NMR Spectroscopy
In
Structural Analysis

exercises – werkcollege 2009

http://nmr.chem.uu.nl/Education/structuralanalysis_notes.php

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Notes.

• Exercises labeled with a “B” are supplementary. Some are a bit more difficult, others are just additional exercises. Good practice material for the exam!
• Answers to questions are included at the end of this book.
• Answer the question yourself, before turning to the answers!
• Ask! The assistants are there to help you.

Chapter 1. Introduction

B1. NMR deals with nuclear magnetism, arising from the magnetic moment of the nucleus of some types of atoms. The everyday form of (macroscopic) magnetism as manifested by permanent magnets and magnetizable materials, has a different source. Find out which.

Chapter 2. Basic NMR Theory

2. a) Draw the energy diagrams and indicate the α and β spin states for the following nuclei:

\[ ^3\text{H} \quad \gamma^3\text{H} = 2.8535 \cdot 10^8 \text{(T·s)}^{-1} \quad (I = 1/2) \]

\[ ^{113}\text{Cd} \quad \gamma^{113}\text{Cd} = 5.9340 \cdot 10^7 \text{(T·s)}^{-1} \quad (I = 1/2) \]

b) At what frequency do these nuclear spins resonate given a 1.5 Tesla (T) magnetic field?
c) What are the corresponding angular frequencies (“hoeksnelheid”) of the precession motion?

3. A proton at 11.7 T resonates at 500 MHz. To what value must the magnetic field be raised in order to obtain resonance at 600 MHz?

4. Prove that the energy of a randomly oriented magnetic moment in the static \( B_0 \) field is only dependent on the \( z \)-component of the magnetic moment (see reader eq. 2.6 and 2.7).

5. Consider the compound chloroform (CHCl₃) where all carbon-atoms are of the \(^{13}\text{C}\)-isotope. The difference in the Larmor frequency of the \(^{13}\text{C}\) spin and \(^1\text{H}\) spin is 675 MHz. What is field strength of the \( B_0 \) field? What is the proton Larmor frequency? Use Table 1 on reader p. 9.

6. The equation of motion (reader eq. 2.11 on p. 10) is crucial to describe NMR phenomena. Derive reader eq. 2.12 from eq. 2.11 and verify that eq. 2.13 is a correct solution (p. 10/11).

3
B7. The earth magnetic field has a strength of roughly 45 μT.
   a) What is the proton resonance frequency in the earth magnetic field?
   b) What is the wavelength of the electromagnetic radiation that can be absorbed at this field?

Chapter 3. An ensemble of Nuclear Spins

8. a) Consider an ensemble of \(^1\)H nuclear spins. What is the ratio of the populations of the upper and lower states at 25 °C and a magnetic field of 2.35 T?
   b) What is the population difference between the two spin states per \(1\cdot10^6\) spins?
   c) What is the population difference for \(^{13}\)C, under the same conditions, per \(1\cdot10^6\) spins?

9. A sample of chloroform is placed in a NMR spectrometer with a field strength corresponding to a proton resonance frequency of 500 MHz.
   a) Sketch the net-magnetization vector of the proton spins in the laboratory frame under equilibrium conditions.
      We want to give a 90° x-pulse to create transverse magnetization.
   b) What should be the frequency of the B1-pulse?
   c) Assume that strength of the B1-field is 0.60 mT. What should be the duration \((t_p)\) of the 90° pulse?
   d) Sketch the effect of the 90°-pulse in the rotating frame using a vector diagram. What is the rotation frequency of the rotating frame?

10. Sketch in a vector diagram (rotating frame, on resonance) the effect on the magnetization of a certain proton for the following cases.
    a) Start with equilibrium \((\pm M_z)\) magnetization. Apply a 90° x-pulse.
    b) Repeat the same for a 90° y-pulse.
    c) and a 180° y-pulse
    d) and a 90° x-pulse
    e) and a 270° x-pulse. What is the difference compared with d)? What if the pulse is slightly miscalibrated (e.g. if it is slightly too short)?
    f) Start with +Mx magnetization. Apply a 90° x-pulse.
    g) Start with +Mx. Apply a 90° y-pulse.
    h) Start with +My. Apply a 90° x-pulse.
    i) Start with +My. Apply a 90° y-pulse.

11. You want to excite equilibrium magnetization. Unfortunately your excitation pulse is miscalibrated and is only an 80° pulse instead of a 90° pulse. How much of the equilibrium magnetization will be in the transverse plane?

B12. Use the equation-of-motion (reader eq. 2.11) to calculate the effect of a 90° y-pulse. The magnetic moment \(\mu\) for a single spin can be replaced by the net magnetisation vector \(M\) combining all spins of the ensemble.

B13. Consider exercise 9 again. Suppose you use a B1-pulse with a frequency of 400 MHz. To examine the effect of this pulse, we need to use a rotating frame with a rotation frequency of 400 MHz.
a) Sketch in a vector diagram the net-magnetization vector in equilibrium in this rotating frame.
b) Sketch in a vector diagram the magnetization vector of an individual spin in this rotating frame. What is the precession frequency of the spin in this rotating field?
c) What is then the effective B₀ field in this rotating field? Use Hz as the unit for magnetic field strength.
d) What is the total effective B-field in this rotating field, so B₀ + B₁? Assume a B₁ strength of 25 kHz.
e) Predict the result of a 10 µs B₁-pulse.

**Chapter 4. Spin relaxation**

   a) Draw the path that the net-magnetization vector follows back to equilibrium, once in the laboratory and once in the rotating frame.
   b) Same as a) but now only consider T₂-relaxation.
   c) Repeat this for an 180° x-pulse.

B15. Verify that reader eq. 4.2 and 4.3 are valid solutions of eq. 4.1.

**Chapter 5. Fourier Transform NMR**

16. What is the line width at half height (use reader eq. 5.8) of a signal with a T₂ of:
   a) 1.0 s?
   b) 10 ms?
   c) 10 µs?
   d) Associate the T₂’s of a), b) and c) with either a solid protein, chloroform, or a protein in solution. Explain using the graph on reader p. 20.
   e) Sketch the 1D spectra of compounds containing only two protons, with T₂ similar to those of a dissolved protein, dichloromethane and a solid protein. The difference in the Larmor frequencies of the two protons is 100 Hz.

