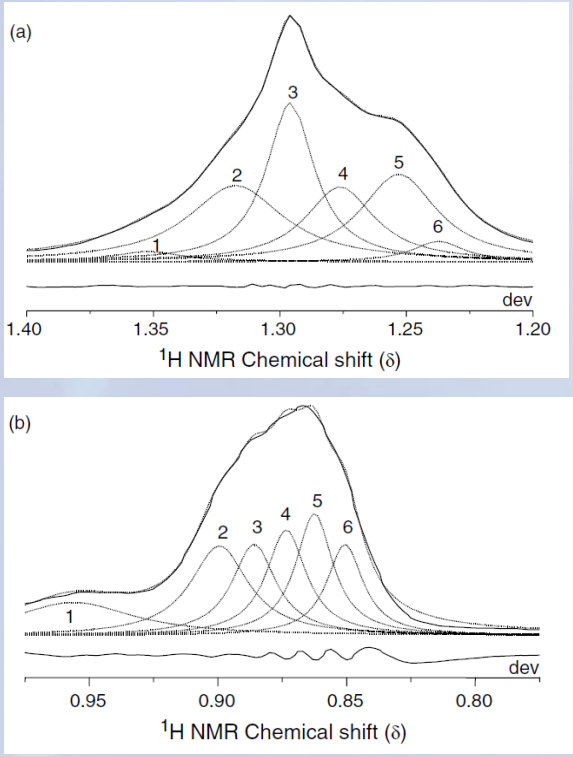


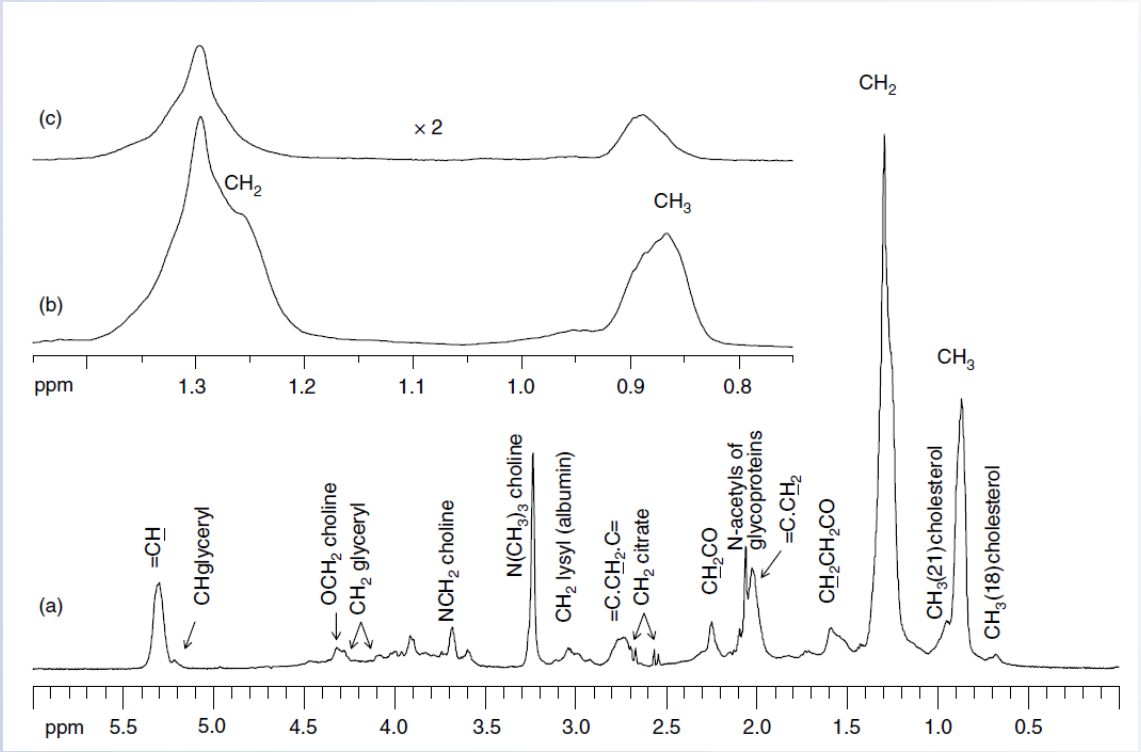




Analysis of Lipoproteins

- 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

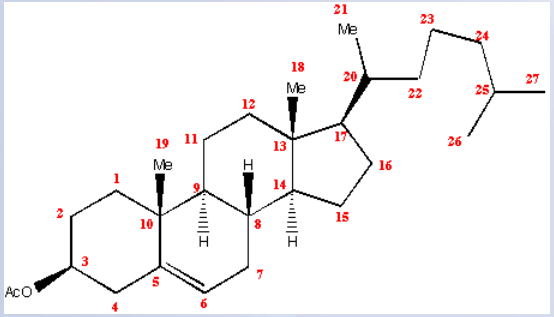


