Introduction to NMR

Lukáš Žídek

CEITEC, Masaryk University, Brno, Czech Republic

Outline

- Nuclei vs. electrons
- NMR spectrometer and NMR experiment
- Chemical shift, dipolar and J-coupling
- Relaxation in NMR spectroscopy
- Signal processing in NMR spectroscopy
- Two- and multi-dimensional NMR spectroscopy
- Structure and dynamics of molecules from NMR data

What is not covered:

- NMR of quadrupolar nuclei
- Solid-state NMR
- Magnetic resonance imaging

Nuclei vs. electrons

Nuclei vs. electrons

The same physics:

$$\vec{\mu} = \gamma \vec{I} = \frac{g}{2}\frac{Q}{m}\vec{I}$$

$$E = -\vec{\mu} \cdot \vec{B}$$

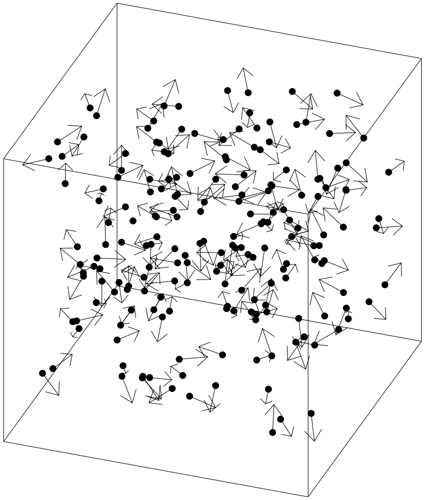
$$\vec{\omega} = -\gamma \vec{B}$$

Nuclei vs. electrons

- **Fundamental differences**
  - electron is a simple particle, well described by QED
    $g = 2.002\,319\,304\,361\,46(56)$ from 2008 measurement
    $g = 2.002\,319\,304\,363\,28(152)$ from QED calculations
  - proton is a complex particle
    $g = 5.585694713(46)$ from experiment
    $g = ?$ from theory (QCD)
- **Technical differences**
  $\gamma(\mathrm{e}^-) \approx 658 \times \gamma(\mathrm{p}^+)$
  - lower frequencies of nuclei
    $\Rightarrow$ different hardware (radio waves vs. microwaves)
  - slower relaxation of nuclei (typically 10^{-2}–10^{0} s)
    $\Rightarrow$ more time for pulsed experiments
  - lower sensitivity
- **Chemical differences**
  - few unpaired electrons in typical compounds
  - 10^0–10^4 protons in organic/biochemical molecules

NMR spectrometer
NMR experiment

Magnetic moments in molecules



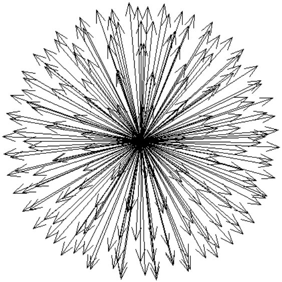
- ● molecule
- ↗ magnetic moment

	S	$\frac{10^{-9}\gamma}{\mathrm{rad\,s^{-1}T^{-1}}}$	%
e^-	1/2	−182.000	100
^{1}H	1/2	0.277	99.98
^{13}C	1/2	0.067	1.1
^{14}N	1	0.019	99.6
^{15}N	1/2	−0.027	0.4
^{17}O	5/2	−0.036	0.04
^{19}F	1/2	0.252	100
^{31}P	1/2	0.108	100
^{129}Xe	1/2	−0.075	24.4

quadrupolar (relax fast)

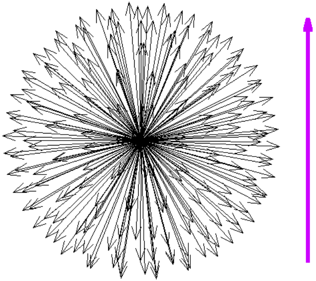
rare isotopes (enrichment)

NMR sample outside magnet



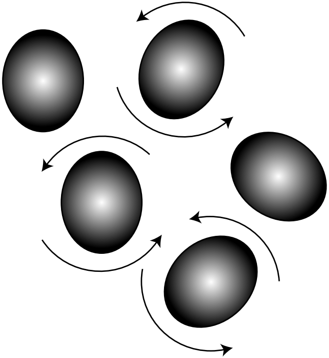
in equilibrium (spherical symmetry)

NMR sample inside magnet

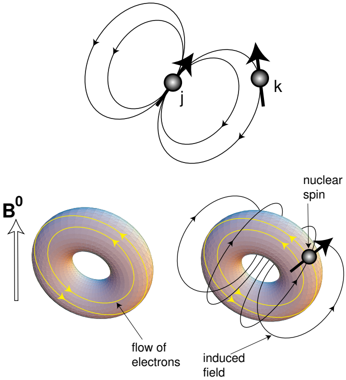


not in equilibrium (vertical force field)

Relaxation via coupling with molecular rotation



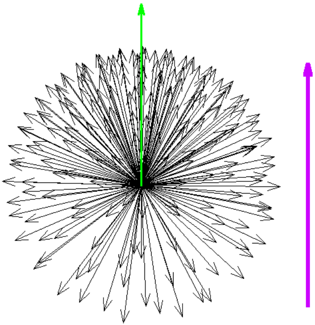
reproduced from M. H. Levitt: Spin Dynamics



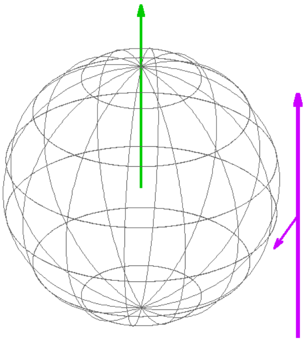
Polarization

Boltzmann distribution: $P(\theta) \propto \mathrm{e}^{-\frac{E}{k_\mathrm{B}T}} = \mathrm{e}^{\frac{\vec{\mu}\cdot\vec{B}}{k_\mathrm{B}T}} \Rightarrow M_z = \frac{N}{V}\frac{\mu^2 B}{3k_\mathrm{B}T}$

Precession (angular momentum in a field): $\vec{\omega} = -\gamma B$



Excitation



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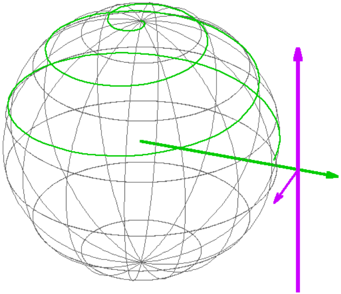
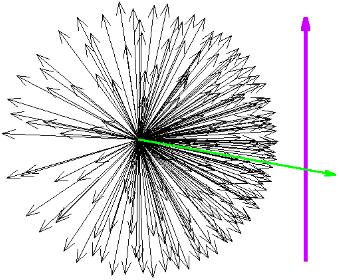
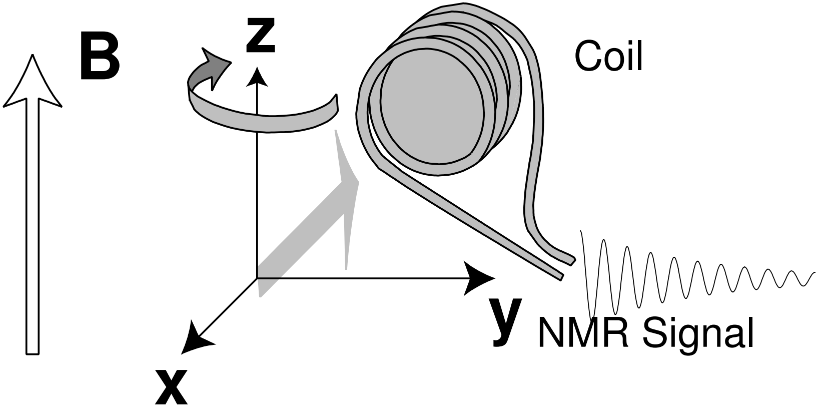


Coherent evolution

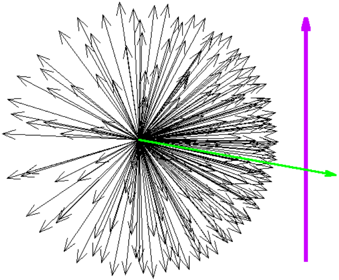


Signal detection

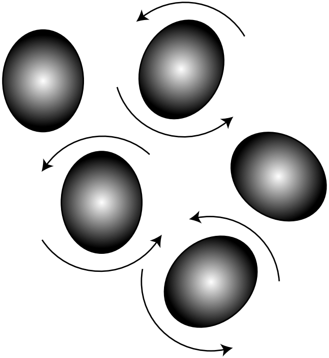


reproduced from M. H. Levitt: Spin Dynamics

Non-equilibrium distribution of magnetic moments



Relaxation via coupling with molecular rotation



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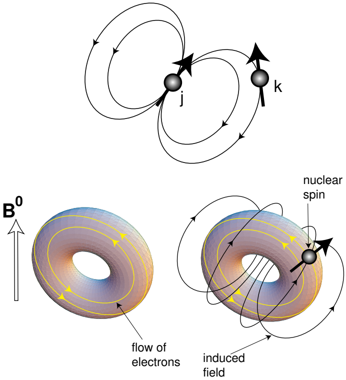
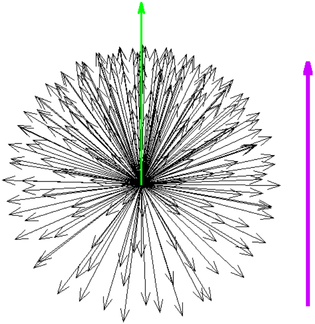
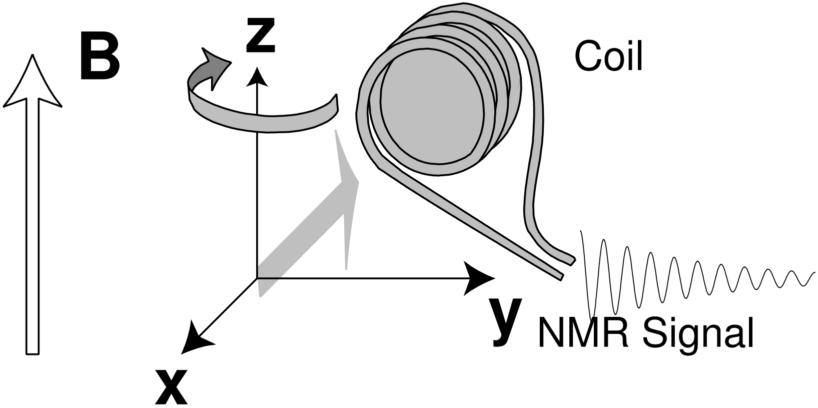