17. Sketch roughly the Fourier transforms of the following FIDs (on the left the Mᵧ part of the FID, on the right the Mₓ part).
   a)
B18. The $T_1$ of a certain sample decreases with increasing temperature. At 20 °C it is necessary to leave 10 seconds between successive scans, while at 40 °C only 5 seconds are required. Suppose that 3 hours of spectrometer time are available and that the instrument is set up for operation at 20 °C. It takes 1 hour to warm the sample to 40 °C and to stabilize the temperature. Assume that the NMR signals are identical at the two temperatures. What is the best strategy for recording the NMR spectrum: running for 3 hours at 20 °C or warming the sample and running for 2 hours at 40 °C? Use eq. 5.9.

Chapter 6. Spectrometer hardware

19. a) What is meant by ‘resolution’?
   b) What are the possibilities to raise the signal-to-noise-ratio (S/N) for a $^1$H experiment?
   c) Compare the S/N in an NMR experiment using $^{13}$C nuclei at natural abundance with the S/N of $^1$H.

20. You want to record a spectrum of compound which has several signals in the range of -12 kHz to +5 kHz. To what value must the dwell-time be set?
B21. If the magnetic field varied by ± 10 nT between the edges of the sample, what level of error would this introduce in the resonance frequency of $^1$H nuclei at a magnetic field strength of 11.7 T (500 MHz)? Compare this error to the typical line width of a $^1$H signal of 1 Hz.

Chapter 7. NMR parameters

22. Consider a compound containing two $^1$H spins that give signals at 2.0 and 7.0 ppm, respectively. You perform a 1D experiment at a 500 MHz spectrometer. Calculate the precession-frequencies in the rotating frame for the two signals, assuming a carrier-frequency (the zero-frequency of the rotating frame) which is at 4.7 ppm.

23. The chemical shift of two equivalent methylene protons is 3.157 ppm. Their NMR signal is split in two lines due to the scalar coupling ($J$-coupling) to the attached $^{13}$C nuclear spin. The value of the scalar coupling constant is 132.15 Hz. The magnetic field strength is 0.5 T. What is the difference when the magnetic field is 10 T?
   a) Calculate the chemical shift difference between the two methylene signals.
   b) Calculate the frequency difference between each methylene signals and the reference signal of TMS at 0 ppm.
   c) What is the chemical shift difference when the field is ten times stronger?

Chapter 8. Nuclear Overhauser effect

24. The following NOEs are observed involving the aromatic protons of a tyrosine (see appendix p.110 for the atom nomenclature):

<table>
<thead>
<tr>
<th>atom pair</th>
<th>η-intensity</th>
<th>R (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$<em>{\delta2}$ - H$</em>{\epsilon3}$</td>
<td>0.125</td>
<td>2.45</td>
</tr>
<tr>
<td>H$<em>{\epsilon5}$ - H$</em>{\delta6}$</td>
<td>0.125</td>
<td>2.45</td>
</tr>
<tr>
<td>H$<em>{\delta2}$ - H$</em>{\beta1}$</td>
<td>0.05</td>
<td>?</td>
</tr>
<tr>
<td>H$<em>{\delta2}$ - H$</em>{\alpha}$</td>
<td>0.02</td>
<td>?</td>
</tr>
<tr>
<td>H$_{\delta2}$ - H?</td>
<td>0.01</td>
<td>?</td>
</tr>
<tr>
<td>H$_{\delta2}$ - H?</td>
<td>0.005</td>
<td>?</td>
</tr>
</tbody>
</table>

The distances between the δ and ε protons are known. Calculate the remaining distances and identify the two unassigned protons.

B25. Estimate the molecular weight where the NOE vanishes on a 500 MHz spectrometer (see reader p. 46).

Chapter 9. Relaxation measurements

26. Derive reader eq. 9.1 from equation 4.2 and prove that at $\tau = \ln(2) \cdot T_1$ no signal will be observed in an inversion-recovery experiment.
27. Assume $M_z$ magnetization. What happens in the following sequence, where the delay is a certain fixed waiting time: $90_x$ — delay — $180_x$ — delay. Give a vectorial representation of the evolution of the magnetization for two spins of unequal frequency (relaxation can be neglected). Use the rotating frame to draw the vector diagrams. Assume that the zero-frequency of this frame (the carrier-frequency) is the average of the frequencies of the two spins. Repeat this for the sequence: $90_x$ — delay — $180_y$ — delay. What is the difference?

28. You measure the $T_2$ of the $^{15}$N-spins in two $^{15}$N-labeled proteins under the exact same conditions. The average $T_2$ of protein A is 120 ms, while the average $T_2$ of protein B is 60 ms. Give an explanation for this difference.

## Chapter 10 Two-dimensional NMR

29. The structure of compound A undergoes a complete change by irradiating with light and is transformed to compound B: $A \rightarrow B$. This reaction can be measured in the SCOTCH 2D experiment (reader p. 56). The light pulse is short enough that we can assume that in the time of the pulse no precession of the spins occurs.

a) A proton A with a resonance $\omega_A$ has a frequency $\omega_B$ after the light pulse. Sketch the 2D-spectrum.

b) What is the difference if the reaction is not complete? Sketch the 2D-spectrum again.

30. The following Figure shows the NMR spectrum of the amino acid tryptophan (W) acquired in D$_2$O.

![Figure 1. $^1$H NMR spectrum of structure of Trp](image-url)
a) Sketch the COSY spectrum without fine structure of the cross-peaks.
b) Sketch the TOCSY spectrum.
c) In the NOESY the β-protons have cross-peaks to just two of the ring protons. To which of them? Sketch the NOESY spectrum.

31. The following Figure shows the NMR spectrum of the nucleotide adenosine-monofosfaat (AMP, see reader p. 81) acquired in H₂O.

![NMR spectrum of AMP](http://www.bmrb.wisc.edu/metabolomics/)

a) Sketch the COSY spectrum without fine structure of the cross-peaks.
b) Sketch the TOCSY spectrum.
c) Sketch the NOESY spectrum.

