Exam: Fundamentals of NMR Spectroscopy

4423FNMRS-1516FWN

October 22nd 2015, 14h-17h, C1

Lecturer(s):
- Dr. H. van Ingen
- Dr. G. Siegal

This examination consists of **6 questions** on **15 pages.**
Answers must be given on the question forms and all sheets must be handed in.
The questions are dived in 2 sections, that are marked separately.
For a pass is required: a 5.5 overall, as well as a 5 for each section.

Weight per question

- Section Siegal
  - 1. (30)
  - 2. (50)
  - 3. (20)
- Section Van Ingen
  - 4. (8)
  - 5. (8)
  - 6. (8)

Allowed information sources and tools:

- Information sheets below.
- Non-graphical calculator

Answers must be given on the question forms and all sheets must be handed in.
Please write with blue or black ink. Don't use a pencil!
Please answer in English, Dutch if you have to.
Write out all intermediate results CLEARLY as you will get partial credit for them if we can understand them.

Clearly indicate on each sheet: name and study number

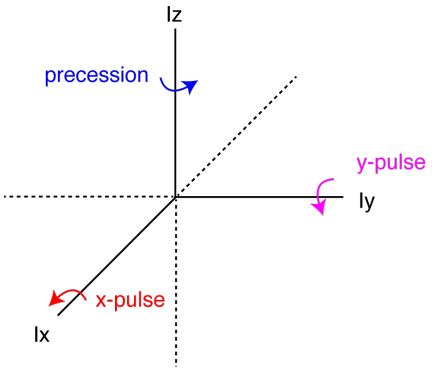
Good Luck!

Constants

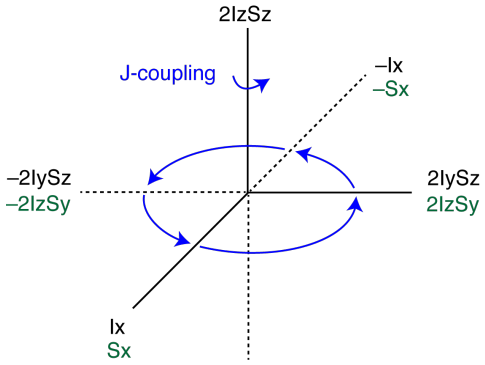
Nuclide	*γ (10⁷ T⁻¹ rad s⁻¹)*	*Natural abundance*
^{1}H	26.75	99.99%
^{13}C	6.73	1.1%
^{15}N	-2.71	0.37%
^{31}P	10.84	100%

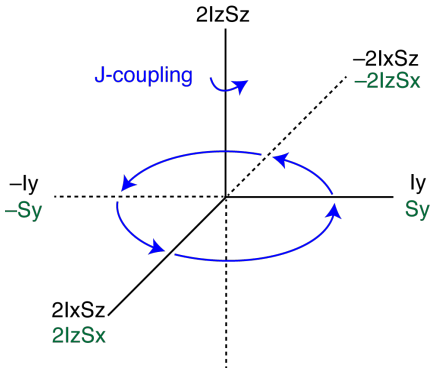
$k = 1.38 \times 10^{-23}$ J/K
$h = 6.63 \times 10^{-34}$ J/s