Return to equilibrium



Signal decay



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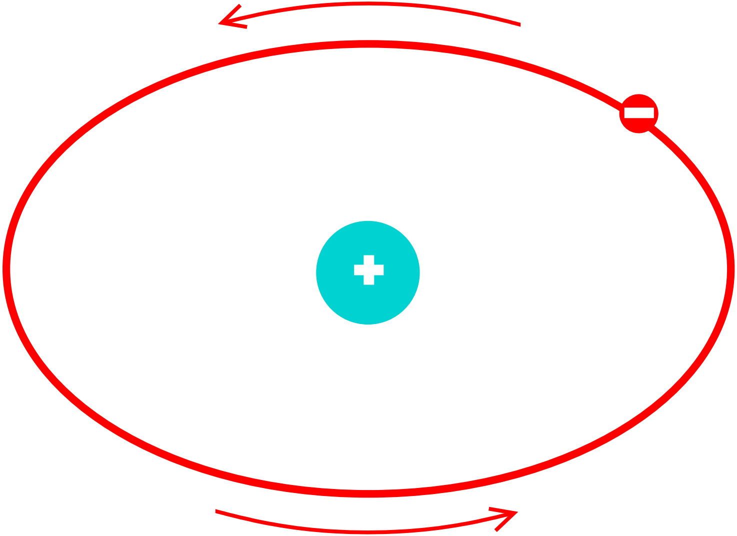
Chemical shift
Dipolar coupling
J-coupling

Shielding of proton in hydrogen atom



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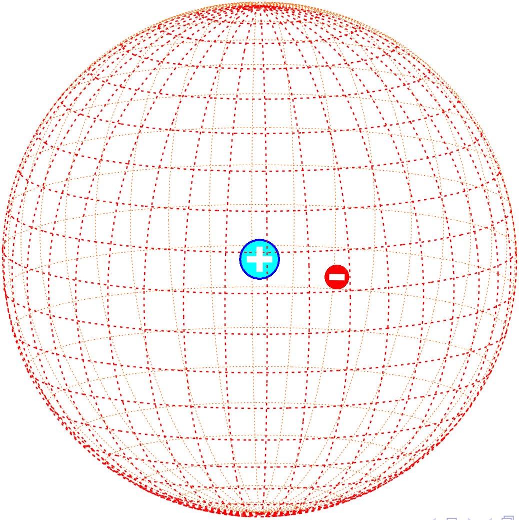

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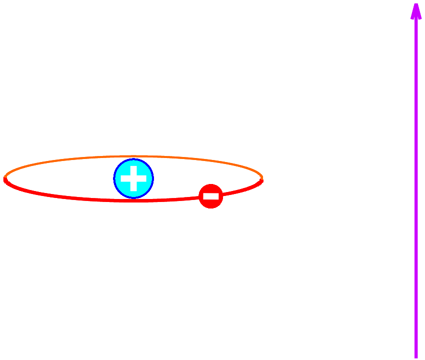

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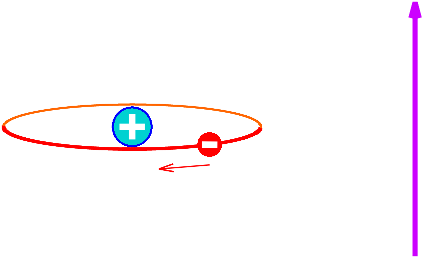

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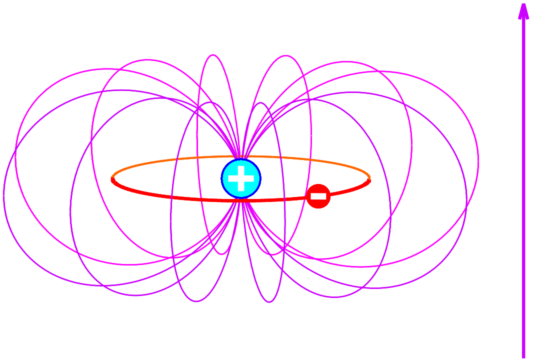

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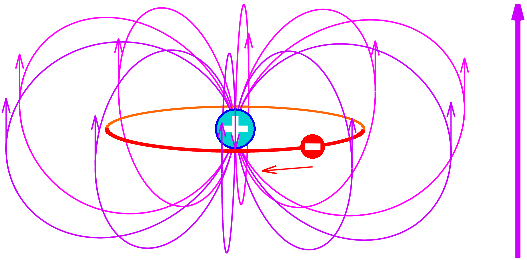
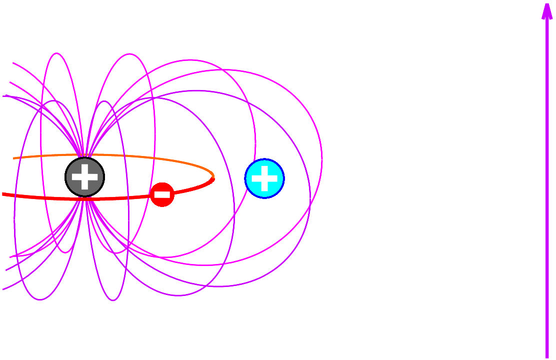


Shielding and deshielding of nuclei in molecules



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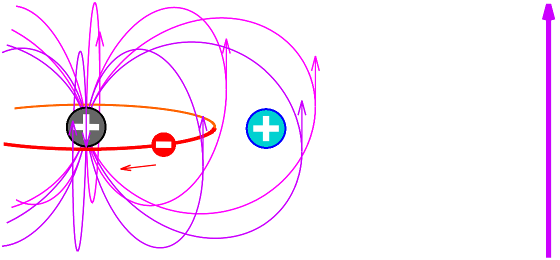

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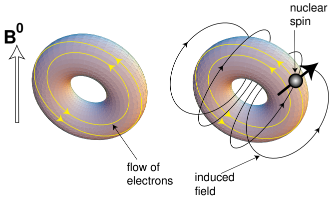

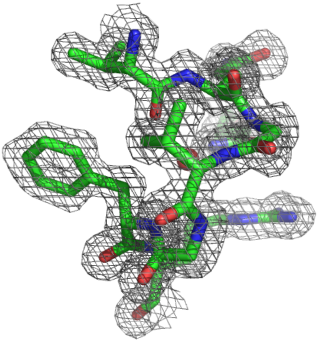

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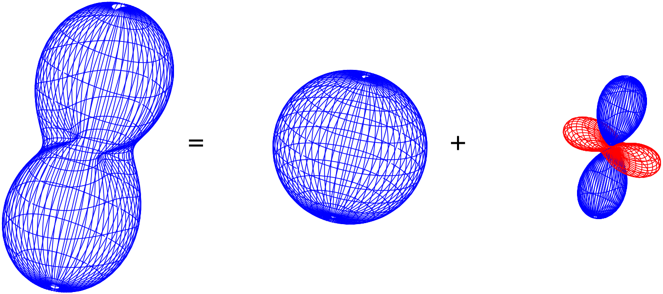
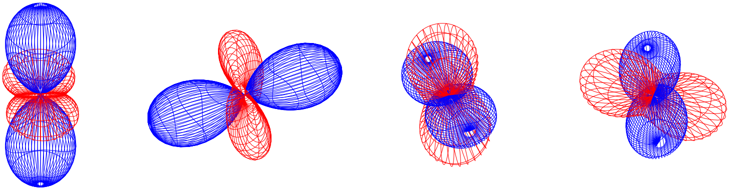


$$\omega = -\gamma \underbrace{(1 - \sigma)}_{\text{shielding}} B_0$$

$$\sigma = \sigma_{\text{isotropic}} + \sigma_{\text{anisotropic}}$$

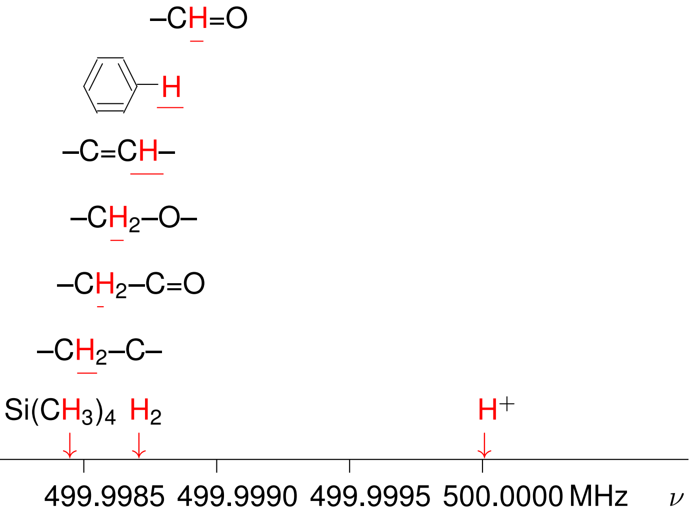
Shielding and deshielding of nuclei in molecules