B32. The three methyl-protons in ethanal are equivalent and are 2.55, 2.78 en 2.88 Å from the aldehyde proton.
a) Calculate the NOE intensity for each of these distances assuming a calibration constant of 1·10⁻⁵⁰.
b) How many cross-peaks will be visible in the 2D NOESY spectrum?
c) Calculate the intensity of one of these cross-peaks.
d) Calculate the distance corresponding to this cross-peak.

**Chapter 11 The assignment problem**

33. Sketch and explain the ¹H-NMR spectrum of the following compounds (take only coupling over 1-3 bonds into account):
a) 1,2-dichloorbenzene
b) 1,3-dichloorbenzene
c) 1,4-dichloorbenzene

34. How many signals and what multiplicity do you expect in the ¹H spectrum for the following compounds (take only coupling over 1-3 bonds into account):
a) methanol
b) 1-bromo-2,2-dimethyl-propane
c) 2-chloro-1-methoxy-propane
35. Assign the proton spectra belonging to propanal (Fig. 3).

Peak data (ppm-value, intensity):

<table>
<thead>
<tr>
<th>ppm-value</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.798</td>
<td>200</td>
</tr>
<tr>
<td>9.793</td>
<td>371</td>
</tr>
<tr>
<td>9.789</td>
<td>203</td>
</tr>
<tr>
<td>2.499</td>
<td>87</td>
</tr>
<tr>
<td>2.495</td>
<td>87</td>
</tr>
<tr>
<td>2.475</td>
<td>265</td>
</tr>
<tr>
<td>2.470</td>
<td>266</td>
</tr>
<tr>
<td>2.451</td>
<td>271</td>
</tr>
<tr>
<td>2.446</td>
<td>275</td>
</tr>
<tr>
<td>2.426</td>
<td>92</td>
</tr>
<tr>
<td>1.137</td>
<td>503</td>
</tr>
<tr>
<td>1.113</td>
<td>1000</td>
</tr>
<tr>
<td>1.089</td>
<td>484</td>
</tr>
</tbody>
</table>

Figure 3. $^1$H NMR spectrum of propanal. (SDBSWeb : http://www.aist.go.jp/RIODB/SDBS/ (National Institute of Advanced Industrial Science and Technology, 07-09-06))

36. Two proton spectra is shown together with the formulas of the corresponding compounds (Fig. 4). Determine the structure.

Figure 4. $^1$H NMR spectra of some organic compounds.
B37. Figure 5 shows the NMR spectrum of the amino acid proline (P) acquired in D$_2$O.

![Figure 5. 1H NMR spectrum and structure of Pro.](image)

a) Draw the COSY spectrum without fine structure of the cross-peaks.
b) Sketch the TOCSY spectrum.
c) Sketch the NOESY spectrum.

Chapter 12 Biomolecular NMR

38. The following peptide segment is part of a protein sequence: . . . YVGLTSA . . . Fig. 6A shows the corresponding TOCSY spectrum of this part. Assign the segment. What is the origin of the peaks around 7 ppm?

39. Assume this segment has a $\beta$-sheet conformation. Add the characteristic peaks you would expect in the NOESY spectrum in Fig. 6B. Use Fig. 12.4 in the reader.

40. Now the segment is located in an $\alpha$-helix. Add the expected peaks in Fig. 6C (use Fig. 12.4).
Figure 6A. $^1$H, $^1$H TOCSY spectrum of -YVGLTSA-
Figure 6B. $^1$H,$^1$H TOCSY spectrum of -YVGLTSA-
Figure 6C. $^1$H,$^1$H TOCSY spectrum of -YVGLTSA-
B41. Consider again the peptide fragment YVGLTSA of exercises 38-40. Construct the NOE-pattern for a type-I turn starting at Gly in Fig. 7 (use Reader Fig. 12.4).

![Type I and Type II NOE patterns](image)

B42. We perform an exchange experiment, replacing H₂O with D₂O. Indicate for Figs. 6B-C (questions 39 and 40) the peaks that are still visible in this solvent (directly after the exchange of solvent).

For 6B, assume that the HN of the Tyrosine is in a H-bond with an opposite β-strand, and the β-strand is the outer strand of a β-sheet.

For 6C, first assume that the segment is located in the middle of a long α-helix. What would be different if the helix starts with the tyrosine(Y) of the segment?
Figure 7. $^1\text{H},^1\text{H}$ TOCSY spectrum of -YVGLTSA-
Answers

Chapter 1. Introduction

B1. The type of magnetism of permanent magnets and magnetizable materials is called ferromagnetism and is caused by a very strong interaction between the magnetic moments of the electron spin. The magnetic moment of the electron spin is roughly 650 times as large as that of a proton. A nuclear version of ferromagnetism can therefore only exist at temperatures close to 0 K.

Chapter 2. Basic NMR Theory

2. a) The energy diagram consists of two energy levels (both are spin $I = 1/2$ nuclei), note that for nuclei with a positive gyromagnetic ratio the $\alpha$-state ($m = 1/2$) has the lowest energy and the $\beta$-state the highest energy. The energy separation between the two levels is larger for the $^3$H spin since it has a higher gyromagnetic ratio and $\Delta E = \hbar \gamma B_z$.

![Energy Level Diagram]

b) The resonance frequency can be calculated using reader eq. 2.10a $\nu = \frac{\gamma}{2\pi} B_0$. Thus, the resonance frequency of $^3$H equals $2.8535 \cdot 10^8 (T \cdot s)^{-1} \times 1.5 T / 2\pi = 0.68122 \cdot 10^8 s^{-1} = 0.68122 \cdot 10^2 MHz = 68.1 MHz$. Likewise, the resonance frequency of $^{113}$Cd is $5.9340 \cdot 10^7 \times 1.5 / 2\pi = 1.4166 \cdot 10^7 s^{-1} = 14.2 MHz$.

Clinical MRI scanners typically operate at 1.5 T. The protons in your brain will then resonate at 63 MHz.

c) The precession has the same frequency as the resonance frequency. The corresponding angular frequencies are obtained by multiplying by $2\pi$ giving $4.28 \cdot 10^8 s^{-1}$ ($^3$H) and $8.90 \cdot 10^7 s^{-1}$ ($^{113}$Cd) (NOTE: the unit has to be $s^{-1}$ and not Hz).