Rotations



Larmor precession angle: Ωt





J-coupling angle: πJt

Formulas

$$M_z(t) = M^0 + \left[M_z(0) - M^0\right]e^{-R_1 t}$$

$$M_{xy}(t) = M^0 e^{-t/T_2}$$

$$j(\omega) = \frac{2\tau_c}{1+\omega^2\tau_c^2}$$

Trigionometric relations

$$\cos^2 A + \sin^2 A = 1$$

$$\sin(A \pm B) = \sin A \cos B \pm \cos A \sin B$$

$$\cos(A \pm B) = \cos A \cos B \mp \sin A \sin B$$

$$\sin A \cos B = \frac{1}{2}[\sin(A+B) + \sin(A-B)]$$

$$\cos A \sin B = \frac{1}{2}[\sin(A+B) - \sin(A-B)]$$

$$\sin A \sin B = \frac{1}{2}[\cos(A-B) - \cos(A+B)]$$

$$\cos A \cos B = \frac{1}{2}[\cos(A+B) + \cos(A-B)]$$

$$\sin 2A = 2 \sin A \cos A$$

$$\cos 2A = \cos^2 A - \sin^2 A$$

$$\sin^2 A = \frac{1}{2}(1 - \cos 2A)$$

$$\cos^2 A = \frac{1}{2}(1 + \cos 2A)$$

$$\cos A = (e^{iA} + e^{-iA})/2$$

$$\sin A = (e^{iA} - e^{-iA})/2i$$

$$e^{iA} = \cos A + i \sin A$$

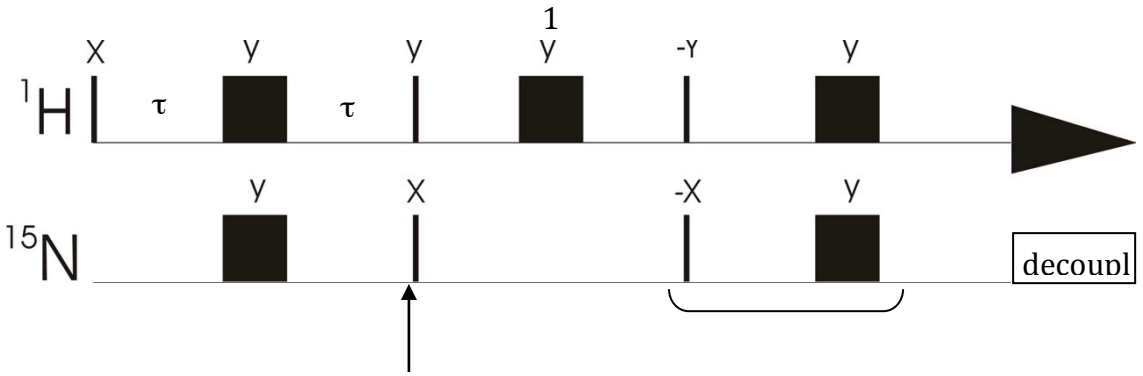
$$e^{-iA} = \cos A - i \sin A$$

Section Siegal

1) Calculate the following values:

   a) Bruker has just developed a fabulous new magnet for me with a field strength of 1.5 GHz for ^{1}H's. What is the strength of the magnet in Tesla (T)?
   b) If my ^{1}H spectrum has peaks at 0.5 ppm and 12 ppm, what is the spectral width in Hz on this fabulous magnet?
   c) Assuming I use quadrature detection, how frequently do I have to sample the ^{1}H spectrum in b in order to assure there is no folding (aliasing) of the peaks. What dwell time does this equate to?
   d) What is the ratio of the population in the high energy state (β) to that in the low energy state (α) at this field strength for ^{1}H's at 298K?

2) NMR Experiments



The figure above presents a schematic view of the HSQC experiment we discussed in the course. Narrow vertical lines indicate 90° pulses and wide ones 180° pulses with the phase of each indicated above the pulse. The black horizontal arrow indicates the acquisition period during which ^{15}N nuclei are decoupled. Please answer the following questions based on this figure.

a) Indicate the points where the *i*) the ^{15}N chemical shift is encoded and *ii*) the ^{1}H chemical shift is detected.
b) What do I have to do with this experiment in order to encode the ^{15}N chemical shift? How do I have to treat the data to have a 2D frequency domain spectrum?
c) What is the purpose of pulse 1?
d) What happens during the time marked τ?
e) Describe the effect of the first pulse on ^{1}H equilibrium magnetization using the product operator formalism.
f) At the point indicated by the arrow, the magnetization has the form:

$$I_y \cos(2\pi J_{IS} t) - I_x S_z \sin(2\pi J_{IS} t)$$

In order to have pure anti-phase magnetization, how long should the period τ be?
g) What is the effect on the operators of the 2 90° pulses immediately after the arrow?
h) What is the net effect of the 4 pulses indicated by the bracket?
i) What operator is present during acquisition?

