Advanced NMR: Metabolite ID by NMR
UAB Metabolomics Training Course
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Wimal Pathmasiri, Rodney Snyder
NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

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

- 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

NMR J-Coupling

J = 7.2 Hz
Dispersion of NMR Signal with Magnetic Field Strength

1D and 2D NMR Methods
COSY: Sucrose

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

TOCSY: Sucrose

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin
HSQC: Sucrose

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin

Chentobiose

HSQC ($J_{	ext{CH}}$)
HMBC ($J_{	ext{CH}}$, $J_{	ext{CH}}$)

NMRGuide 4.3 - TOPSPIN 3.0, Bruker BioSpin
$^{1}H-^{13}C$ HSQC Spectrum of Cell Extract

Spectral Editing
Analysis of Lipoproteins

- Lipoproteins are classified based on their size and density (VLDL, LDL, HDL)
- Lipoproteins can be assigned to these subfractions by deconvoluting the CH₃ and (CH₂)ₙ in diffusion edited spectra
### Diffusion Edited NMR Spectra

<table>
<thead>
<tr>
<th>Peak</th>
<th>$\delta$ (ppm)</th>
<th>Width (Hz)</th>
<th>$D$ (cm$^2$ s$^{-1}$) $\times 10^{7}$</th>
<th>Area (%)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CH$_2$)$_n$ 1</td>
<td>1.353</td>
<td>19.7</td>
<td>2.72</td>
<td>2.3</td>
<td>LDL + VLDL</td>
</tr>
<tr>
<td>(CH$_2$)$_n$ 2</td>
<td>1.317</td>
<td>28.0</td>
<td>1.98</td>
<td>26.4</td>
<td>VLDL</td>
</tr>
<tr>
<td>(CH$_2$)$_n$ 3</td>
<td>1.296</td>
<td>14.0</td>
<td>1.85</td>
<td>27.9</td>
<td>VLDL</td>
</tr>
<tr>
<td>(CH$_2$)$_n$ 4</td>
<td>1.276</td>
<td>17.8</td>
<td>3.15</td>
<td>15.5</td>
<td>LDL</td>
</tr>
<tr>
<td>(CH$_2$)$_n$ 5</td>
<td>1.255</td>
<td>20.5</td>
<td>5.19</td>
<td>19.9</td>
<td>HDL(60.6%) + LDL(39.4%)</td>
</tr>
<tr>
<td>(CH$_2$)$_n$ 6</td>
<td>1.240</td>
<td>18.4</td>
<td>5.96</td>
<td>7.9</td>
<td>HDL</td>
</tr>
<tr>
<td>CH$_3$ 1</td>
<td>0.956</td>
<td>33.9</td>
<td>3.77</td>
<td>15.8</td>
<td>VLDL + HDL</td>
</tr>
<tr>
<td>CH$_3$ 2</td>
<td>0.899</td>
<td>16.4</td>
<td>1.70</td>
<td>20.6</td>
<td>VLDL</td>
</tr>
<tr>
<td>CH$_3$ 3</td>
<td>0.886</td>
<td>12.4</td>
<td>1.84</td>
<td>15.9</td>
<td>VLDL</td>
</tr>
<tr>
<td>CH$_3$ 4</td>
<td>0.873</td>
<td>11.3</td>
<td>3.07</td>
<td>16.7</td>
<td>LDL</td>
</tr>
<tr>
<td>CH$_3$ 5</td>
<td>0.862</td>
<td>10.5</td>
<td>4.51</td>
<td>17.9</td>
<td>HDL(40.8%) + LDL(59.2%)</td>
</tr>
<tr>
<td>CH$_3$ 6</td>
<td>0.851</td>
<td>10.2</td>
<td>7.11</td>
<td>13.1</td>
<td>HDL</td>
</tr>
</tbody>
</table>

### DEPT: Cholesterol

The DEPT spectra show the chemical shifts and carbon types in cholesterol. The DEPT-45, DEPT-90, and DEPT-135 experiments provide information on the carbon types (CH, CH$_2$, CH$_3$) and their corresponding chemical shifts. The spectra highlight the structural features of cholesterol with peaks corresponding to different carbon types.
NMR Libraries, Software, and Databases

- Continuously emerging databases
  - HMDB (http://www.hmdb.ca/)
  - Birmingham Metabolite Library (http://www.bml-nmr.org/)
  - BMRB (http://www.bmrbr.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
  - 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