3. Reader eq. 2.10a shows that the resonance frequency depends linearly (“recht evenredig”) on the strength of the magnetic field. Thus, the field should be raised to $11.7 / 500 \times 600 = 14.04 T$.

4. Reader eq. 2.6 denotes the dot-product (“in-produkt”) of the magnetic moment and the static magnetic field (two vectors), resulting in the scalar energy: $E = \vec{\mu} \cdot \vec{B}$. The orientation of the magnetic field is normally taken to be parallel with the z-axis, thus $\vec{B} = (0,0,B_z)$. The magnetic moment will have non-zero xyz-projections. Thus:
$$E = - \bar{\mu} \cdot \vec{B} = - \begin{pmatrix} \mu_x & \mu_y & \mu_z \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ B_0 \end{pmatrix}$$

$$= - \begin{pmatrix} \mu_x \cdot 0 + \mu_y \cdot 0 + \mu_z B_0 \end{pmatrix}$$

$$= - \mu_z B_0$$

5. Use reader eq. 2.10a: the resonance frequency (= Larmor frequency) of the $^{13}C$ spins is

$$\nu_{13C} = \frac{\gamma_{13C}}{2\pi} B_0.$$ For the $^1H$ spins $\nu_{1H} = \frac{\gamma_{1H}}{2\pi} B_0$. The difference in the Larmor frequency is thus:

$$\nu_{1H} - \nu_{13C} = \frac{\gamma_{1H}}{2\pi} B_0 - \frac{\gamma_{13C}}{2\pi} B_0 = \left(\gamma_{1H} - \gamma_{13C}\right) B_0 \quad \Rightarrow \quad B_0 = \frac{2\pi}{\gamma_{1H} - \gamma_{13C}} \nu_{1H} - \nu_{13C}.$$ Thus, the field strength is $2\pi \times 675 \cdot 10^6$ Hz / $(2.6752 \cdot 10^8 - 6.7266 \cdot 10^7$ (T·s)$^{-1}) = 4.2411500 \cdot 10^9$ Hz / $2.00254 \cdot 10^8$ (T·s)$^{-1} = 21.18$ T. The Larmor frequency of the protons is

$$\nu_{1H} = \frac{\gamma_{1H}}{2\pi} B_0 = 2.6752 \cdot 10^8$ (T·s)$^{-1}$ × $21.18$ T / $2\pi = 901.7$ MHz.

6. Reader eq. 2.11 denotes the cross-product (“uite-produkt”) of the magnetic moment and the magnetic field. The cross-product equals the determinant of the matrix shown on the right-hand side of reader eq. 2.11. The determinant is calculated by expansion by minors (“ontwikkeling naar de eerste rij”):

$$\frac{d\mu}{dt} = -\gamma \begin{vmatrix} e_x & e_y & e_z \\ B_x & B_y & B_z \\ \mu_x & \mu_y & \mu_z \end{vmatrix} = -\gamma \begin{vmatrix} e_x & B_y & B_z \\ \mu_y & \mu_z & \mu_x \\ e_y & B_x & B_y \end{vmatrix} + \begin{vmatrix} B_x & B_y \mu_z - B_z \mu_y \\ \mu_x & \mu_z \\ B_z & B_x \end{vmatrix} + \begin{vmatrix} B_x \mu_y - B_y \mu_x \end{vmatrix}$$

$$= -\gamma \begin{vmatrix} e_x \left(B_x \mu_z - B_z \mu_y \right) - e_y \left(B_y \mu_z - B_z \mu_x \right) + e_z \left(B_z \mu_y - B_y \mu_x \right) \end{vmatrix}$$

Note that $e_x$ is the unit vector $(1,0,0)$. Similarly, $e_y=(0,1,0)$ and $e_z=(0,0,1)$. That means that the $e_x$ term shows the change of $d\mu/dt$ in the x-direction, etc.

In the last line, it was used that $\vec{B} = (0,0,B_0)$. Thus,

$$\frac{d\mu}{dt} = -\gamma B_0 \mu_z$$

$$\frac{d\mu}{dt} = \gamma B_0 \mu_x$$

$$\frac{d\mu}{dt} = -\gamma B_0 \mu_x$$

$$\frac{d\mu}{dt} = 0$$

Note that the orientation of the resulting vector is given by the right-hand rule (see Figure on reader p. 10).

Now to verify that reader eq. 2.13 is a correct solution, we back substitute the solution in the differential equation and differentiate:
\[
\frac{d\mu_x}{dt} = \frac{d}{dt} \left( \mu_x(0) \cos(\gamma B_z t) + \mu_y(0) \sin(\gamma B_z t) \right) \\
= -\mu_x(0) \gamma B_z \sin(\gamma B_z t) + \mu_y(0) \gamma B_z \cos(\gamma B_z t) \\
= \gamma B_z \left( -\mu_x(0) \sin(\gamma B_z t) + \mu_y(0) \cos(\gamma B_z t) \right) = -\gamma B_z \mu_x \\
\frac{d\mu_y}{dt} = \frac{d}{dt} \left( -\mu_x(0) \sin(\gamma B_z t) + \mu_y(0) \cos(\gamma B_z t) \right) \\
= -\mu_x(0) \gamma B_z \cos(\gamma B_z t) - \mu_y(0) \gamma B_z \sin(\gamma B_z t) \\
= -\gamma B_z \left( \mu_x(0) \cos(\gamma B_z t) + \mu_y(0) \sin(\gamma B_z t) \right) = -\gamma B_z \mu_x \\
\frac{d\mu_z}{dt} = \frac{d}{dt} \mu_z(0) = 0
\]

Here you have to use the chain-rule ("ketting-regel"): \( d/dt \left( f(g(t)) = d/dg f \times d/dt g \right) \). Note that you have to assume that the answer is correct to establish the correctness of the answer!

B7. The resonance frequency can be calculated using reader eq. 2.10a \( \nu_0 = \frac{\gamma}{2\pi} B_z \). Thus, the resonance frequency of \(^1\text{H}\) equals \( 2.6752 \cdot 10^8 \, \text{T}^{-1} \times 45 \cdot 10^{-6} \, \text{T} / 2\pi = 1916.0 \, \text{s}^{-1} = 1.92 \, \text{kHz} \). The corresponding wavelength can be calculated using \( \lambda = c/\nu \) (\( c = 2,99792458 \cdot 10^8 \, \text{m/s} \)), resulting in a wavelength of \( 156,470 \, \text{m} = 1.56 \cdot 10^2 \, \text{km} \).