3) Varia

a) Draw the way the peak in an NMR spectrum would look for magnetization described by the following types of operators at the start of the acquisition time:

I. I_x

II. $2I_xS_z$

III. $2I_xS_y$

b) Assume you have one spin that is not coupled. Using a vector diagram similar to those presented in the class, show the effects of the following pulse sequences on the spin. Assume all magnetization starts from equilibrium.

A) π_y

B) $\pi/2_x$ –τ- π_y –τ- $\pi/2_x$

Section Van Ingen

4. Measuring ^{15}N R_1-relaxation times in proteins (8p)

The amide backbone ^{15}N nucleus is often used as a probe of protein backbone dynamics. A drawback of the amide nitrogen (N) is that it is close to the amide-proton (H_N). There is also a significant *J*-coupling between N and H_N, $^1J_{NH}$
Now consider the inversion-recovery experiment, applied to N:

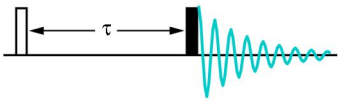


Fig. 1 . The inversion recovery experiment.
The open bar represents a 180° pulse, the filled bar a 90° pulse.

4a. What is the effect of the $^1J_{NH}$-coupling during period τ? Explain briefly. (3p)

The H_N and N nuclei form a two-spin system (labeled H and N), of which the longitudinal relaxation during the inversion recovery experiment is described by the Solomon-equations:

$$\frac{dH_z(t)}{dt} = -R_{1,H}\left(H_z - H_z^0\right) - \sigma\left(N_z - N_z^0\right)$$
$$\frac{dN_z(t)}{dt} = -R_{1,N}\left(N_z - N_z^0\right) - \sigma\left(H_z - H_z^0\right) \qquad [1]$$

4b. On which parameters of Eq. [1] will the inversion-recovery curve of the amide nitrogen depend? Briefly explain. (2p)

While not trivial, it is possible to determine residue-specific $R_{1,N}$-values for the amide nitrogen. Fig. 2 shows the $R_{1,N}$ values obtained for a protein domain.

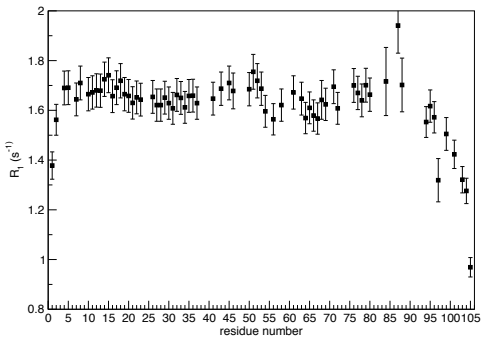


Fig. 2 . $R_{1,N}$ values for a protein domain.

4c. What is your response to someone who says: "That data for the terminal regions of the protein must be wrong, since it deviates so much from the rest." (3p)

5. DNA intercalators in exchange (8p)

The NOESY pulse-sequence (Fig. 3) is often used to study chemical exchange experienced by ^{31}P nuclei in DNA. In this context, it is called Exchange Spectroscopy (EXSY). EXSY spectra of ^{31}P in DNA do not show cross-peaks due to the NOE effect.

5a. Give two reasons why ^{31}P-^{31}P NOEs will be so small that they can be ignored. (2p)

EXSY is used for instance to study the binding of a drug to DNA:

$$DNA + drug \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} DNA:drug$$

^{31}P nuclei in the binding interface may experience different chemical environments in these two states and thus have different chemical shifts in free and bound states.

EXSY (Fig. 4) works the same way as the NOESY, except that magnetization transfer is now brought about by the chemical exchange between free and bound DNA.