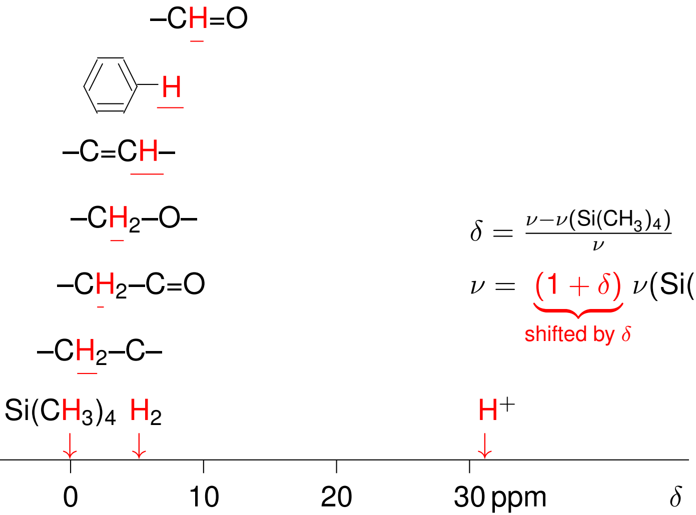
Molecular tumbling in solution $\Rightarrow$ $\langle \sigma_{\text{anisotropic}} \rangle = 0$

$$\Rightarrow \quad \langle \sigma \rangle = \sigma_{\text{isotropic}}$$

Chemical shifts



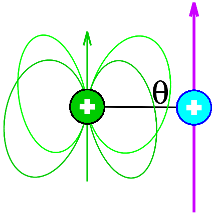
Chemical shifts



$$\delta = \frac{\nu - \nu(\mathrm{Si(CH_3)_4})}{\nu}$$

$$\nu = \underbrace{(1+\delta)}_{\text{shifted by } \delta} \nu(\mathrm{Si(CH_3)_4})$$

Dipolar coupling



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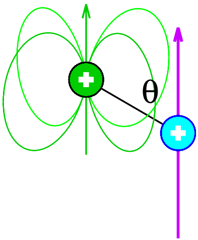

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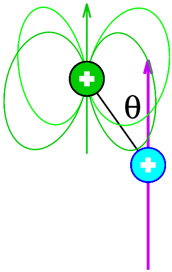

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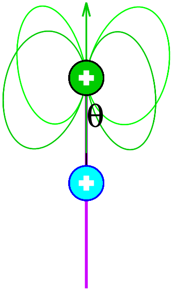


Dipolar coupling

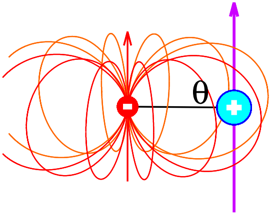
$$\nu(\theta) = \nu_0 + D(\theta)$$

$$D = -\frac{\gamma_1 \gamma_2 \mu_0 h}{8\pi^3 r_{12}^3} \left(\frac{3}{2} \cos^2 \theta - \frac{1}{2} \right)$$

In isotropic solution:

$$\left\langle \frac{3}{2} \cos^2 \theta - \frac{1}{2} \right\rangle_\theta = 0 \quad \Rightarrow \quad \langle D \rangle_\theta = 0$$

Dipolar coupling



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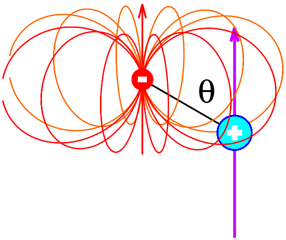

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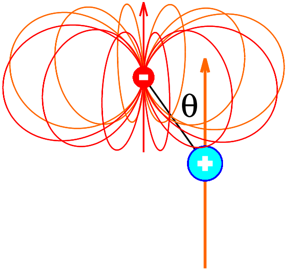

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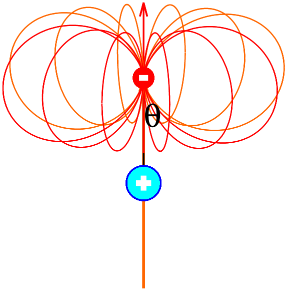

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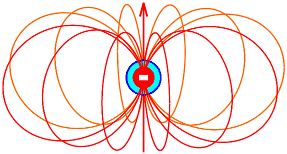
Fermi contact interaction



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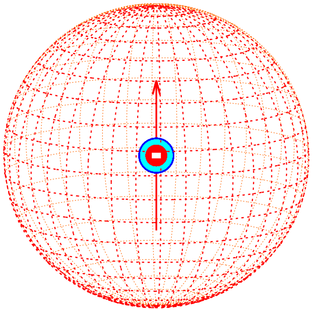
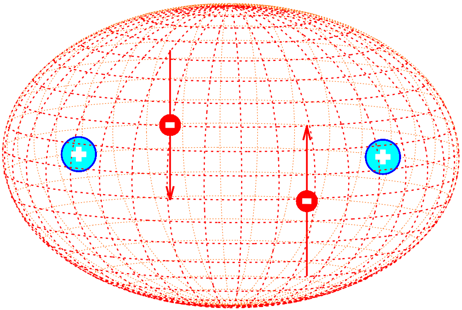


Fermi contact interaction

$$E = -\frac{2\mu_0}{3} \langle \vec{\mu}_\mathrm{n} \cdot \vec{\mu}_\mathrm{e} \rangle \, |\psi(\text{inside nucleus})|^2$$

Does not depend on orientation (scalar product $\vec{\mu}_\mathrm{n} \cdot \vec{\mu}_\mathrm{e}$)

J-coupling



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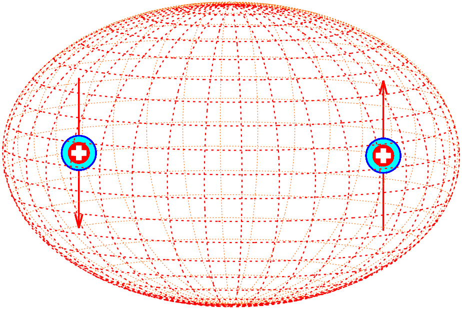

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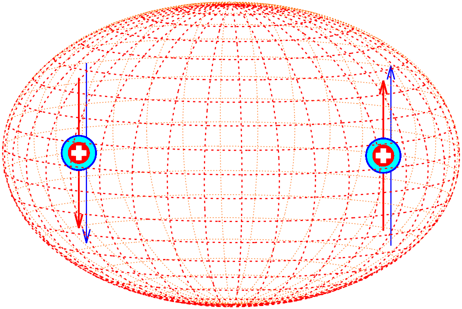


J-coupling

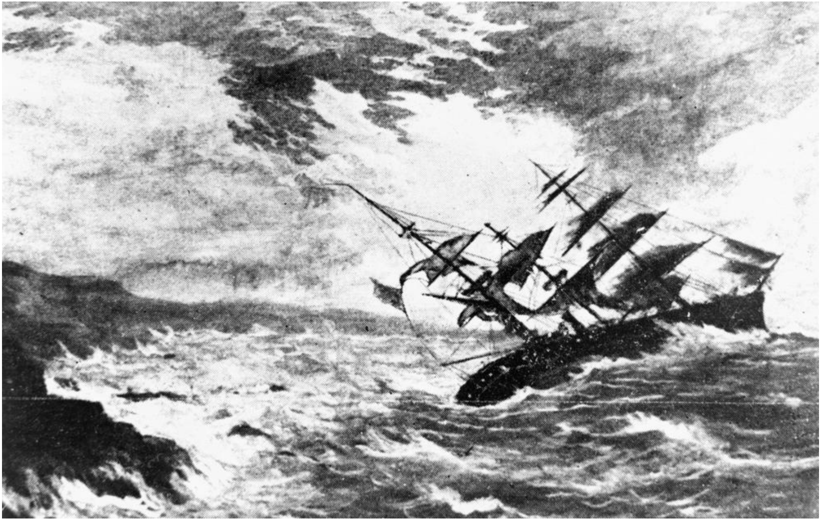
$$\nu = \nu_0 \pm J$$

J (C–H)	130–230 Hz	
J (N–H)	90 Hz	
J (C–C)	35–55 Hz	
J (N–C)	10–15 Hz	
J (H–C–H)	14 Hz	
J (H–C–C–H)	0–14 Hz	depends on torsion angle

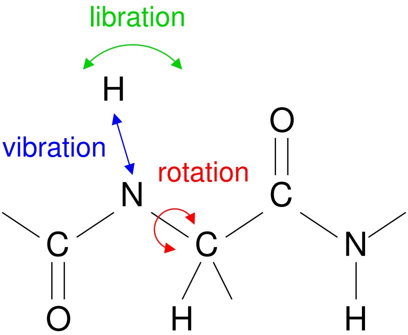
Relaxation

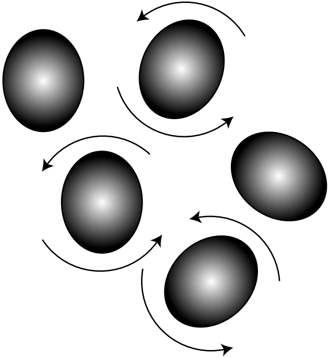
Relaxation is essential for NMR

Relaxation limits NMR



Molecular motions

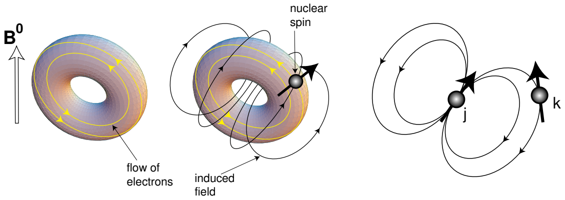


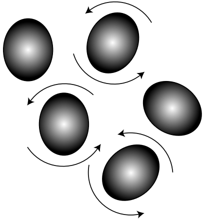


reproduced from M. H. Levitt: Spin Dynamics

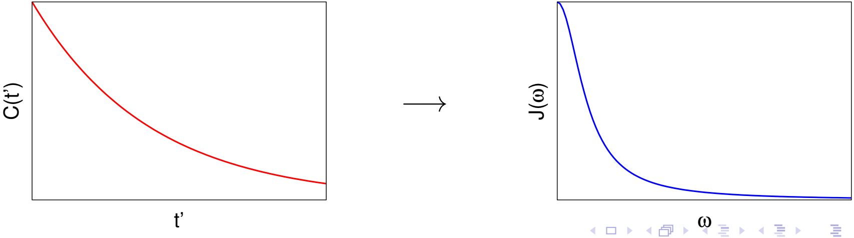
$$C(t) = \left\langle \frac{(3\cos^2\theta(t_0)-1)(3\cos^2\theta(t_0+t)-1)}{4r^3(t_0)r^3(t_0+t)} \right\rangle_{\text{all } t_0,\text{all molecules}} = \sum_i a_i e^{-t/\tau_i}$$

Fluctuations of local fields





$$J(\omega) = \int_0^\infty e^{-t'/\tau_i} \cos \omega t' \mathrm{d}t' = \frac{\tau_i}{1+(\omega\tau_i)^2}$$