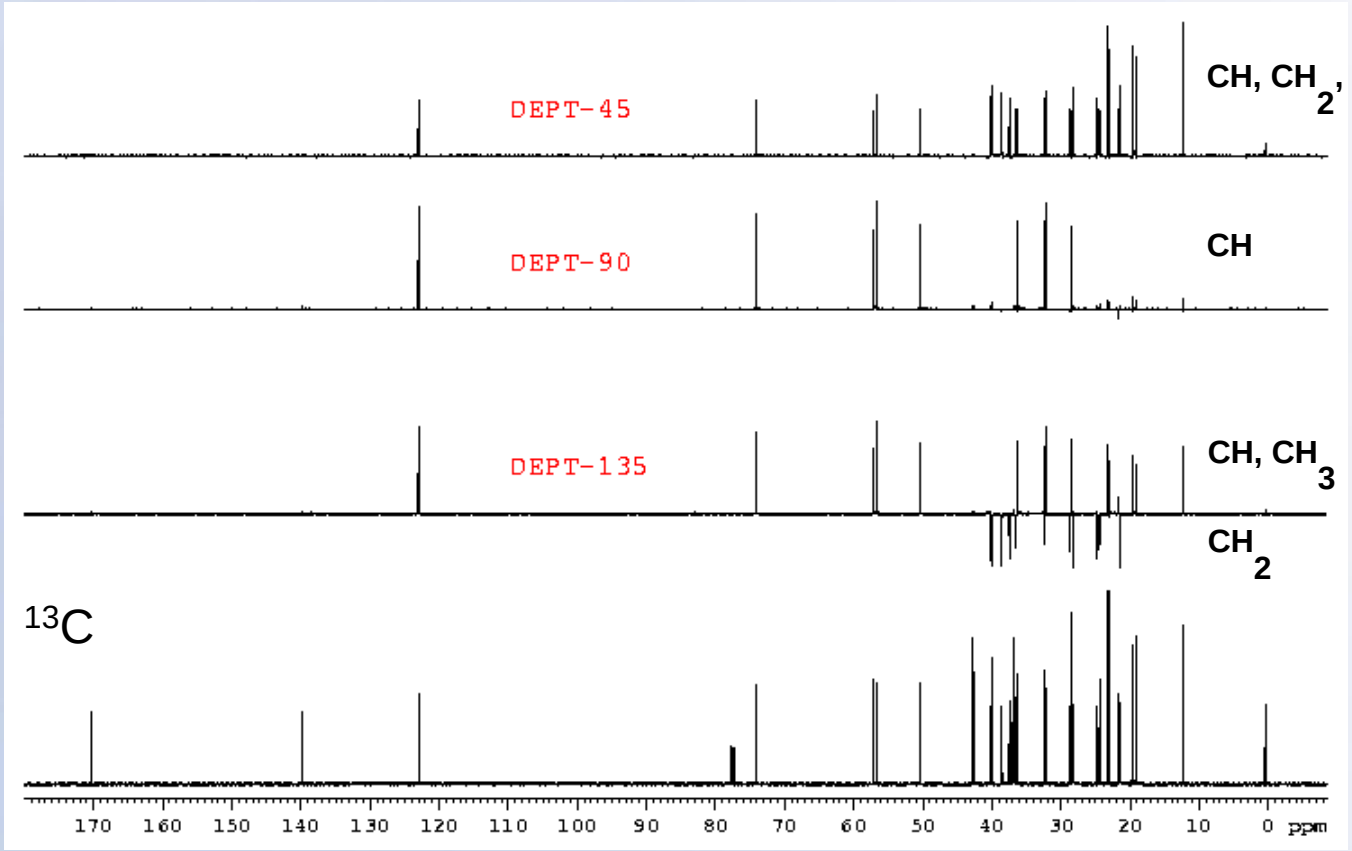


Diffusion Edited NMR Spectra

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

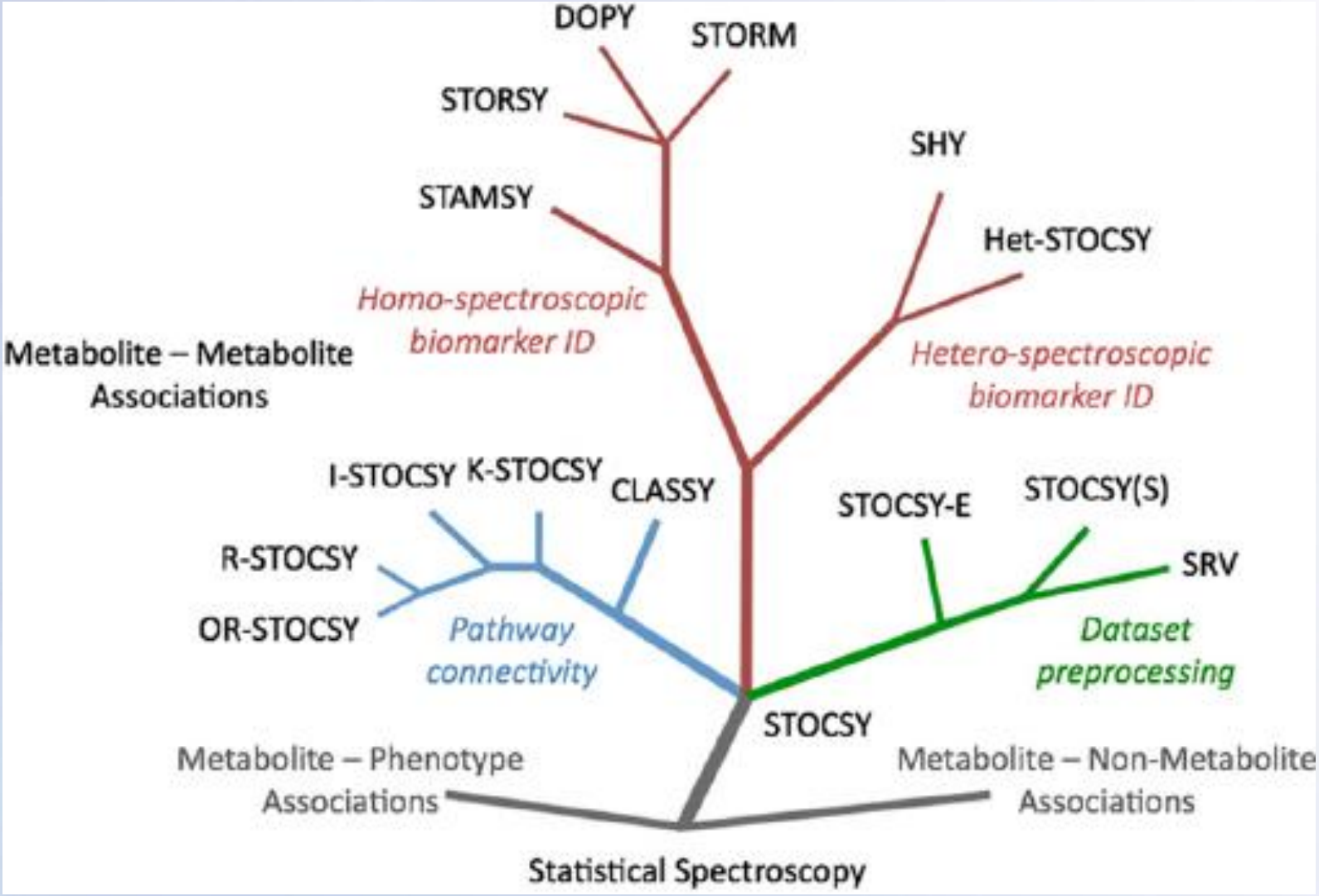
NMR Libraries and Databases

- 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/), Bayesil (http://bayesil.ca/)
- 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 (http://rnmr.nmrfam.wisc.edu/)

