Homework 3 #1 (Due 9/20): Explain what the following operation or function means. What is the purpose for each item?

(1) Shimming
(2) Lock
(3) Spinning
(4) Window Function
(5) Fourier Transform
(6) Phasing
(7) Probe Tuning
Homework 3 #2

Q 1. Plot $\omega$ vs $A(\omega)$ for $\Omega = 100$ and $\lambda = 10$.
Q 2. Plot $f$ vs $D(\omega)$ for $\Omega = 100$ and $\lambda = 10$.
Q 3. Plot $f$ vs $A(\omega)$ for $\Omega/2\pi = 200$ and $\lambda = 10$.
Q 4. Plot $f$ vs $A(\omega)$ for $\Omega/2\pi = 200$ and $\lambda = 40$.
Q 5. Plot $f$ vs $D(\omega)$ for $\Omega = 200$ and $\lambda = 40$.

\[ S(\omega) = \frac{\lambda}{(\Omega - \omega)^2 + \lambda^2} + i\frac{(\Omega - \omega)}{(\Omega - \omega)^2 + \lambda^2} \]
\[ A(\omega) = \frac{\lambda}{(\Omega - \omega)^2 + \lambda^2} \]
\[ D(\omega) = \frac{(\Omega - \omega)}{(\Omega - \omega)^2 + \lambda^2} \]

Calculation of FT (p139-140)

- $S'(t) = \exp(i\Omega t)e^{-\lambda t}\exp(i\alpha)$ ($\lambda = 1/T_2$)
- $[3.14b]$

\[ S(\omega) = \int_0^\infty dt s(t) \exp{\{i\omega t\}} \int_0^\infty dt \exp{\{i(\Omega - \omega)t - \lambda t\}} \]

Calculate FT of $s'(t)$ in [3.14b] for $\alpha = \pi/2$ as $S'(\omega)$ and Calculate $\text{Real}(S'(\omega))$ and $\text{Imag}(S'(\omega))$.

\[ S(\omega) = \frac{1}{i(i(\Omega - \omega) - \lambda)} \exp{\{i(\Omega - \omega)t - \lambda t\}} \bigg|_0^\infty \]
\[ = \frac{1}{i(i(\Omega - \omega) - \lambda)} - 1 \]
\[ = \frac{i(\Omega - \omega) - \lambda}{(\Omega - \omega)^2 + \lambda^2} + i\frac{(\Omega - \omega)}{(\Omega - \omega)^2 + \lambda^2} \]

$A(\omega)$ $D(\omega)$
Q 1. Plot $\omega$ vs $A(\omega)$ for $\Omega = 100$ and $\lambda = 10$

Q 2. Plot $f$ vs $D(\omega)$ for $\Omega = 100$ and $\lambda = 10$

Q 5. Plot $f$ vs $D(\omega)$ for $\Omega = 200$ and $\lambda = 40$

Announcement 1

- How is the preliminary analysis of the unknown ($^1$H & $^{13}$C NMR) for Homework 4 (Due 9/22)?
Chemical Shifts

- Electron near nuclei creates extra field \(-\gamma B_0 \delta\)
  \(-\gamma B_0 (1 + \delta)\)

Higher Frequency

Lower Frequency

Induction Effects in $^1$H shifts

$^1$H chemical shifts of $\text{CH}_n\text{Cl}_{4-n}$(ppm)

<table>
<thead>
<tr>
<th>$\text{CH}<em>n\text{Cl}</em>{4-n}$</th>
<th>ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_4$</td>
<td>0.23</td>
</tr>
<tr>
<td>$\text{CH}_3\text{Cl}$</td>
<td>3.05</td>
</tr>
<tr>
<td>$\text{CH}_2\text{Cl}_2$</td>
<td>5.3</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>7.27</td>
</tr>
</tbody>
</table>

$^1$H shifts of $\text{CH}_3X$ (ppm)

<table>
<thead>
<tr>
<th>$\text{X}$</th>
<th>OH</th>
<th>NH$_2$</th>
<th>H</th>
<th>Me$_3$Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>4.26</td>
<td>3.38</td>
<td>2.47</td>
<td>0.23</td>
</tr>
<tr>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>NH$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me$_3$Si</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analysis Example: $^{13}$C and $^1$H NMR of diethyl ether $(\text{CH}_3\text{CH}_2)_2\text{O}$

Q1. $^{13}$C NMR: Which is CH$_3$?