Chapter 3. An ensemble of Nuclear Spins

8. a) Using the Boltzmann equation (reader eq. 3.1), and reader eq. 2.9 and 2.10 to relate the magnetic field strength to an energy difference and \( k = 1.38066 \cdot 10^{23} \, \text{J/K} \), one finds:

\[
\frac{n_\beta}{n_\alpha} = e^{\frac{\Delta E}{kT}} = e^{\frac{\gamma \hbar B_z}{kT}} = e^{\frac{2.6752 \cdot 10^8 \, \text{T}^{-1} \times 6.62607 \cdot 10^{-34} \, \text{Js} \times 2.35 \, \text{T}}{2\pi \cdot 1.38066 \cdot 10^{23} \, \text{JK}^{-1} \cdot 298 \, \text{K}}} = e^{41.6562 \cdot 10^{-26}} = e^{41.6562 \cdot 10^{-26}} = e^{41.6562 \cdot 10^{-26}} = e^{41.6562 \cdot 10^{-26}} = e^{0.016113 \cdot 10^{-3}} = 0.99998389
\]

b) \( \frac{n_\beta}{n_\alpha} = (1 - n_\alpha) \)

\[
\Rightarrow 0.99998389 n_\alpha = 1 - n_\alpha \Rightarrow n_\alpha = \frac{1}{0.99998389} = 0.500004
\]

Thus, for an ensemble of \( 1 \cdot 10^6 \) spins there are \( 1 \cdot 10^6 \times 0.500004 = 500004 \) spins in the \( \alpha \)-state and \( 1000000 - 500004 = 499996 \) spins in the \( \beta \)-state. The population difference for a real sample, expressed as a fraction of \( 10^6 \) spins, is thus 8.06 spins.

c) For \(^{13}\text{C}\) spins, this is 2.02 spins.
9. a) The net-magnetization vector is aligned with static $B_0$-field under equilibrium conditions:

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

\[ B_0 \]

b) The frequency of the $B_1$-pulse should match the energy-difference between the two energy-levels, which means that it should be identical to the Larmor-frequency (see reader eq. 2.9 and 2.10). Thus, the frequency should be 500 MHz.

c) The magnetization vector precesses around the $B_1$-field with \[ \nu = \frac{\gamma}{2\pi} B_1 \] (see reader eq. 3.2). The rotation frequency is: \[ 2.6752 \times 10^8 \text{ (T·s)}^{-1} \times 0.60 \times 10^{-3} \text{ T} / 2\pi = 25546 \text{ Hz} \]. A $90^\circ$-pulse corresponds to a quarter of a rotation. Thus, the length should be \[ \frac{1}{4} \times \frac{1}{25546} = 9.79 \mu \text{s} \].

d) To describe the effect of the $90^\circ$-pulse, the rotating frame must have the same frequency as the $B_1$-pulse, 500 MHz.

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

10. a) $+ M_y$

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

\[ 90x \]

b) $- M_x$

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

\[ 90x \]

c) $- M_z$

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

\[ 180y \]

d) $- M_y$

```
\[ \mathbf{z} \]
```

\[ \mathbf{x} \]

\[ \mathbf{y} \]

\[ 90x \]
e) $-M_y$

The magnetization vector now travels in the opposite direction to end up at the same point. If the pulse is slightly too short, a $90^\circ$ $-x$-pulse will rotate the magnetization vector to some point above the $xy$-plane, whereas a $270^\circ$ $x$-pulse will rotate the vector to some point under the $xy$-plane. Note that because of the three times longer pulse, this 'mismatch' will be thrice as large, i.e. the effects of miscalibration are more pronounced for longer pulses.

f) $+M_x$

g) $+M_z$

h) $-M_z$

i) $+M_y$.

Note that all rotations are clock-wise.

11. The projection of the magnetization vector onto the $y'$-axis is given by $\sin(\alpha)$, where $\alpha$ is the flip-angle. Thus, 98.5% is in the transverse plane.
B12. Again we use the equation of motion, reader eq. 2.11. In the rotating frame \( \mathbf{B} = (0, B_1, 0) \):

\[
\begin{vmatrix}
    e_x & e_y & e_z \\
    B_x & B_y & B_z \\
    M_x & M_y & M_z \\
\end{vmatrix}
= e_x \begin{vmatrix}
    B_y & B_z \\
    M_y & M_z \\
\end{vmatrix}
- e_y \begin{vmatrix}
    B_x & B_z \\
    M_x & M_z \\
\end{vmatrix}
+ e_z \begin{vmatrix}
    B_x & B_y \\
    M_x & M_y \\
\end{vmatrix}
\]

\[
= e_x B_1 M_z - e_z B_1 M_x 
\]

\[
\frac{dM_x}{dt} = -\gamma B_1 M_z 
\]

Thus,

\[
\frac{dM_y}{dt} = 0 
\]

\[
\frac{dM_z}{dt} = \gamma B_1 M_x 
\]

The solution of the differential equation is analogous to reader eq. 2.13 (just swap the \( x, y, z \) indices):

\[
M_z(t) = M_z(0) \cos(\gamma B_1 t) + M_z(0) \sin(\gamma B_1 t) 
\]

\[
M_x(t) = -M_x(0) \sin(\gamma B_1 t) + M_x(0) \cos(\gamma B_1 t) 
\]

\[
M_y(t) = M_y(0) = 0 
\]

Which simplifies to: \( M_x(t) = -M_0 \sin(\gamma B_1 t) \) when starting from equilibrium \( M_x(t) = 0 \)

magnetization. Thus we see a simple rotation around the \( y \)-axis going from +z to –x.