Relaxation rates

$$
\begin{aligned}
R_2 &= 4\left(c^2+d^2\right)\left(a_0\frac{\tau_0}{1+(0\cdot\tau_0)^2}+a_1\frac{\tau_1}{1+(0\cdot\tau_1)^2}+\cdots\right)\\
&+ 3\left(c^2+d^2\right)\left(a_0\frac{\tau_0}{1+(\omega_2\tau_0)^2}+a_1\frac{\tau_1}{1+(\omega_2\tau_1)^2}+\cdots\right)\\
&+ 6d^2\left(a_0\frac{\tau_0}{1+(\omega_1\tau_0)^2}+a_1\frac{\tau_1}{1+(\omega_1\tau_1)^2}+\cdots\right)\\
&+ 6d^2\left(a_0\frac{\tau_0}{1+((\omega_1+\omega_2)\tau_0)^2}+a_1\frac{\tau_1}{1+((\omega_1+\omega_2)\tau_1)^2}+\cdots\right)\\
&+ d^2\left(a_0\frac{\tau_0}{1+((\omega_1-\omega_2)\tau_0)^2}+a_1\frac{\tau_1}{1+((\omega_1-\omega_2)\tau_1)^2}+\cdots\right)
\end{aligned}
$$

$$
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R_2 \;=\;& 4\left(c^2+d^2\right)\left(a_0\frac{\tau_0}{1+(0\cdot\tau_0)^2}+a_1\frac{\tau_1}{1+(0\cdot\tau_1)^2}+\cdots\right)\\
+\;& 3\left(c^2+d^2\right)\left(a_0\frac{\tau_0}{1+(\omega_2\tau_0)^2}+a_1\frac{\tau_1}{1+(\omega_2\tau_1)^2}+\cdots\right)\\
+\;& 6d^2\left(a_0\frac{\tau_0}{1+(\omega_1\tau_0)^2}+a_1\frac{\tau_1}{1+(\omega_1\tau_1)^2}+\cdots\right)\\
+\;& 6d^2\left(a_0\frac{\tau_0}{1+((\omega_1+\omega_2)\tau_0)^2}+a_1\frac{\tau_1}{1+((\omega_1+\omega_2)\tau_1)^2}+\cdots\right)\\
+\;& d^2\left(a_0\frac{\tau_0}{1+((\omega_1-\omega_2)\tau_0)^2}+a_1\frac{\tau_1}{1+((\omega_1-\omega_2)\tau_1)^2}+\cdots\right)
\end{aligned}
$$

small/flexible molecules $\Rightarrow$ fast motions $\Rightarrow$ short τ's $\Rightarrow \omega_j\tau_i \ll 1$

$\Rightarrow \sum\limits_i a_i \frac{\tau_i}{1+(\omega_j\tau_i)^2} \rightarrow \sum\limits_i a_i\tau_i = \overline{\tau}$

$\Rightarrow\; R_2 \rightarrow \left(7c^2+20d^2\right)\overline{\tau}$ **(small)**

Large rigid molecules relax rapidly

$$\tau_0 = \frac{4\pi\eta}{3k_\mathrm{B}T} r^3$$

$$\begin{aligned}
R_2 &= 4\left(c^2+d^2\right)\left(a_0\tau_0 + a_1\tau_1 + \cdots\right)\\
&+ 3\left(c^2+d^2\right)\left(a_0\frac{\tau_0}{1+(\omega_2\tau_0)^2} + a_1\frac{\tau_1}{1+(\omega_2\tau_1)^2} + \cdots\right)\\
&+ 6d^2\left(a_0\frac{\tau_0}{1+(\omega_1\tau_0)^2} + a_1\frac{\tau_1}{1+(\omega_1\tau_1)^2} + \cdots\right)\\
&+ 6d^2\left(a_0\frac{\tau_0}{1+((\omega_1+\omega_2)\tau_0)^2} + a_1\frac{\tau_1}{1+((\omega_1+\omega_2)\tau_1)^2} + \cdots\right)\\
&+ d^2\left(a_0\frac{\tau_0}{1+((\omega_1-\omega_2)\tau_0)^2} + \cdots\right)
\end{aligned}$$

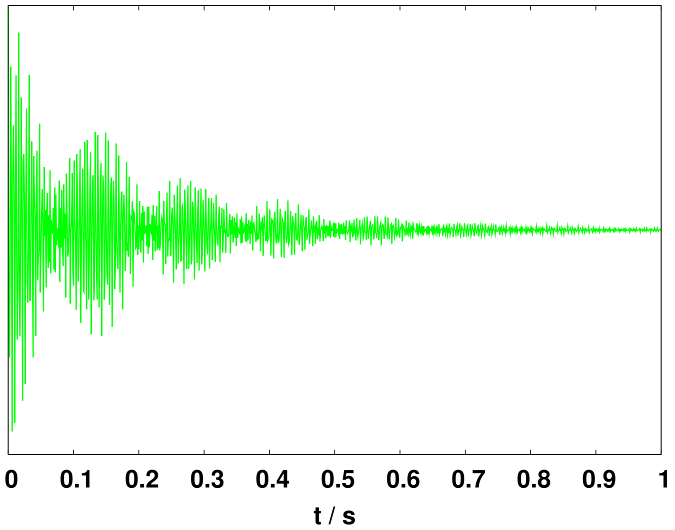
spherical rigid molecules $\Rightarrow$ slow tumbling $\Rightarrow$ long $\tau_0 \gg \tau_{i\neq 0} \Rightarrow$

$\Rightarrow \sum_i a_i \frac{\tau_i}{1+(\omega_{j\neq 0}\tau_i)^2} \to 0, \sum_i a_i\tau_i \approx a_0\tau_0$

$\Rightarrow$ $R_2 \to 4\left(c^2+d^2\right) a_0\tau_0$ **(large)**

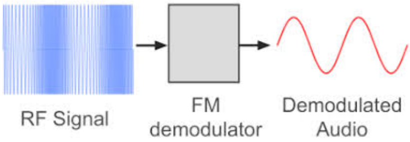
Signal processing

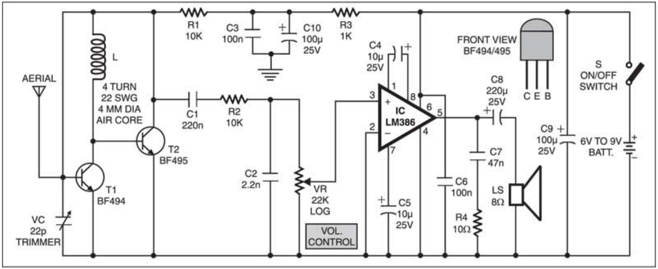
NMR signal: acetaldehyde $O{=}CH{-}CH_3$



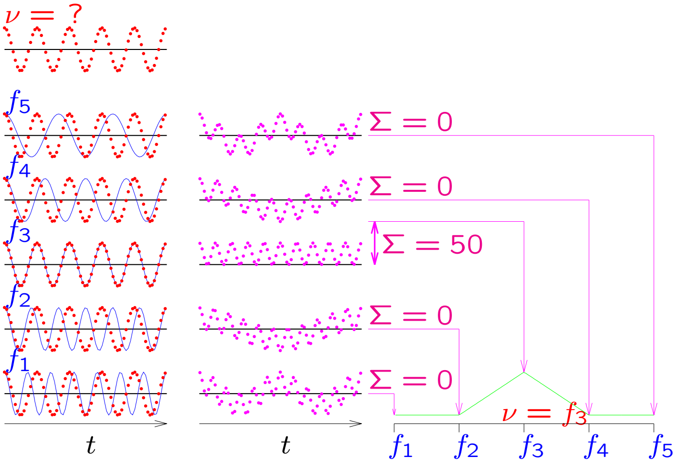
Demodulation

Like in FM radio receiver:

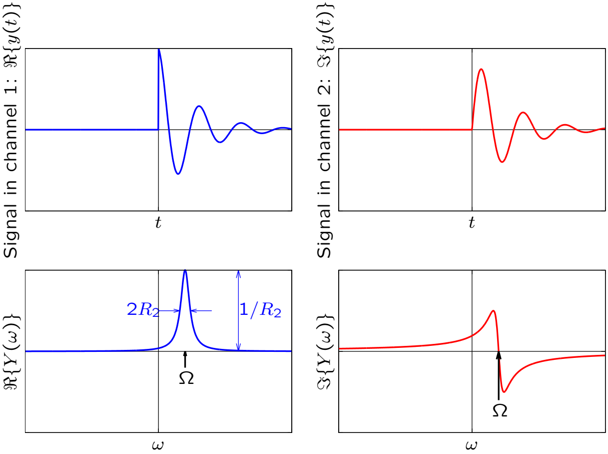




Fourier transformation

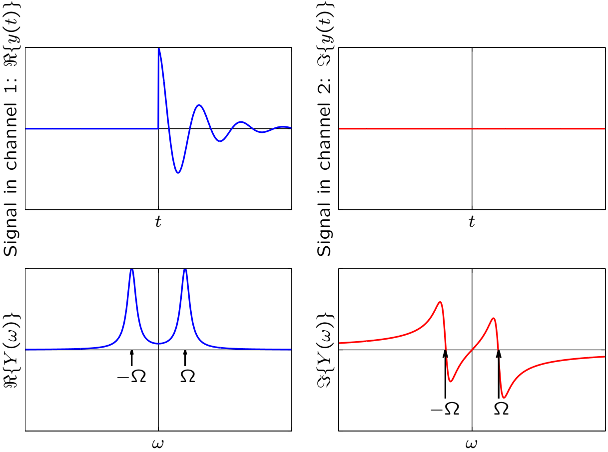


Complex Fourier transformation



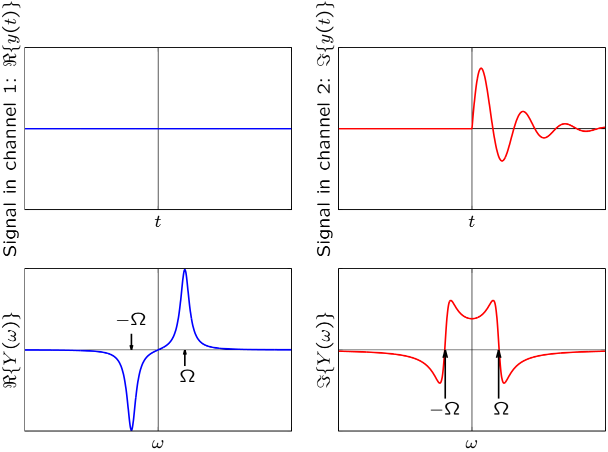
Fourier transformation of ideal signal.