$\delta$ (Ppm) = \frac{\text{Shift from TMS (Hz)}}{\text{NMR Frequency (MHz)}}$

Q2. $^1$H NMR: Which is CH$_3$?

$\delta$ (Ppm) = \frac{\text{Shift from TMS (Hz)}}{\text{NMR Frequency (MHz)}}$

$^{13}$C Chemical shift additivity principle

• $\delta = -2.5 + \sum A_j \eta_j$

• $A_j$ denotes a substituent parameter for neighbouring chemical groups

| Substituent parameters for C and CH, CH$_2$, CH$_3$, |  
|---|---|---|---|
| $\alpha$ | $\beta$ | $\gamma$ |  
| 9.1 | 9.4 | -2.5 |  

$\text{CH}_3$-$\text{CH}_2$-$\text{CH}_2$-$\text{CH}_2$-$\text{CH}_3$ Exp

$\text{CH}_3$: $-2.5 + 9.1 + 9.4 - 2.5 = 13.4$ (13.9)

$\text{CH}_2$: $-2.5 + 2 \times 9.1 + 9.4 - 2.5 = 22.6$ (22.8)

$\text{CH}_2$: $-2.5 + $ (34.7)
\[ ^{13}C \text{ aliphatic shifts of ethyl-ether} \]

- \( \text{CH}_3-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_3 \)

\[ \text{CH}_3 : -2.5 + 9.1 + \boxed{8} - 2.5 = 12.1 \text{ ppm} \]

\[ \text{CH}_2 : -2.5 + 9.1 + \boxed{58} + 9.4 - 2.5 = 71.5 \text{ ppm} \]

---

\[ ^{13}C \text{ aliphatic shifts of ethyl-ether} \]

- \( \text{CO}_2\text{H-CH}_2-\text{NH}_2 \) (Glycine)

\[ \text{CH}_2 : -2.5 + 29 + 21 = 47.5 \text{ ppm} \]

- \( \text{CO}_2\text{H-CH(\text{CH}_3)}-\text{NH}_3 \) (Alanine)

\[ \text{CH} : -2.5 + 29 + 21 + 9.1 = 56.6 \text{ ppm} \]

---

\[ \text{Table 12: Carbon-13 Substituent Parameters for Functional Groups} \]

<table>
<thead>
<tr>
<th>X</th>
<th>Terminal X (3-30)</th>
<th>Internal X (3-31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \alpha )</td>
<td>( \beta )</td>
</tr>
<tr>
<td>F</td>
<td>68</td>
<td>9</td>
</tr>
<tr>
<td>Cl</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Br</td>
<td>20</td>
<td>11</td>
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<tr>
<td>I</td>
<td>-6</td>
<td>11</td>
</tr>
<tr>
<td>OH</td>
<td>-8</td>
<td>10</td>
</tr>
<tr>
<td>OR</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>GAc</td>
<td>51</td>
<td>6</td>
</tr>
<tr>
<td>NH_2</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>NR_3</td>
<td>43</td>
<td>6</td>
</tr>
<tr>
<td>CN</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>NO_2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>CH==CH_3</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>CH_2</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>C==CH</td>
<td>4.5</td>
<td>5</td>
</tr>
<tr>
<td>C==OOR</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>C==OOR</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>C==OOR</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>C==OOR</td>
<td>22</td>
<td>5</td>
</tr>
</tbody>
</table>

2D NMR
A. 2D Heteronuclear Correlation NMR (HETCOR)
- The basic idea of 2D HETCOR NMR is to specify a peak with two frequencies $\Omega_I$ and $\Omega_S$.
- The sequence is given as an extension of polarization transfer experiment, but there is the $t_1$ period for indirectly detecting $^1$H signal.

\[
I_x \cos(\Omega_I t_1) + I_y \sin(\Omega_I t_1)
\]

\[
S_x \cos(\Omega_I t_1)
\]