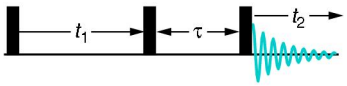
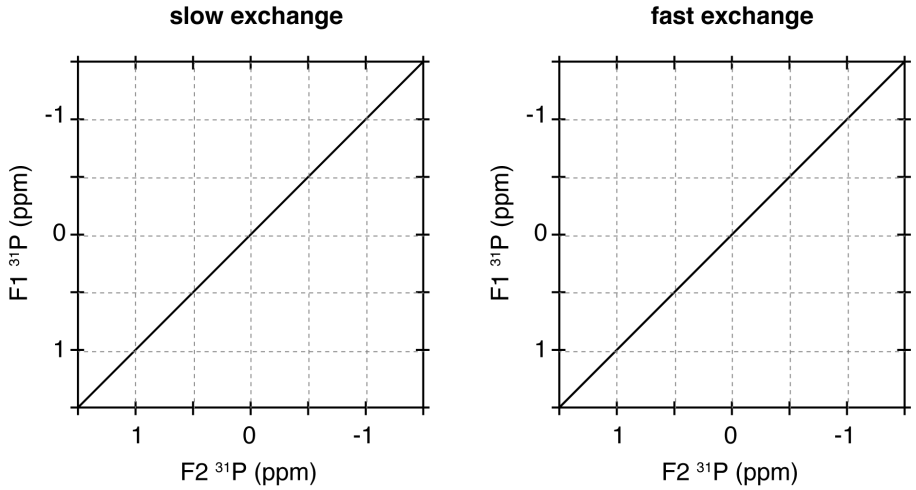


Fig. 3 . The EXSY experiment.
The filled bars are 90° pulses, mixing time τ is typically tens of milliseconds.

5b. Sketch in the figures below the ^{31}P EXSY spectra observed for both indicated exchange regimes. Assume chemical shifts of –1 ppm in the free and +1 ppm in the bound state. Assume 50% of the DNA is bound. (4p)



Mazzini et al. used this experiment to determine the average life-time ($1/k_{off}$) of the doxorubicin–DNA complex. They found k_{off} is 0.8 ± 0.3 s^{-1}.

5c. Explain how the EXSY experiment can be used to determine k_{off}. (2p)

6. A constant-time {^{1}H,^{13}C}-HSQC (8p + BONUS)
Fig. 4 shows the pulse-sequence for a 2D {^{1}H,^{13}C}-HSQC.

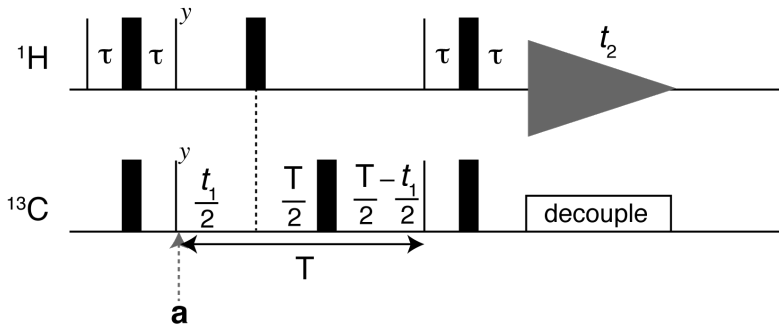


Fig. 4 . Constant-time HSQC pulse sequence. Narrow bars are 90° pulses, wide bars 180°. All pulses are along *x*, unless indicated otherwise.

The difficulty with the ^{13}C-HSQC is that $^1J_{CH}$ varies a lot between different chemical groups. Methyl groups have $^1J_{CH}$ = 125 Hz, while for an aromatic group $^1J_{CH}$ =165 Hz.
We will compare the efficiency of this pulse-sequence for a CH_3 and an aromatic CH group. Assume τ is set to achieve full polarization transfer for the average coupling constant, 145 Hz.

6a. Calculate how much anti-phase ^{13}C magnetization is generated at point **a** for the CH_3 group, and for the CH group. (6p)

6b. Name two benefits of using decoupling during detection of the FID. (2p)

BONUS (4p):
Another problem with the ^{13}C-HSQC is that for molecules with all carbon atoms labeled as ^{13}C, there will many that have a $^1J_{CC}$ coupling. This limits the resolution in the ^{13}C dimension. This particular pulse sequence is designed to suppress this J-coupling.

- Analyze what happens to an antiphase 13C 8magnetization term during the constant-time period T. Consider ^{13}C Larmor-precession of ^{13}C, J-coupling to its directly bonded proton and J-coupling to an adjacent ^{13}C. (3p)
- What should be the value of T to suppress $^1J_{CC}$? (1p)