B13. a). The net-magnetization is aligned with the \( B_0 \)-field, same as in 9a).

b) The magnetic moment of an individual spin will make an angle with \( z \)-axis and precesses around the \( B_0 \)-field with 500 MHz in the laboratory frame. When transforming into the rotating frame, the new precession frequency is \( \Omega = \omega - \omega_{\text{rot}} \), where \( \omega \) is the lab-frame Larmor frequency and \( \omega_{\text{rot}} \) the rotation frequency of the rotating frame. The spin thus precesses with 500 - 400 = 100 MHz.
c) As the spin still precesses in this rotating field, it must still feel a $B_0$-field. This field is now much smaller as the precession frequency is also smaller. When we express the strength of the $B_0$-field in MHz, the strength is 500 MHz in the lab-frame and 100 MHz in the rotating frame.

d) The total effective field is the vector-sum of the reduced $B_0$ and $B_1$. So the strength is:

$$B_{\text{eff}} = \sqrt{B_{0,\text{reduced}}^2 + B_1^2} = \sqrt{(100 \cdot 10^6)^2 + (25 \cdot 10^3)^2} \approx 100 \text{ MHz.}$$

The angle of this effective field with the z-axis is:

$$\alpha = \arctan \left( \frac{B_1}{B_{0,\text{reduced}}} \right) = \arctan \left( \frac{25 \cdot 10^3}{100 \cdot 10^6} \right) = \arctan(0.25 \cdot 10^{-3}) \approx 0.$$ 

e) Since the frequency of the $B_1$-pulse (400 MHz) is much lower than the resonance frequency of the spins (500 MHz), the spins are not affected by the pulse. So the orientation of the net-magnetisation vector will not change.

**Chapter 4. Spin relaxation**

14. a) In the laboratory frame: the magnetization vector starts somewhere in the $xy$-plane and precesses around the $z$-axis. Due to $T_2$-relaxation, the component in the $xy$-plane will shrink. Simultaneously, the vector starts moving back to the positive $z$-axis due to $T_1$-relaxation. In the rotating frame: now there is no precession around $z$-axis since the spin is on resonance. So after the pulse, the vector is along the $+y$-axis and the vector rotates around the $x$-axis back towards the $z$-axis. Check for yourself that the Figure is only correct if $T_1 = T_2$. What does it look like if $T_2 < T_1$?

b) In the laboratory frame: the magnetization vector starts somewhere in the $xy$-plane and precesses around the $z$-axis. Simultaneously, the length of the vector becomes smaller due to $T_2$-relaxation. In the rotating frame, the magnetization starts at the $+y$-axis and simply shrinks to eventually vanish.
c) After a 180° pulse the magnetization is along $-z$. As there is no $x$- or $y$-component, there is no $T_2$-relaxation, only $T_1$ relaxation. The magnetization vector starts at $-M_z$, will shrink, at some point be zero, and then start growing again to become $+M_z$. The description in the lab and rotating frame are the same since there will be no precession since there is no transverse magnetization, only longitudinal magnetization.

\[
\frac{dM_z}{dt} = \frac{d}{dt} \left( M_{eq} + [M_z(0) - M_{eq}] e^{-\frac{-t}{T_1}} \right)
\]

\[
= \frac{d}{dt} M_{eq} + \frac{d}{dt} M_z(0) e^{-\frac{-t}{T_1}} - \frac{d}{dt} M_{eq} e^{-\frac{-t}{T_1}}
\]

\[
= 0 + M_z(0) \frac{-1}{T_1} e^{-\frac{-t}{T_1}} - M_{eq} \frac{-1}{T_1} e^{-\frac{-t}{T_1}}
\]

\[
= -\frac{1}{T_1} [M_z(0) - M_{eq}] e^{-\frac{-t}{T_1}} = -\frac{M_z(t) - M_{eq}}{T_1}
\]

and

\[
\frac{dM_x}{dt} = \frac{d}{dt} \left( M_x(0) e^{-\frac{-t}{T_2}} \right)
\]

\[
= M_x(0) \frac{-1}{T_2} e^{-\frac{-t}{T_2}}
\]

\[
= -\frac{1}{T_2} M_x(0) e^{-\frac{-t}{T_2}} = -\frac{M_x(t)}{T_2}
\]

Chapter 5. Fourier Transform NMR

16. The line width (\(l.w. or \Delta\nu_{l.w.}\)) is related to the $T_2$ via: $l.w. = \frac{1}{\pi T_2}$. a) 0.318 Hz; b) 31.8 Hz; c) 31.8 kHz; d) chloroform = a, dissolved protein = b, solid protein = c. The $T_2$ becomes shorter as the tumbling time of a molecule becomes longer (= slower tumbling). Chloroform is a very small molecule so tumbles very rapidly, resulting in very slow $T_2$. 
relaxation = large T₂ values. A solid protein does not tumble at all, resulting in very fast T₂ relaxation = small T₂ values.

e)

17 Realize that in the observed FID the present frequency is \( \Omega = \omega_j - \omega_{rf} \) (reader page 23). \( \Omega \neq 0 \) when \( \omega_j \neq \omega_{rf} \), so when \( \nu_j \neq \nu_{rf} \). In a), transverse magnetisation starts at +y, then turns to +x, so turns faster than the rotating frame. Therefore in the spectrum the frequency will have a positive value. Also, when in and FID intensity goes to zero, T₂ relaxation occurs and line-widths are not zero (reader eq. 5.8). In the FID shown in b) the visible wavelength is longer, so the visible frequency \( \Omega \) is smaller than in a). The direction of rotation is the same as for a), so frequency is positive. The signal decays with similar speed as in a), so the T₂ and line width is the same. In c) the frequency and sign of the FID are the same as in b), but the signal decays to zero faster. That means that T₂ is shorter and the signal gets broadened. In d), the magnetisation starts at +y and then turns to –x. This means that is rotates slower than the rotating frame and gives a negative frequency.
B18. At 40 °C you can record twice as many scans within the same time as at 20 °C. In 2 hours, you thus record $2 \times \frac{2}{3} = \frac{4}{3}$ as much scans at 40°C as in 3 hours at 20 °C. The signal-to-noise is thus $\sqrt{\frac{4}{3}}$ better at 40 °C.