Complex Fourier transformation



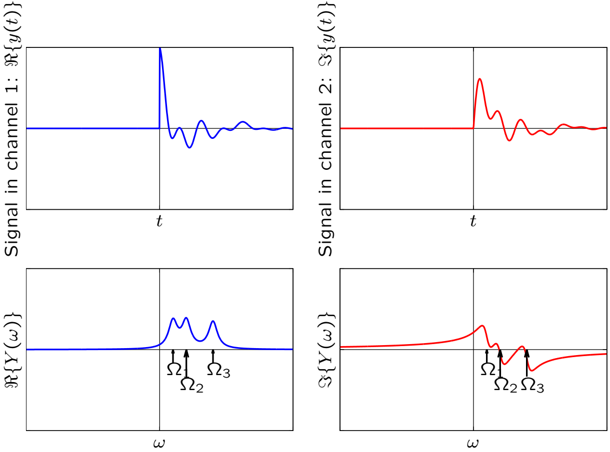
Fourier transformation of cosine.

Complex Fourier transformation



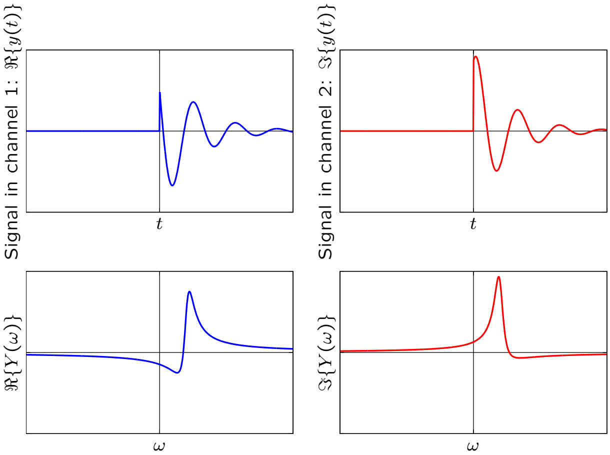
Fourier transformation of sine.

Complex Fourier transformation



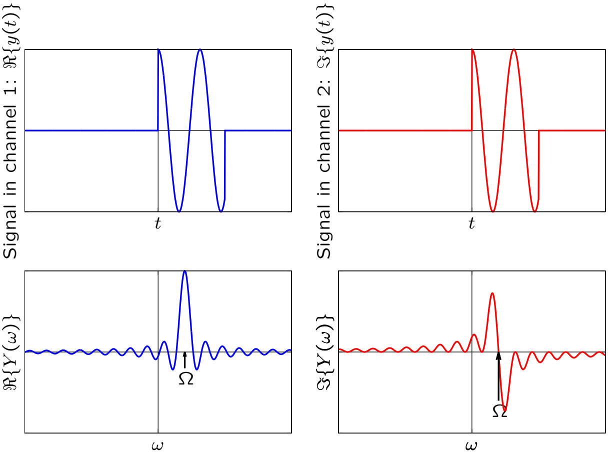
Three Larmor frequencies.

Phase is unknown



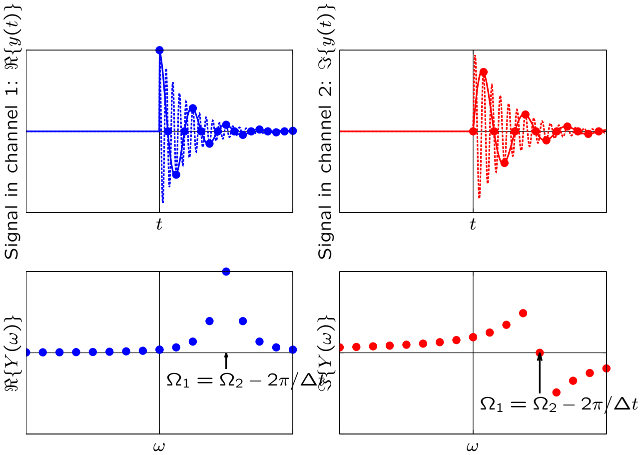
Phase.

Acquisition is finite



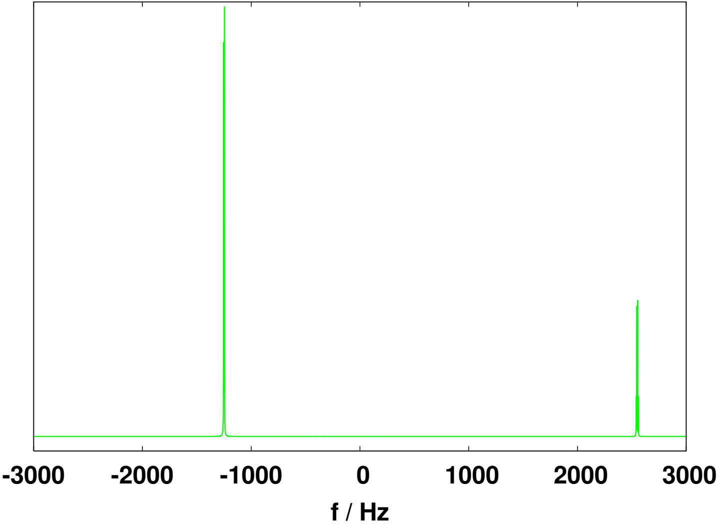
Truncation artifact.

Signal is digitized

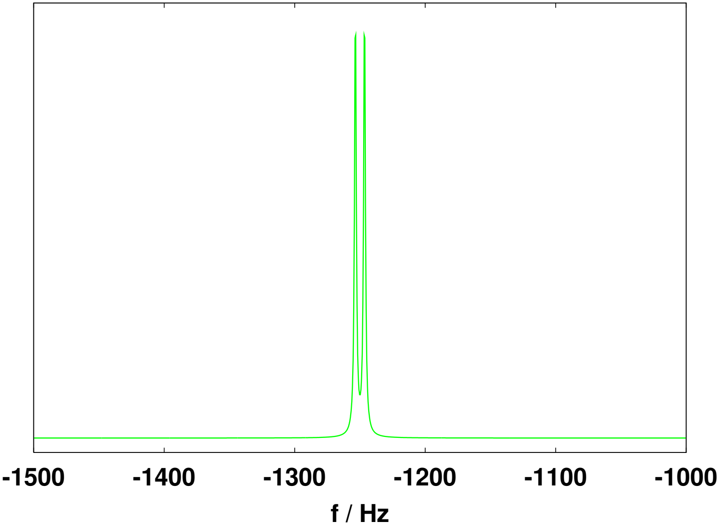


Aliasing.

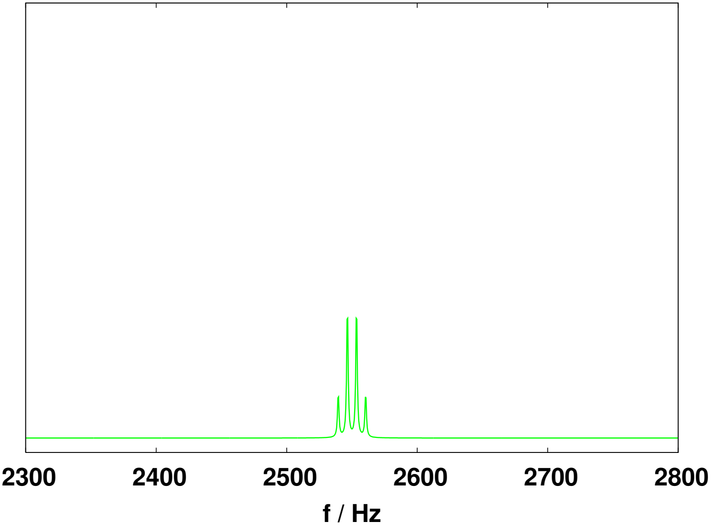
NMR spectrum: acetaldehyde $O{=}CH{-}CH_3$



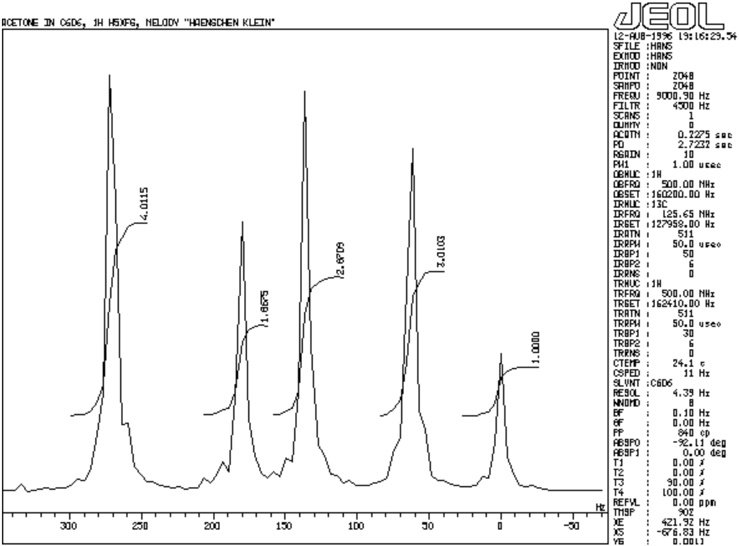
NMR spectrum: acetaldehyde $O{=}CH{-}CH_3$



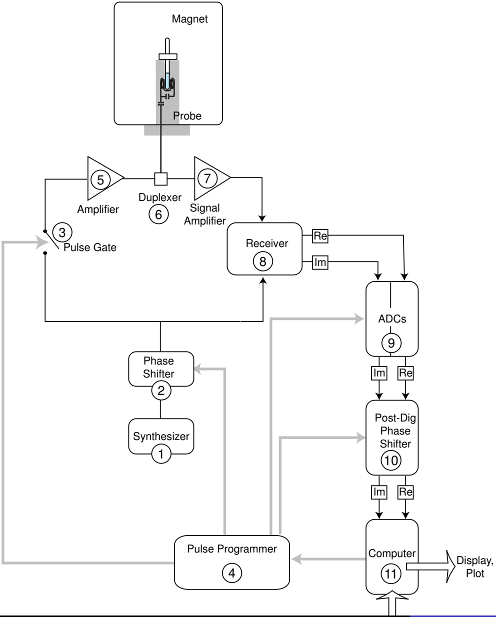
NMR spectrum: acetaldehyde $O{=}CH{-}CH_3$