**Tagging**

**Smart Tagging**


References

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'-Deoxynosine, 2'-Aminoadipate, 2'-Aminobutyrate, 2'-Ethylcysteine, 2'-Hydroxymethylvalerate, 2'-Hydroxybutyrate, 2'-Hydroxylglutamate, 2'-Hydroxyscaproate, 2'-Hydroxysorbose, 2'-Hydroxythreonate, 2'-Methylglutamate, 2'-Octanate, 2'-Oxoglutarate, 2'-Phosphohexonate, 3,4-Dihydroxymandelate, 3,5-Dibromotyrosine, 3,6-Hydroxylysine, 3-Hydroxy-3-methylglutarate, 3-Hydroxybutyrate, 3-Hydroxyisovalerate, 3-Hydroxyphenylacetic acid, 3-Indoxylsulfate, 3-Methyl-2-oxovalerate, 3-Methyladipate, 3-Methylbutyrate, 3-Phenylacetate, 3-Phenylpropionate, 4-Aminobutyrate, 4-Aminoisobutyrate, 4-Hydroxy-3-methyloxazolidine, 4-Hydroxy-6-methylpyrones, 4-Hydroxyphenylacetic acid, 4-Pyridoxal, 5,7-Dihydroxybenzoic acid, 5-Hydroxyphenylacetate, 5-Hydroxy-5-Methoxysalicylate, Acetyl-CoA, Acetamide, Acetaminophen, Acetate, Acetocetate, Acetone, Acetylsalicylate, Adenine, Adenosine, Adipate, Alanine, Allantoin, Allolactone, Anserine, Arginine, Argininosuccinate, Asparagine, Aspartate, Benzamide, Benzaldehyde, Caffeine, Caprate, Caprylate, Carnitine, Carnosine, Choline, Cinnamate, Citrate, Citrulline, Cystine, Cystidine, Cysteine, DSS (Chemical Shift Indicator), Dimethylamine, Epicatechin, Ethanol, Ethanolamine, Ethylene glycol, Ethylmalonate, Ferulate, Formate, Fructose, Fucose, Fumarate, Galactarate, Galactitol, Galactonate, Galactose, Germitenate, Glucarate, Glucose, Glutamate, Glutamine, Glutathione, Glycin, Glycol, Glycine, Glycerol, Glycolate, Glycylglycine, Guanidocacete, Guanine, Hippurate, Histidine, Homocitrulline, Homocysteine, Homogentisate, Homoserine, Homovanillate, Hypoxanthine, Ibutrofen, Idoxazol, Isotope-3-acetate, Inosine, Isobutyrate, Isocaproate, Isocitrate, Isoureic acid, Isolevulinate, Kynurenate, Kynurenine, Lactate, Lactoside, Leucine, Leluvinate, Lysine, Malate, Maleate, Malonate, Mannonol, Mannose, Methanol, Methionine, Methylamine, Methylglycine, Methylglycine, Methylmalonate, Methylmalonate, Methylsuccinate, N,N-Dimethylformamide, N,N-Dimethylglycine, N-Acetylglutamate, N-Acetylglutamine, N-Acetyltyrosine, N-Carbanoyl-β-alanine, N-Carbamoylaspartate, N-Isovalerylglutamine, NAD+, Nicotinamide, Nicotinate, O-Acetylcarnitine, O-Phosphoethanolamine, O-Phosphoethyleneglycol, Ornithine, Oxalacetate, Oxyurinol, Pantothenate, Phenol, Phenylacetate, Phenylacetylglycine, Phenylalanine, Pimelate, Proline, Propionate, Propylene glycol, Protocatechuic acid, Pyridoxal, Pyruvate, Quintulenate, Riboflavine, Ribose, S-Adenosylhomocysteine, S-Fumarate, Salicylate, Salicylurate, Sarcosine, Serine, Sulfate, Succinate, Succinylacetone, Succrose, Tartarate, Tartaric acid, Theophylline, Threonate, Threonine, Thymine, Thymol, Tigoniluric acid, Trimethylamine, Trimethylamine N-oxide, Tryptophan, Tyrantine, Tyrosine, Uracil, Urea, Uridine, Uric acid, Valerate, Valine, Valproate, Valine, Xanthine, Xanthosine, Ylose, cis-Aconitate, m-O-Cresol, o-Cresol, trans-4-Hydroxy-L-proline, trans-Aconitate, β-Alanine, n-Methylhistidine, t-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
and GC-MS

Jessica Gooding
LC-MS

Kelly Mercier
NMR

Susan McRitchie
Data Analysis

Bob Clark
Genetics

Zach Acuff
Biostatistics

Anita Patil
LC/MS

Aurora Cabrera
LC-MS/MS

Jocelin Spruill
GC-MS

Neurotransmitter

Tammy Cavalo
Biology and OC

Delissa Stewart
Cell Biology

Ninell Mortensen
Microbiology

Maria Moreno
Biology

Rose Ewald
Intern

Yayi Li
LC-MS

Red Snyder
LC-MS

Sherry Black
In vivo and in vitro Metabolism

Scott Watson
Neurotransmitter LC/MS

Puxi Patel
In vitro metabolism

Yan Lan
LC-MS

Mocky Markley Model Systems

Sue Clark
Administrative Support

Tim Fennell
Metabolism

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