Array of FIDs are taken for different $t_1$ period

Ch4.1 (See p275)
2D FT Processing

• So we obtained
  \[ s(t_1, t_2) = \cos(\Omega t_1)\exp(i\Omega_s t_2). \]

• First step is Complex FT on \( t_2 \)
  \[ s(t_1, \omega_2) = \cos(\Omega t_1)A(\omega_2 - \Omega_S) \]

• Second, Cosine FT on \( t_1 \)
  \[ s(t_1, \omega_2) = A(\omega_1 - \Omega_I)A(\omega_2 - \Omega_S) \]

2D COSY for Coupled A-X system
Q1. Do you expect a cross peak between A and B if A and B form a dipeptide like A-B?

Q2. Do you expect a cross peak between A and B if A and B are a mixture of amino acids?
3.7.1 Presaturation (p223)

- Irradiate weakly ($\omega_1 / 2\pi \sim 50$ Hz) for a long time (1-2 s) $\rightarrow$ Q. What would happen?

- Advantage: Simple
- Disadvantage:
  - Requires a good shimming
  - Exchangeable $^1$H (such as amide $^1$H) may be also suppressed
3.2 Data Acquisition

- FID Generated \( \cos(\omega_0 t) \)
- Mixing to lower frequency (Real/Imag+Filter)
  \[ \cos(\omega_0 - \omega_{RF}) \sin(\omega_0 - \omega_{RF}) \]
- Digitization
- Store to the computer
- Display

3.2.3 Quadrature Detection (p132)

Q. Explain how the experimental scheme for data acquisition & detection (So called quadrature detection) works
3.2.1 Sampling (p124)

After digitization, a time-domain signal is digitized as
\[ s(t_n) = s(n\Delta t) \leftarrow \text{A/D Conversion} \]

Nyquist frequency: \( f_n = 1/(2\Delta t) \) defines the highest-frequency sinusoidal that can be reproduced from the digitized data.

\[ S(t) = \sum s(kt\Delta) \text{sinc}[2\pi f_n(t-k\Delta t)], \]

where \( \text{sinc}(x) = \sin(x)/x \) \( \leftarrow \text{D/A Conversion} \)
What happens if the frequency is higher than $f_n$?

- First note that $2\pi(2f_n\Delta t) = 2\pi$
  From this,
  \[
  \cos[2\pi(2f_nM + \nu_0)k\Delta t] = \\
  \cos[2\pi 2f_nMk\Delta t + 2\pi \nu_0k\Delta t] = \\
  \cos[2\pi Mk + 2\pi \nu_0k\Delta t] = \\
  \cos[2\pi \nu_0k\Delta t] \quad (k \text{ is an integer})
  \]

$\rightarrow (2f_nM + \nu_0)$ and $\nu_0$ can’t be distinguished by the digital sampling.

Hence, the signal shows up at $\nu_a$

$\nu_a = (2f_nM + \nu_0)$, where $-f_n \leq \nu_a \leq f_n$

Aliasing

Q. Where does the signal $\nu_a$ show up?
3.2.3 Quadrature Detection (p132)

\[
\cos(\omega_{RF}t)\cos(\omega_0t) = \cos\left(\left(\omega_{RF} + \omega_0\right)t\right)/2 + \cos\left(\left(\omega_{RF} - \omega_0\right)t\right)/2
\]

\[
\sin(\omega_{RF}t)\cos(\omega_0t) = \sin\left(\left(\omega_{RF} + \omega_0\right)t\right)/2 + \sin\left(\left(\omega_{RF} - \omega_0\right)t\right)/2
\]

Digital resolution & Zero Filling

- Actual digital resolution in the obtained spectrum is
  \[
  \frac{1}{(N*\Delta t)} = \frac{1}{t_{\text{max}}}
  \]
  \[\Delta t = \text{sampling interval and N is data points in the FID}\]

  Hence, the digital resolution can be enhanced by extending \(t_{\text{max}}\).
Ch 3.4 Pulse Effects

• We will cover
  – \(^1\)H Decoupling (3.4.3)
  – Off-Resonance Effects (3.4.1)
  – Composite Pulse (3.4.2)
  – Selective Pulse (3.4.4)
  – Water Suppression (3.4.5)

Q. Why are there no \(^1\)H-\(^{13}\)C J splitting?
**J coupling**

The local field that the S spin experiences due to a J coupling between I and S spins is denoted by

\[ 2\pi J l_z = 2\pi J m_i. \]

The energy due to the J coupling is

\[ E_J = 2\pi J m_i S_Z = 2\pi J l_z S_Z. \]

---

**What is $^1H$ decoupling?**

- The idea is to exchange $\alpha$ and $\beta$ states rapidly for the I spin so that the splitting is averaged.