NMR signal is an audio signal

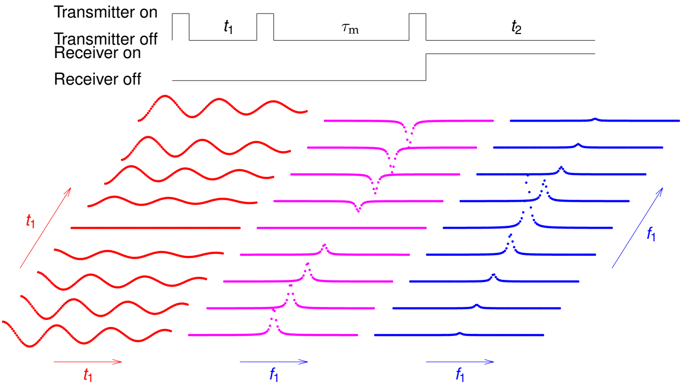


NMR spectrometer

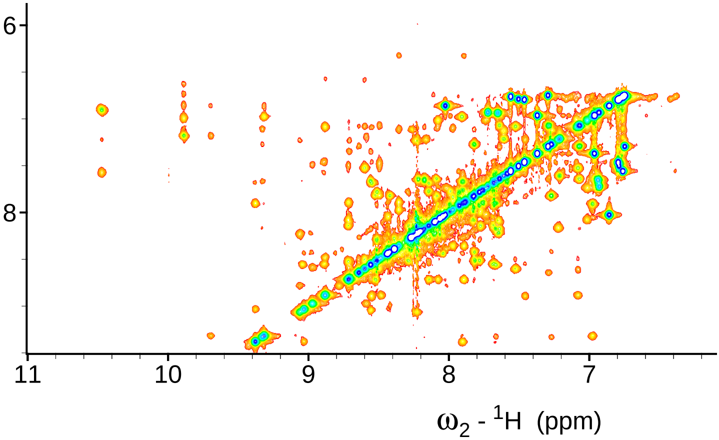


2D, 3D (nD) NMR experiments

2D NMR experiment

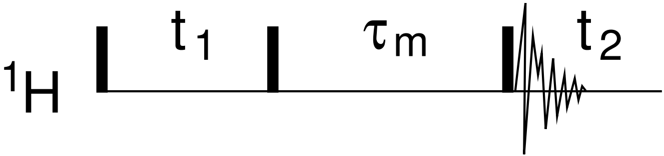


2D correlation via nuclear Overhauser effect

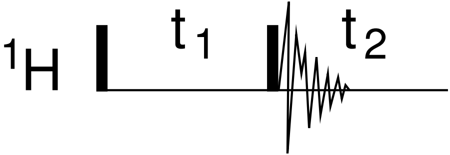


Homonuclear 2D correlation

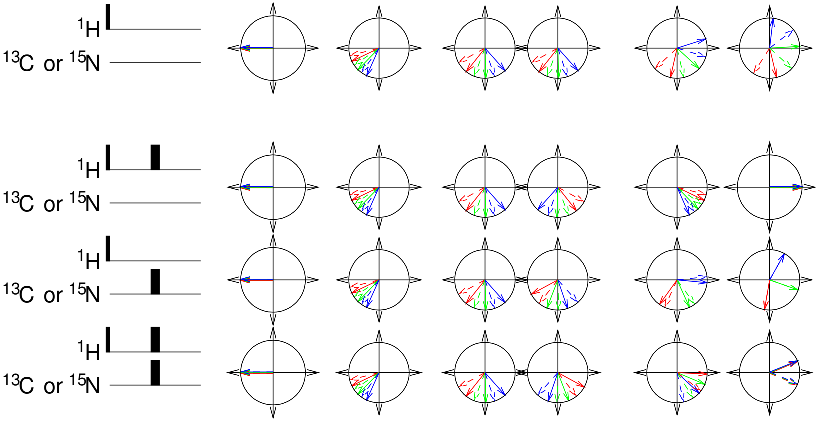
NOESY:



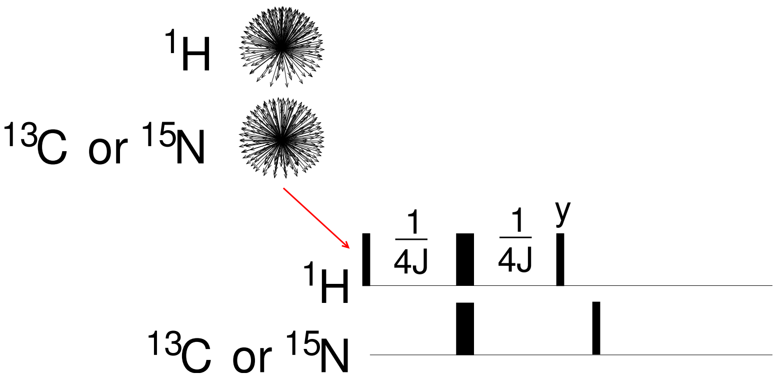
COSY:



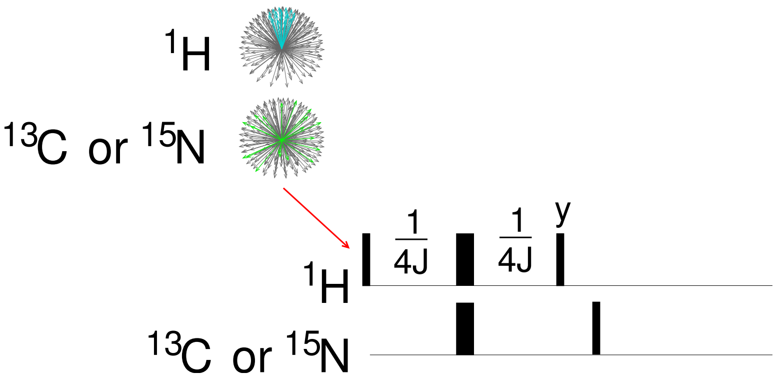
Heteronuclear spin echoes



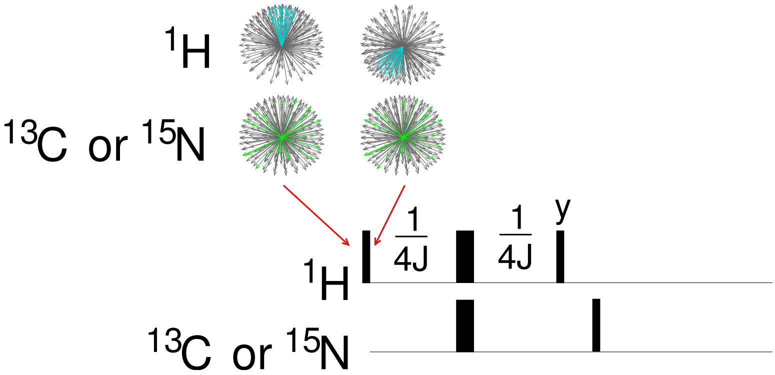
Heteronuclear polarization transfer



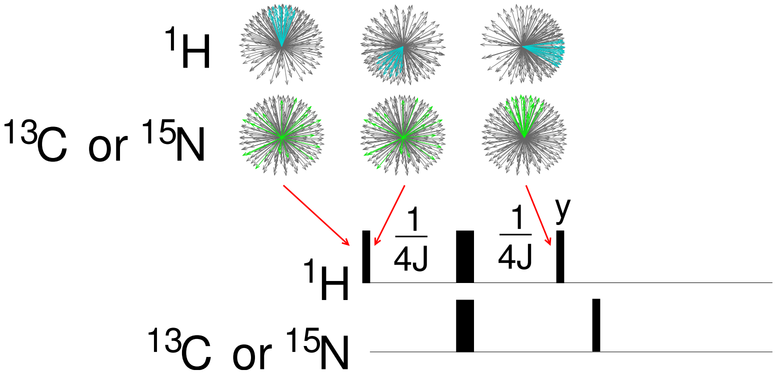
Heteronuclear polarization transfer



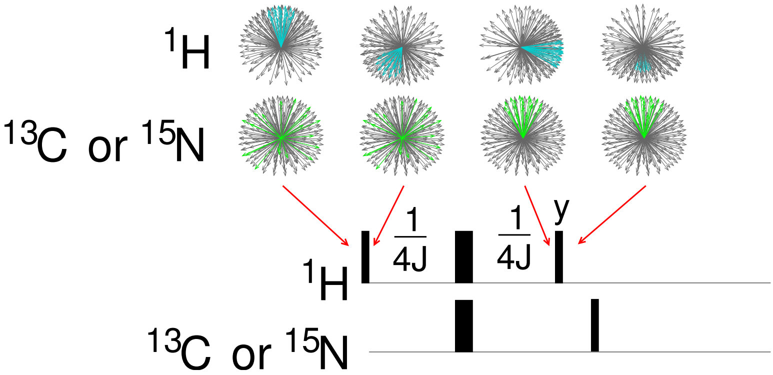
Heteronuclear polarization transfer



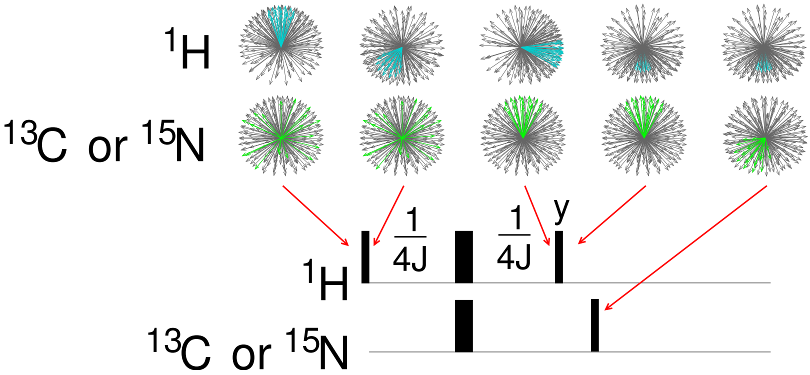
Heteronuclear polarization transfer



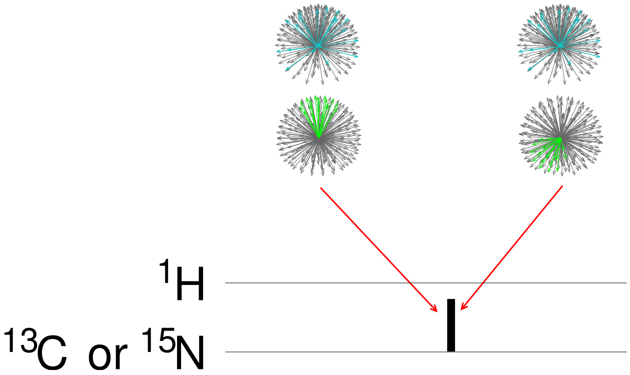
Heteronuclear polarization transfer



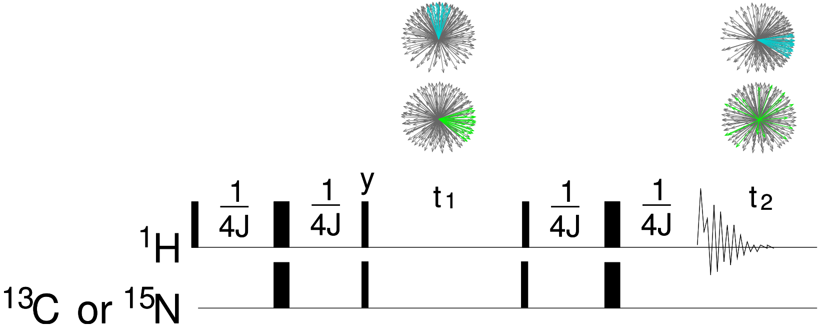
Heteronuclear polarization transfer



Direct excitation of ^{13}C or ^{15}N

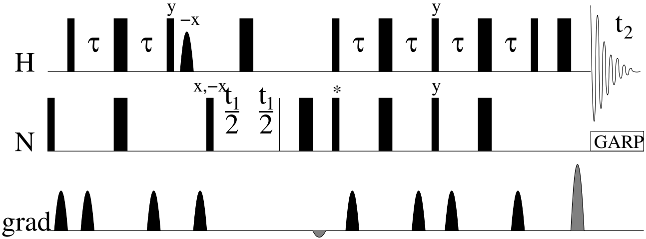


Basic principle:

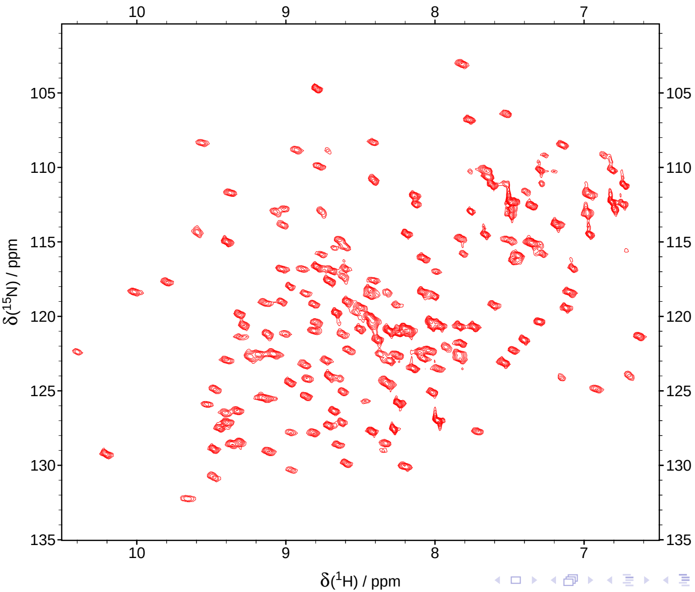


Real example:

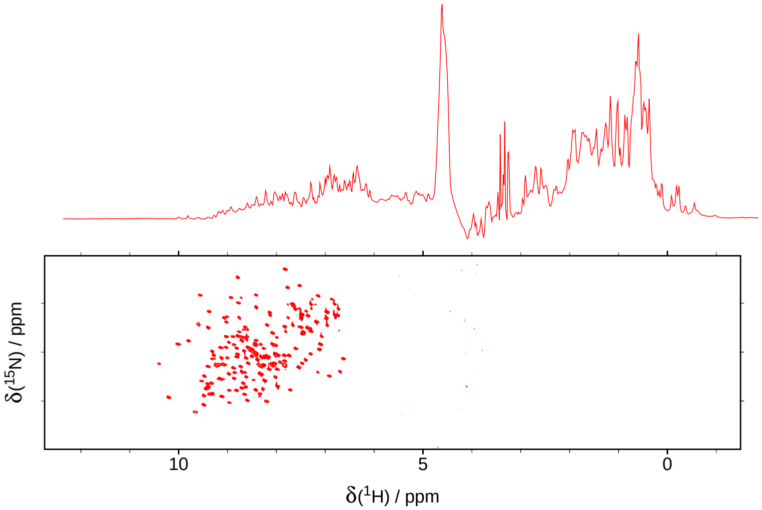
HSQC



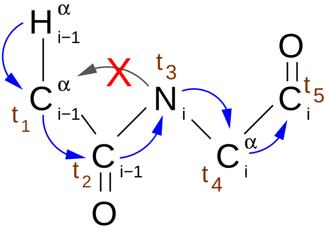
Heteronuclear 2D correlation

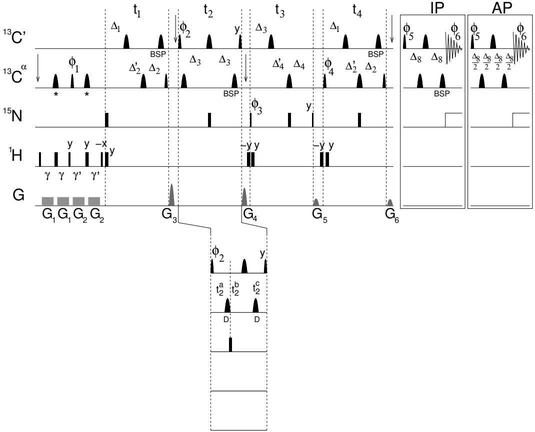


Advantage of 2D vs. 1D experiment



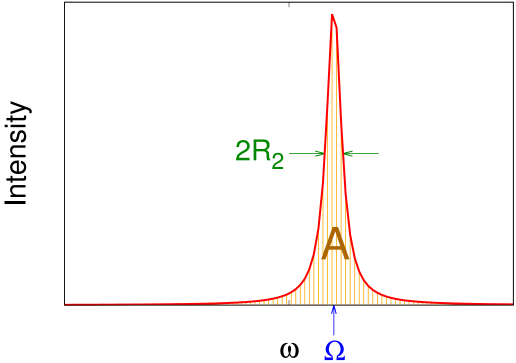
Example of a 5D experiment





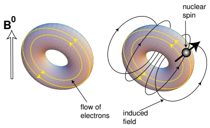
Structure and dynamics from NMR data

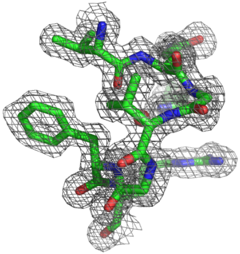
Information in the peak parameters

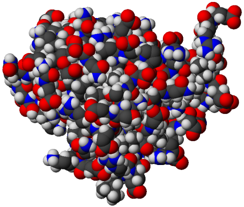


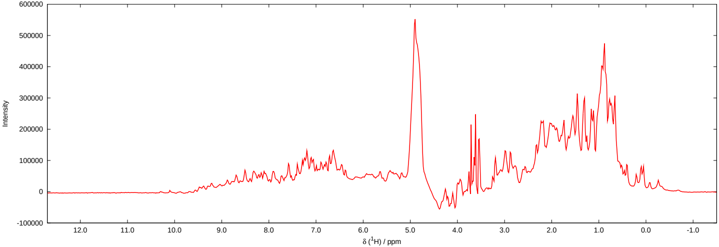
- Peak position (chemical shift) ⟶ (local) conformation
- Peak width ⟶ dynamics (not affecting chemical shift)
- Peak area ⟶ quantity (for ideal signal)