Other Complementary Methods

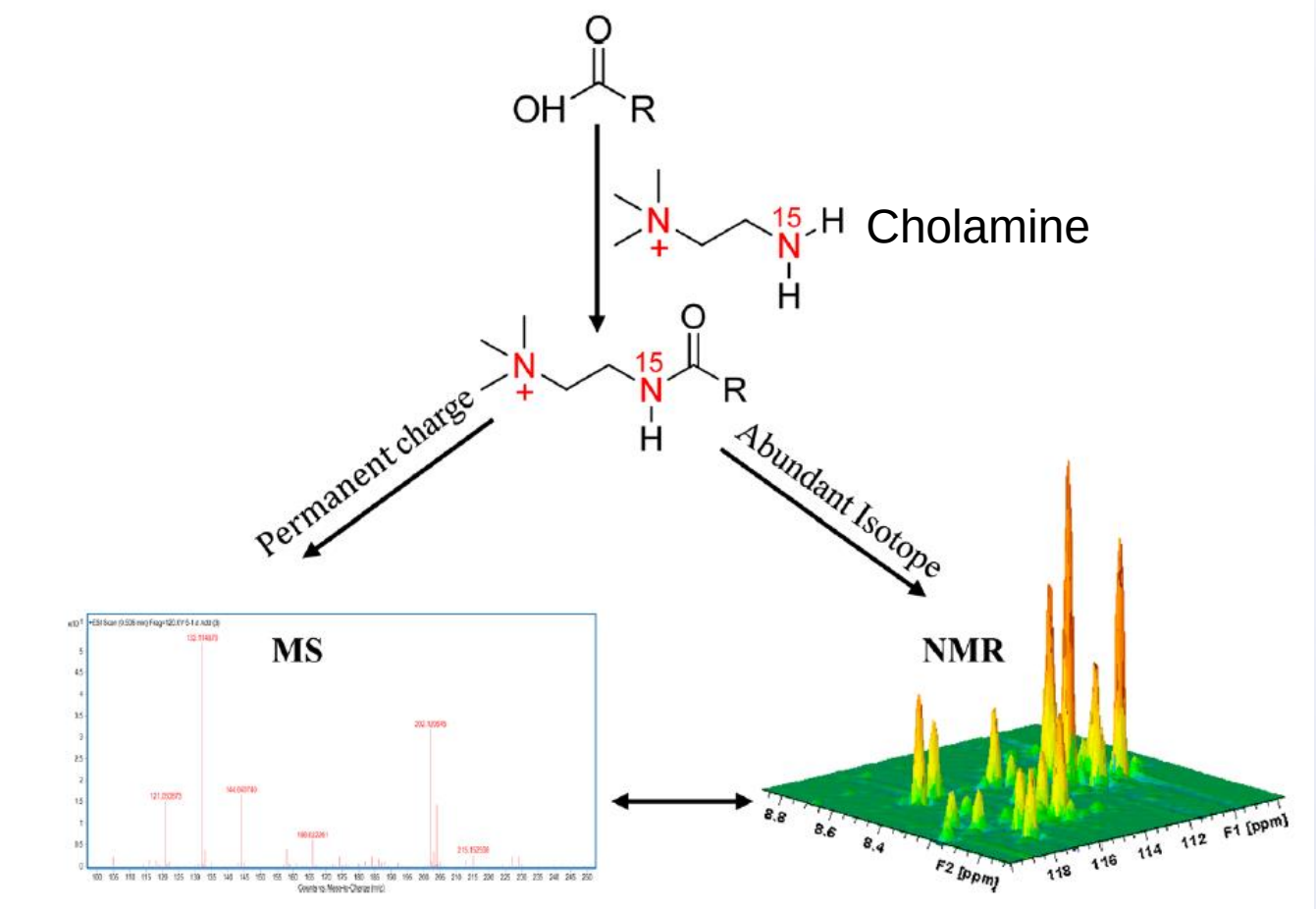
Statistical Spectroscopic Tools



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

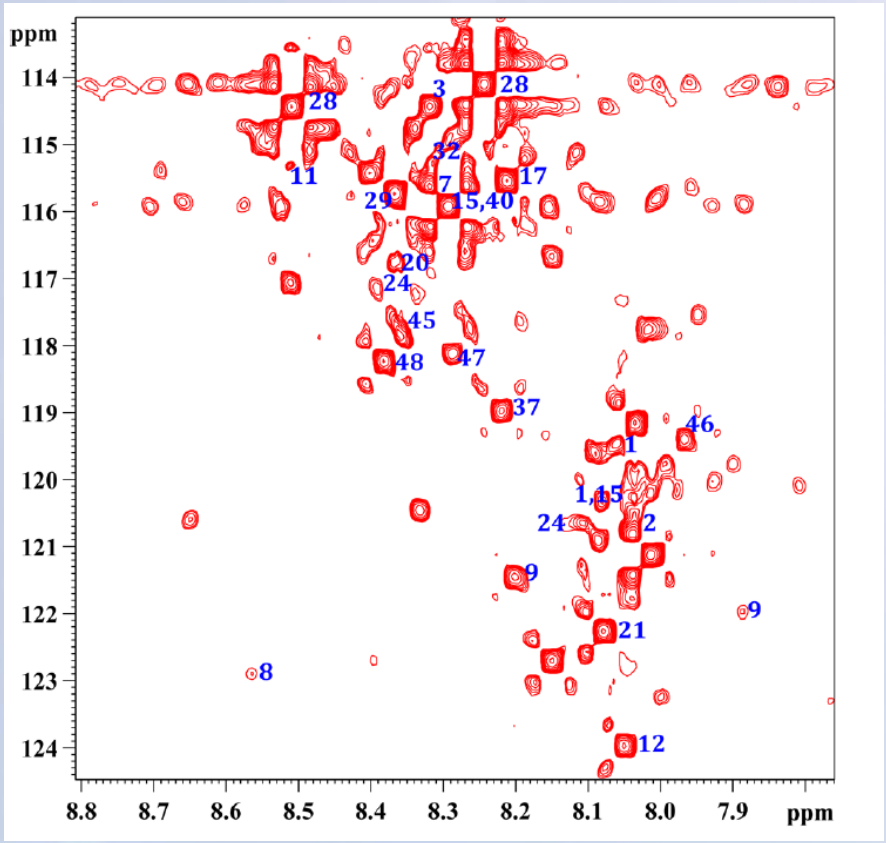


Tayyari, F., G. A. Gowda, H. Gu and D. Raftery (2013). "15N-cholamine--a smart isotope tag for combining NMR- and MS-based metabolite profiling." Anal Chem **85**(18): 8715-8721