Chapter 6. Spectrometer hardware

19. a) In the context of NMR resolution is used to refer to the resolving power of the NMR spectrum, i.e. the degree to which peaks with small differences in resonance frequency can be distinguished.

b) According to reader eq. 6.1 one can: raise the number of spins (higher concentration), raise the field, increase the number of scans, lengthen the relaxation time (higher temperature, lower viscosity medium) or lower the temperature (notably of the detection circuit, not of the sample).

c) The S/N of a $^{13}\text{C}$ experiment will be much lower due to the lower number of spins and the lower gyromagnetic ratio. In most cases there will be slightly more favorable $T_2$ for the $^{13}\text{C}$ spins than the $^1\text{H}$ spin. Disregarding this last effect, the relative S/N is:

$$\frac{N_{^{13}\text{C}}}{N_{^1\text{H}}} \cdot \left( \frac{\gamma_{^{13}\text{C}}}{\gamma_{^1\text{H}}} \right)^2 \left( \frac{6.7266 \cdot 10^7}{2.6752 \cdot 10^8} \right) = 0.0111 \cdot 0.25144^2 = 0.0003519$$

So the $^1\text{H}$ experiment is roughly 3000 times as sensitive!

20. The dwell-time has be set according to the Nyquist-equation (see reader eq. 6.2). The largest absolute value of frequencies is 12 kHz. This frequency has to be sampled twice per period, so the dwell-time must be $0.5 \times 1/12000 = 0.0416$ ms.

B21. The relative error in the resonance frequency will be equal to the relative error in the magnetic field, which is $10 \text{ nT} / 11.7 \text{ T} \approx 1 \cdot 10^{-9}$. This creates an absolute error of $5 \cdot 10^8 \text{ Hz} \times 1 \cdot 10^{-9} = 0.5 \text{ Hz}$, roughly half of the natural line width. This is unacceptable and must be improved by shimming.

Chapter 7. NMR parameters

22. The precession-frequency in the rotating frame ($\Omega$) is given by: $\Omega = \omega - \omega_{rt}$, where $\omega$ is the frequency of the signal of interest and $\omega_{rt}$ the rotation frequency of the rotating frame (in Hz this would be $\nu - \nu_{rt}$).

In this case it is easy to calculate the frequencies with respect to an reference signal at 0 ppm. Thus, we need to translate ppm to Hz: at 500 MHz, 1 ppm corresponds to 1 millionth of 500 MHz is 500 Hz.

Thus, the frequency $\nu$ of signal at 2 ppm is $2 \times 500 = 1000$ Hz (with respect to a signal at 0 ppm), the frequency $\nu$ of the signal at 7 ppm is $7 \times 500 = 3500$ Hz (with respect to a signal at 0 ppm) and the frequency $\nu_{rt}$ of the rotating frame is $4.7 \times 500 = 2350$ Hz.
Thus, the frequency difference for the signal at 2 ppm is 1000 - 2350 = -1350 Hz (a counter clockwise rotation) and for the signal at 7 ppm is 3500 - 2350 = +1150 Hz (a clockwise rotation).

23. a) The frequency difference between the components of the doublet is determined by the scalar coupling constant and is not influenced by the magnetic field strength. The frequency difference is thus 132.15 Hz. At 0.5 T, the resonance frequency of \( ^1\text{H} \) equals \( 2.6752 \times 10^8 \frac{(\text{T} \cdot \text{s})^{-1}}{2\pi} \times 0.5 \, \text{T} = 21.3 \, \text{MHz} \), 1 ppm is thus about 21.3 Hz, and 132.15 Hz = 6.207557 ppm (due to \( 2 \times 3.103778926 \)).

b) The reference signal of TMS is at 0 ppm. The difference to the center of the methylene doublet is thus 3.157 ppm. At 0.5 T, the resonance frequency of \( ^1\text{H} \) equals \( 2.6752 \times 10^8 \frac{(\text{T} \cdot \text{s})^{-1}}{2\pi} \times 0.5 \, \text{T} = 21.3 \, \text{MHz} \). 3.157 ppm thus equals \( 3.157 \times 10^{-6} \times 21.3 \, \text{MHz} = 3.157 \times 21.3 \, \text{Hz} = 67.2 \, \text{Hz} \). The two lines are thus at 67.2 +/- 132.15/2 Hz from 0 ppm, which makes the shortest frequency distance equal to 1.13 Hz.

c) Ten times smaller, i.e. 0.6207557 ppm. Therefore the signals appear much closer to each other in the spectrum (3.46737785 and 2.84662215). So, the \( J \)-coupling constant is in Hz independent of the field strength, but leads to signals at different positions when the ppm-scale is considered.

**Chapter 8. Nuclear Overhauser effect**

24.

\[
\begin{align*}
H_{\delta_2} - H_{\beta_1} & \quad r = r_{ref} \times \left( \frac{V_{ref}}{V} \right)^{1/6} = 2.45 \times (0.125/0.05)^{**(1/6)} = 2.85 \, \text{Å}. \\
H_{\delta_2} - H_{\alpha} & \quad 2.45 \times (0.125/0.02)^{**(1/6)} = 3.32 \, \text{Å}. \\
H_{\delta_2} - H' & \quad 3.73 \, \text{Å}. \\
H_{\delta_2} - H? & \quad 4.19 \, \text{Å}.
\end{align*}
\]

The distance between the \( \delta_2 \) and \( \varepsilon_5 \) proton is \( 2 \times \cos(60) \times 2.45 + 2.45 = 4.9 \, \text{Å} \). The distance between the \( \delta_2 \) and \( \delta_6 \) proton is \( 2 \times \cos(30) \times 2.45 = 4.24 \, \text{Å} \). So the first distance, must be to the \( \beta_2 \)-proton, and the second is to the \( \delta_6 \) proton. (Use the molecular structure on reader p. 110 to prove these relations).
25. The NOE vanishes for $\omega \tau_c = 1.18$. Thus, $\tau_c = 1.18 / (500 \times 2 \times \pi \times 10^6) = 3.75 \cdot 10^{-10}$ s. This corresponds to a molecular weight of $\tau_c \times 2.4 \cdot 10^{12} = 900$ Dalton.