If $<m_i> = 0$

\[ <E_J> = 2\pi J <l_z> S_Z \]
\[ = 2\pi J <m_i> S_Z = 0 \]

- If you remember $l_z - \pi i x \rightarrow -l_z$

Under continuous RF field matched at $^1H$ frequency,

$<l_z> = <m_i> = 0$
Chemical Exchange (Ch5. p400)

\[ A \xrightarrow{k} B \]
\[ \Omega_A \xleftarrow{} \Omega_B \]

![Diagram of chemical exchange](image)

$10 \text{ s}^{-1}$
$100 \text{s}^{-1}$
$450 \text{s}^{-1}$
$5000 \text{s}^{-1}$

$160 \text{ Hz}$

$\approx 10$

![Diagram of 1H Spectrum of Leu-enkephalin in DMSO](image)

\[ ^1 \text{H Spectrum of Leu-enkephalin in DMSO} \]
\[ (\text{Tyr-Gly-Gly-Phe-Leu}) \]

From "high-resolution NMR techniques in organic chemistry" by Claridge
How strong $^1$H decoupling must be?

- The idea is to exchange $\alpha$ and $\beta$ states rapidly for the I spin so that the splitting is averaged.

From the chemical exchange example,

$\rightarrow I_Z - \pi I_X \rightarrow -I_Z$

must be faster than J coupling

However, there is a problem in $^1$H decoupling $\rightarrow$

![1H spectrum of L tryptophan in 400 MHz](image)
Off-resonance Effects & Effective Field

How is the magnetization evolved by the effective field?

\[ \omega_{\text{eff}} = -\gamma B_{\text{eff}} \]

\[ \omega_{\text{eff}} = (\Omega^2 + \omega_1^2)^{1/2} \]

Near On-Resonance

\[ \omega_{\text{eff}} = -\gamma B_{\text{eff}} \]

Off-Resonance

\[ \omega_{\text{eff}} = (\Omega^2 + \omega_1^2)^{1/2} \]
3.4.1 Off-Resonance Effects

Off-resonance Effects in $^1H$ Decoupling

- Scaled coupling is $J \lambda_c$, where $\lambda_c = \Omega/\{\Omega^2 + (\gamma B_1)^2\}^{1/2}$

\[
\lambda_c = 0 \text{ for the on-resonance case } \Omega=0. \\
\text{However, when } \Omega \sim \gamma B_1, \lambda_c = 0.707.
\]

On the other hand, if you use a strong $B_1$ field, you can bake your sample because of the heat generated by a decoupling RF field. ($W \propto B_1^2$)

What is solution?
3.4.3 Composite Pulse (p174)

- Composite pulse compensates non-ideal effects of off-resonance and RF-inhomogeneity over sample {i.e. $B_1(1\pm\delta)$}.

- One idea is to add an extra pulse that does not function in the ideal case, but compensates for the non-ideal effects.
Off-resonance dependence of composite rotation

\[
P = (\beta_0(\beta')_\pi/2(\beta)_0
\]
\[
\beta = 90^\circ \quad \beta' = 240^\circ
\]

From "Principles of NMR in one and two dimension" by Ernst et al.

3.5.2 Super cycle for Composite Pulse Decoupling (p204)

- \( R = 90\cdot180\cdot x270 \cdot x \)
- \( R = 90\cdot x180\cdot x270\cdot x \)
- If 90 = 1, \( R = 1\cdot 2\cdot x3 \cdot x \)

RRRRR WALTZ4
RRRRRRRRR WALTZ8
Offset (Ω) dependence of effective coupling constants

Fig. 3.28

Effects of Super cycle
Computer optimized sequence
Garp-1

Figure 3.22 Scalar coupling scaling factor for GARP-I decoupling sequence.