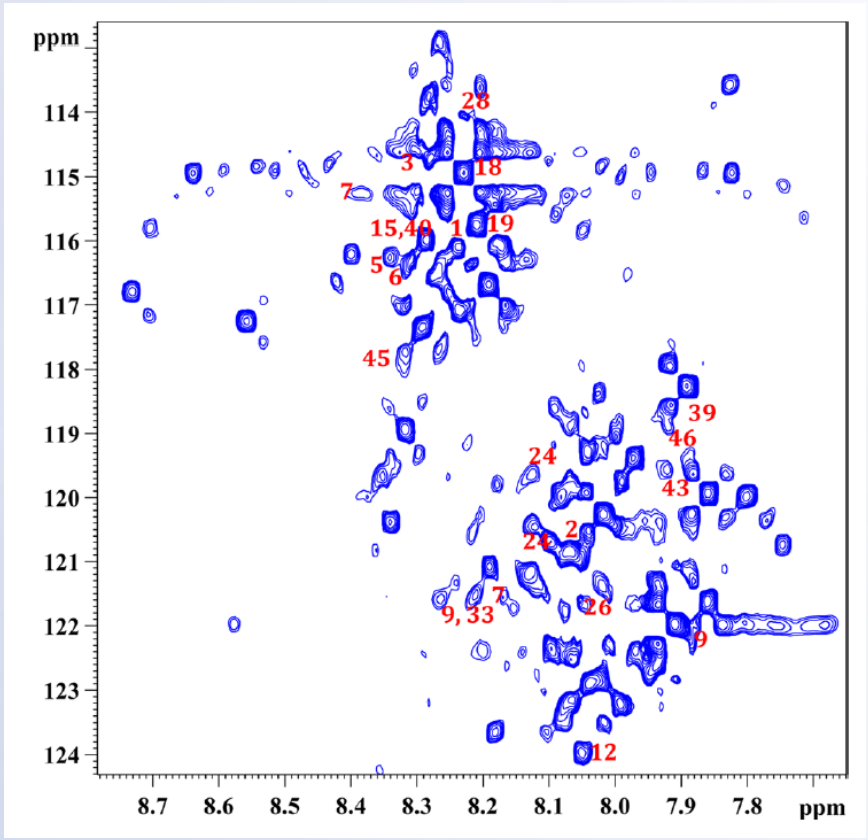
Smart Tagging

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

Human Serum



Human Urine



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References

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- 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.
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- 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

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-Oxoisocaproate, 2-Oxovalerate, 2-Phosphoglycerate, 3,4-Dihydroxymandelate, 3,5-Dibromotyrosine, 3-Aminoisobutyrate, 3-Chlorotyrosine, 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-Hydroxybenzoate, 4-Hydroxybutyrate, 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, Biotin, Butanoyltransferase, 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 onto 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

ERCMRC at UNC Chapel Hill

UNC NUTRITION OBESITY RESEARCH CENTER

Yuanyuan Li
LC-MS/MS
LC-TOF-MS

Wimal Pathmasiri
NMR & GC-TOF-MS

Delisha Stewart
NMR and LC-TOF-MS

Maria Moreno
NMR and LC-MS/MS

Reza Ghanbari
Postdoctoral Fellow

Rose Ewald
Graduate Studies

Susan Sumner
PI, ERCMRC

Susan McRitchie
Program Coordinator
Data Analysis

NCRC

Nick Gillitt
Dole
700 MHz NMR
6500 Sciex LC-MS

Debby Reed
GC-MS
GC-TOF-MS

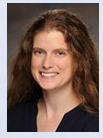
Stephen Orena
LC-MS/MS

Martin Kohlmeier
Training

Tim Fennell
Director,
Analytical Chemistry &
Pharmaceutics

RTI

Yan Lan Yueh
LC-MS



Jessica Gooding
LC-MS

Rod Snyder
NMR and LC-MS

Courtney Whitaker
LC-MS

Scott Watson
Neurotransmitter
LC/MS

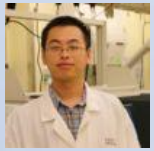
Colin Kaye
NCSU
6500 Sciex
Triple Quad

DHMRI

Kevin Knagge
700 and 950 MHz NMR

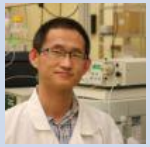
Jason Winnike
NMR
2D-GC-TOF-MS

David Kirchner
LC-MS/MS



Huadong Chen
LC-MS
LC-TOF-MS

Huiyuan Chen
GC-MS
GC-TOF-MS



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