Chapter 9. Relaxation measurements

26. For the inversion-recovery experiment $M_z(0) = -M_{eq}$, thus:

$$M_z = M_{eq} + [M_z(0) - M_{eq}] e^{-t/T_1}$$

$$= M_{eq} + [M_{eq} - M_{eq}] e^{-t/T_1}$$

$$= M_{eq} - 2M_{eq} e^{-t/T_1}$$

$$= M_{eq} \left[ 1 - 2e^{-t/T_1} \right]$$

and

$$M_z(\ln(2)T_1) = M_{eq} \left[ 1 - 2e^{-\ln(2)T_1} \right] = M_{eq} (1 - 2e^{-\ln(2)}) = M_{eq} (1 - 2 \cdot \frac{1}{2}) = 0.$$  

(Using the rule that $-\ln(x) = \ln(1/x)$).

27. $+M_x$ - precession creates an $-x$ component – 180$^\circ$ pulse rotates the $+y$ component to $-y$ but the leaves the $x$-component unaffected: effectively the vector is reflected in the $xz$-plane – precession rotates the vector along the same angle as in the first part and in the same direction, thus leaving the vector aligned the $-y$-axis. This sequence is called a spin-echo (note that the last three Figures show projections on the $x' y' z'$-plane).

Using a 180$^\circ$ pulse the $y$-component is unaffected but the $-x$ component is rotated to $+x$: a reflection in the $yz$-plane. Now the same precession-angle ends up at $+y$.

28. The $T_2$ relaxation time depends on the rotational correlation time which is a measure for the tumbling time of the molecule. Large molecules have long tumbling times (tumble very slowly) and have very short $T_2$’s (see reader Figure p. 20). Thus protein B must be larger than protein A.
Chapter 10 Two-dimensional NMR

29.  
   a) a peak at coordinate \((t_1, t_2) (\omega_A, \omega_B)\).
   b) two peaks: one at \((\omega_A, \omega_A)\) and one at \((\omega_A, \omega_B)\). Their relative intensities will reflect their relative concentrations.

30. Diagonal peaks are present, but not shown here!

The \(\beta\)-protons only ‘see’ the H4 and H2. Note that the H2 is more than 5 Å from the H4 or H7.

31.  
   a) COSY: cross peaks between \(H1'\times H2'; H2'\times H3'; H3'\times H4'; H4'\times H5'/H5''\)
   b) TOCSY: cross peaks between all sugar protons. No cross peak between H2 and H8
c) NOESY: cross peaks between all sugar protons. $H1' \times H2$; $H1' \times H8$; $H2' \times H8$; $H3' \times H8$, $H4' \times H8$; $H5'/H5'' \times H8$, etc. Signal intensities between sugar and base depend on the base orientation.

B32. a) $V = C \times r^{-6}$. Substituting the values results in $3.63 \times 10^7$, $2.16 \times 10^7$ and $1.75 \times 10^7$, respectively.
b) Only 1 since the methyl protons are dynamically averaged to equivalence.
c) It will be the sum of the volumes above, so $7.56 \times 10^7$.
d) The corresponding single distance is 2.25 Å, shorter than the three actual distances! When calculating protein structures from NOESY cross-peak volumes this effect must be taken into account to prevent distorted structures.

Chapter 11 The assignment problem

33. The chemical shift of the protons in these compounds will be roughly the same, around 6-7 ppm. The number of signals and the splitting will differ.
a) 1,2-dichlorobenzene has two sets of chemically equivalent protons, $H3/H6$ and $H4/H5$. Within the $H3$ is coupled to the $H4$ and the $H6$ to the $H5$, thus they have the same coupling pattern and they are magnetically equivalent. For the $H4/H5$ is the same thing. So one expects two signals, each split in a doublet (1:1).
b) 1,3-dichlorobenzene has three different sets of protons: the $H2$, the $H4/H6$ and the $H5$. The $H5$ is coupled to the $H4$ and the $H6$ so will be a triplet (integral 1/4:1/2:1/4). The $H4$ and $H6$ are split in a doublet (1:1). The $H3$ is a singlet (1).
c) 1,4-dichlorobenzenen is fully symmetric, so all 4 protons experience the same chemical environment. The protons are only coupled to the adjacent proton and both have the same coupling pattern, so they are also magnetically equivalent, so one signal without splitting is observed (integral 4).

34. a) CH$_3$OH
   -CH$_3$ doublet (1:1); -OH: quartet(1:3:3:1)
b) (CH$_3$)$_3$C–CH$_2$Br
   -CH$_3$ singlet; -CH$_2$ singlet
c) CH$_3$–CHCl–CH$_2$–O–CH$_3$
   -CH$_3$ doublet; -CH quartet of triplets (1:2:1) in case $J_{CH3-CH} >> J_{CH2-CH}$ or a triplet of quartets in case $J_{CH3-CH} << J_{CH2-CH}$. If the two J’s are equal, then a sextet results with relative intensities 1:5:10:10:5:1; -CH$_2$ doublet; -OCH$_3$ singlet.

35. The triplet at 9.7 ppm –COH; the quartet of doublets at 2.4 ppm –CH$_2$, the triplet at 1.1ppm –CH$_3$.

36. a) 2-chloro-propane; c) diethyl-para-benzenedicarboxylate.
Chapter 12 Biomolecular NMR

38. see Figure below. The peaks around 7 ppm are the aromatic $\delta,\varepsilon$ protons of Y.

39. add $d_{\alpha N(i,i+1)}$ strong peak and $d_{NN(i,i+1)}$ weak peak. Note that the $d_{NN(i,i+1)}$ contacts should be symmetrical with respect to the diagonal.
40. add $d_{\alpha N}(i,i+1)$ weak peak, $d_{NN}(i,i+1)$ strong peak, $d_{NN}(i,i+2)$ weak peak, $d_{\alpha N}(i,i+3)$ medium peak, $d_{\alpha N}(i,i+4)$ weak peak,
B42. If the β-strand is on the outside of a β-sheet, the amide proton of every second residue is exposed to the solvent. E.g. if Y’s HN is in a H-bond, the amide protons of V,L,S will be unprotected and will exchange rapidly disappear from the spectrum. If the segment is in the middle of a long helix, all peaks remain visible because the amide protons are protected by hydrogen bonds (at least initially, they will be exchanged only very slowly). If the helix starts at Y, the amide protons Y,V,G will be unprotected and will exchange rapidly and disappear from the spectrum.