Local conformation from chemical shift

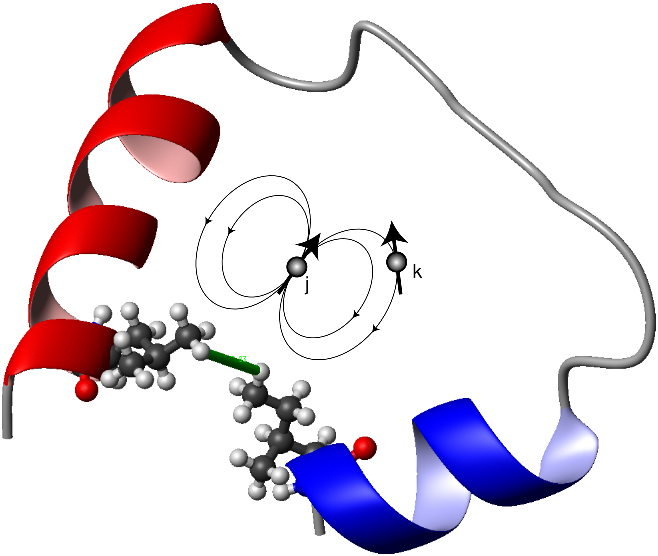




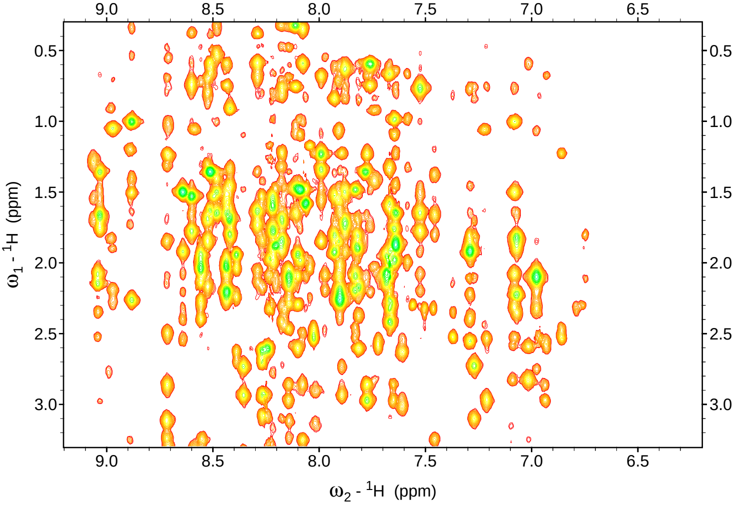




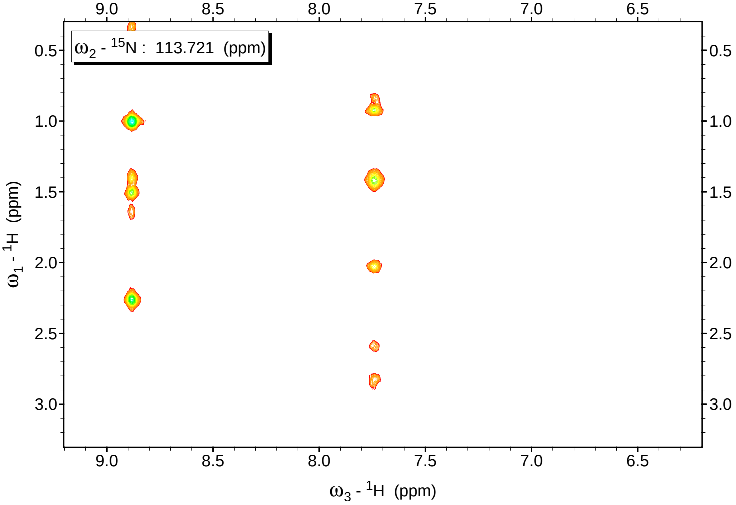
Global fold from nuclear Overhauser effect



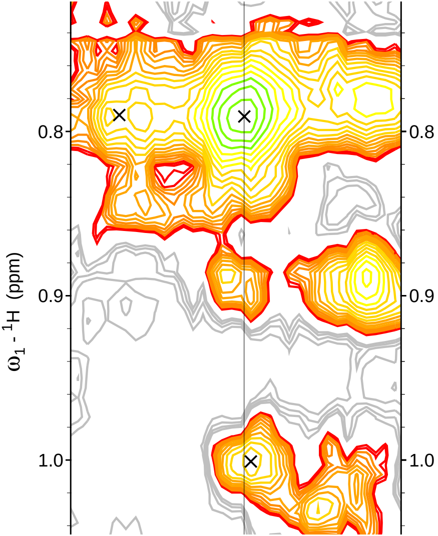
2D NOESY spectra



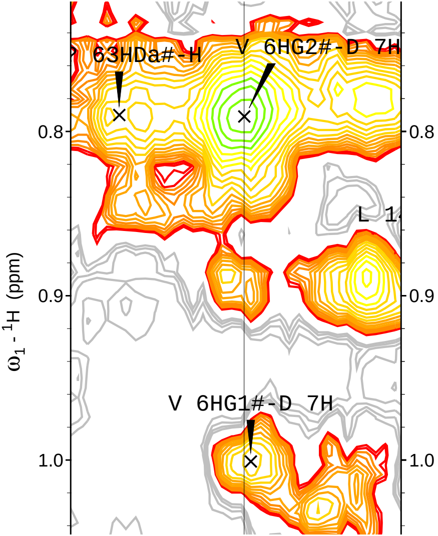
3D NOESY-HSQC spectra



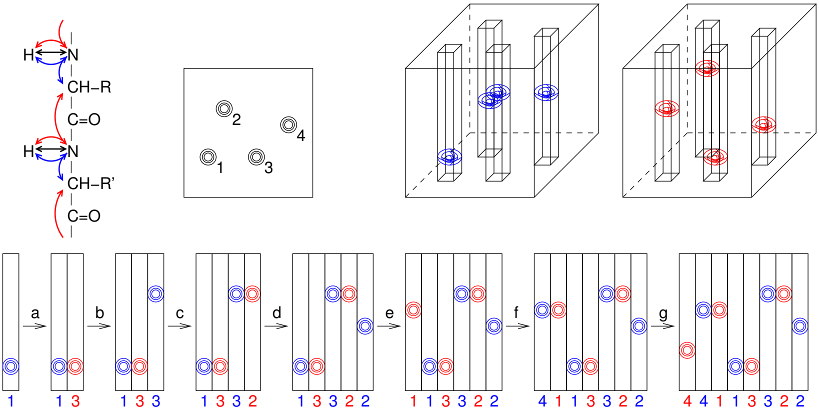
Assignment needed



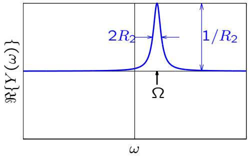
Assignment needed



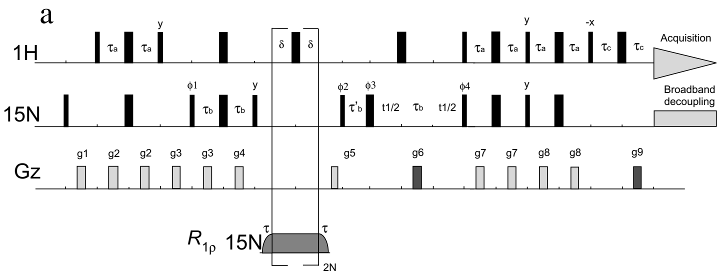
Protein backbone assignment



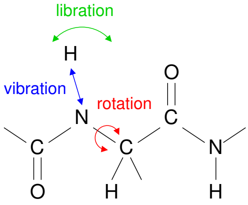
Relaxation rates from special experiments

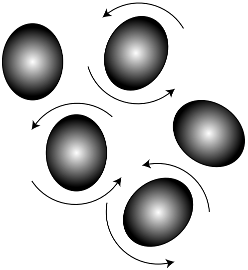


216 *D.M. Korzhnev et al. / Progress in Nuclear Magnetic Resonance Spectroscopy 38 (2001) 197–266*



Relaxation rates: motions not affecting chemical shift

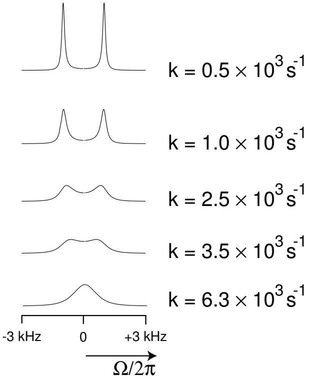




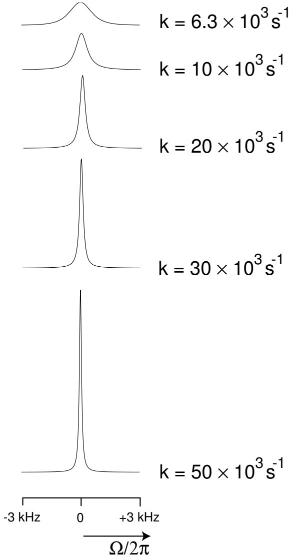
$$J(\omega) = \int_0^{\infty} e^{-t'/\tau_i} \cos \omega t' \mathrm{d}t' = \frac{\tau_i}{1 + (\omega \tau_i)^2}$$

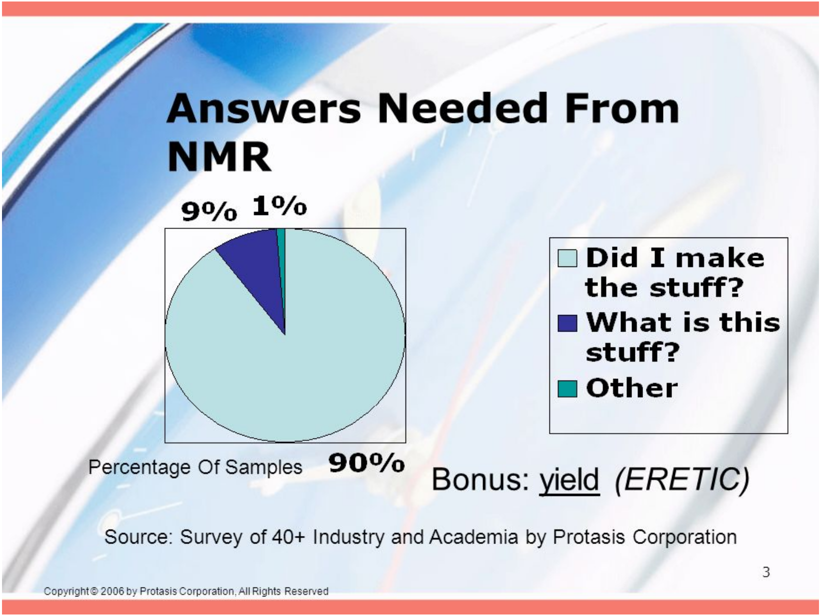
$$\begin{aligned} R_2 &= 4\left(c^2 + d^2\right) \sum_i a_i \frac{\tau_i}{1 + (0 \cdot \tau_i)^2} \\ &+ 3\left(c^2 + d^2\right) \sum_i a_i \frac{\tau_i}{1 + (\omega_2 \tau_i)^2} \\ &+ 6d^2 \sum_i a_i \frac{\tau_i}{1 + (\omega_1 \tau_i)^2} \\ &+ 6d^2 \sum_i a_i \frac{\tau_i}{1 + ((\omega_1 + \omega_2)\tau_i)^2} \\ &+ d^2 \sum_i a_i \frac{\tau_i}{1 + ((\omega_1 - \omega_2)\tau_i)^2} \end{aligned}$$

Chemical/conformational exchange



reproduced from M. H. Levitt: Spin Dynamics




Answers Needed From NMR
9% 1%
Did I make the stuff?
What is this stuff?
Other
Percentage Of Samples
90%
Bonus: yield (ERETIC)
Source: Survey of 40+ Industry and Academia by Protasis Corporation
3
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