3.4.4 Selective Pulse (p179)

Ideal Selective $\pi/2$-pulse

Ideal Selective $\pi$-pulse

$\Omega/\gamma B_1$
Typical Shaped Pulse for Selective Excitation

Figure 3.23 (a) Gaussian, (b) half-Gaussian, (c) sinc, and (d) Hermitean amplitude-modulated selective pulse shapes. Amplitudes are proportional to \( \exp(-at^2) \), \( \sin(\pi at)/(\pi at) \), and \((1 - 2at^2) \exp(-at^2)\) for the Gaussian, sinc, and Hermitean pulses, respectively. Gaussian, half-Gaussian, and Hermitean pulse shapes are truncated at the 1% amplitude level. The sinc pulse is truncated at the first zero of the sinc function.

Selective 90° Pulse

Figure 3.24 Selective 90° pulses. Resulting (a–c) \( M_x \), (d–f) \( M_y \), and (g–i) \( M_z \) magnetization components obtained following application of (a, d, g) rectangular, (b, e, h) Gaussian, and (c, f, i) half-Gaussian 90° pulses to equilibrium \( M_z \) magnetization. All pulses have \( x \) phase.
3.7.1 Presaturation (p223)

- Irradiate weakly ($\omega_1/2\pi \sim 50$ Hz) for a long time (1-2 s) → Q. What would happen?
  
  - Advantage: Simple
  
  - Disadvantage:
    - Requires a good shimming
    - Exchangeable $^1$H (such as amide $^1$H) may be also suppressed
3.7.2 Jump-Return

$90_y \quad \tau \quad 90-y$

In case of on-resonance (H$_2$O signal)

$I_Z - 90_y \rightarrow I_X - \tau \rightarrow I_Z - 90_y \rightarrow I_Z$

Water Signal Undetected!

In case of some-what off-resonance (Other signals)

$I_Z - 90_y \rightarrow I_X - \tau \rightarrow I_X \cos \Omega \tau + I_y \sin \Omega \tau$

$\quad - 90_y \rightarrow I_Z \cos \Omega \tau + I_y \sin \Omega \tau$

Other Signals Detected!

Spin Lock & Field Gradient for Water Suppression

Non-selective $\pi/2_x$  
Water $\pi/2_x$  
Non-selective $\pi/2_x$

Non-selective $\pi_x$  
Non-selective $\pi_x$

FG  
FG
H exchange rate $k_{ex}$ (p 224)

From "NMR of Proteins and Nucleic Acids"
By K. Wuthrich

Log($k_{ex}$) (min$^{-1}$)

Indole, (Trp)

pH and Temperature dependence of $k_{ex}$

$k_{ex}$ (sec$^{-1}$) $t_{1/2}$ (min)

<table>
<thead>
<tr>
<th>$k_{ex}$ (sec$^{-1}$)</th>
<th>$t_{1/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>00:00:00.69</td>
</tr>
<tr>
<td>1.0</td>
<td>00:00:10.69</td>
</tr>
<tr>
<td>0.1</td>
<td>00:01:06.9</td>
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<tr>
<td>0.01</td>
<td>00:01:09</td>
</tr>
<tr>
<td>0.001</td>
<td>00:11:33</td>
</tr>
<tr>
<td>0.0001</td>
<td>01:55:33</td>
</tr>
</tbody>
</table>

From:
http://www.hxms.com/
H/D Exchange of BPTI

pH = 4.1  25 °C

3 min
12 min
38 min
600 min
16,000 min

2D 1H/15N HSQC spectrum of β2m amyloid fibril in 95 % DMSO/5 % D₂O
Goto et al. Nature Structural Biology 2002

Use of DMSO
Dissolved into DMSO
After 8 days incubation of amyloid in D₂O

H/D Exchange Pattern

Amyloid Fibril  Native
Fast 2D NMR and Real-time Monitoring of H/D exchange in protein folding

PNAS 2007
Brutscher et al.