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Frans Mulder

## PhD course Jyväskylä 2017

### Where are the protons? Measuring and modelling proton equilibria in complex macromolecular systems.

✓ ✓ ✓

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## Lecture Overview

- Lecture 1: NMR spectroscopy: 1D and 2D NMR
- Lecture 2: NMR of peptides and proteins
- Lecture 3: Electrostatics,  $pK_a$  and proton binding curves
- Lecture 4: Electrostatics in intrinsically disordered proteins

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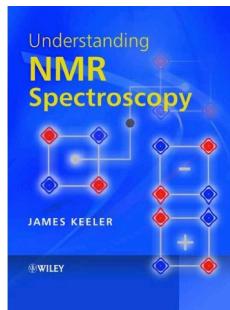
## NMR Introduction

If the information on NMR spectroscopy given in this class gets you interested in the technique, but the material is not sufficient, or if you wish to see some of it explained in other words, please go to:

<https://www.youtube.com/user/ANZMAG>, where you can find NMR lectures taught by James

Keeler.

You can also buy his outstanding book.  
(there's a new version of it)



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## Lecture 1 Nuclear spin angular momentum and NMR spectroscopy



## Spin angular momentum

### ›Stern-Gerlach experiment

measurement of spin angular momentum quantum number  $S = 1/2$



credit: Julien Bobroff

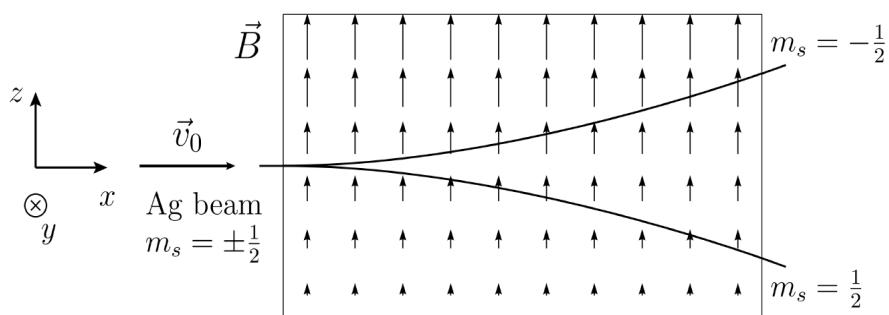
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## Spin angular momentum

### ›Stern-Gerlach experiment

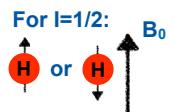
measurement of spin angular momentum quantum number  $S = 1/2$



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## Nuclear spin angular momentum

- › Nuclei with a spin angular momentum number  $I \neq 0$  can be exploited in an NMR experiment
  - › We measure the z-component,  $\hat{I}_z$ :
- For  $I=1/2$ : 
- $$\hat{I}_z \Psi_m = \hbar m \Psi_m \quad \text{with} \quad m = -I, I+1, \dots, I-1, I$$
- › Total number of energy levels:  $2I+1$

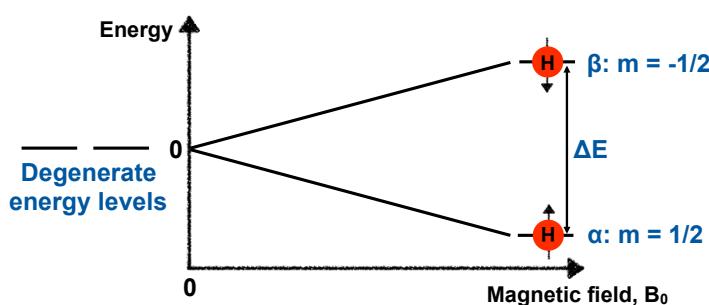
Nucleus	Spin quantum number, I	Natural abundance [%]	Magnetogyric ratio, g [ $10^6$ rad/T]	Larmor freq. ( $B_0 = 9.4$ T) [MHz]	Sensitivity (absolute)
$^1\text{H}$	1/2	99.98	267.5	400.0	very high
$^{13}\text{C}$	1/2	1.10	67.2	100.6	low
$^{14}\text{N}$	1	99.63	19.3	28.9	no (in liquid)
$^{15}\text{N}$	1/2	0.37	-27.1	40.5	very low
$^{19}\text{F}$	1/2	100	251.8	376.4	very high
$^{31}\text{P}$	1/2	100	108.4	161.9	high

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## Energy splitting and Larmor freq.

- › Energy:  $E_m = -\hbar m \gamma B_0$
- › Energy difference ( $I=1/2$ ):  $\Delta E_{\alpha \rightarrow \beta} = E_\beta - E_\alpha = \frac{1}{2} \hbar \gamma B_0 - \left( -\frac{1}{2} \hbar \gamma B_0 \right) = \hbar \gamma B_0$

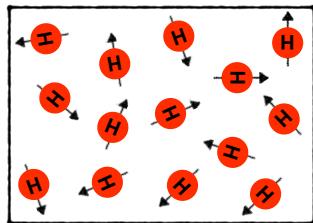


NMR is about  
measuring tiny  
differences in  $\omega_o$ !

› Larmor frequency:  $\omega_o = -\frac{\Delta E_{\alpha \rightarrow \beta}}{\hbar} = -\gamma B_0$

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## How many spins are in each state?



› Equilibrium population for spin-½ system:

$$\text{Ratio between states: } \frac{N_\beta}{N_\alpha} = \exp\left(\frac{-\Delta E}{k_B T}\right) \approx 1 - \frac{\Delta E}{k_B T}$$

› Excess magnetization:

$$N_\alpha - N_\beta = \frac{N_\alpha - N_\beta}{N} N \approx \frac{N_\alpha - N_\beta}{2N_\alpha} N = \frac{1}{2} \left(1 - \frac{N_\beta}{N_\alpha}\right) N ; \frac{1}{2} \frac{\Delta E}{k_B T} N = \frac{1}{2} \frac{\gamma h B_0}{k_B T} N$$

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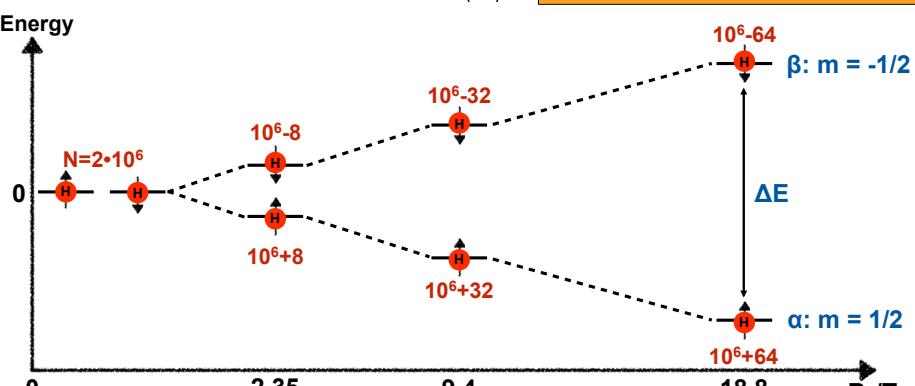
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## The bulk magnetization

› NMR measures the bulk magnetization  $\vec{M}_0$  of a sample...  
but how many spins contribute?

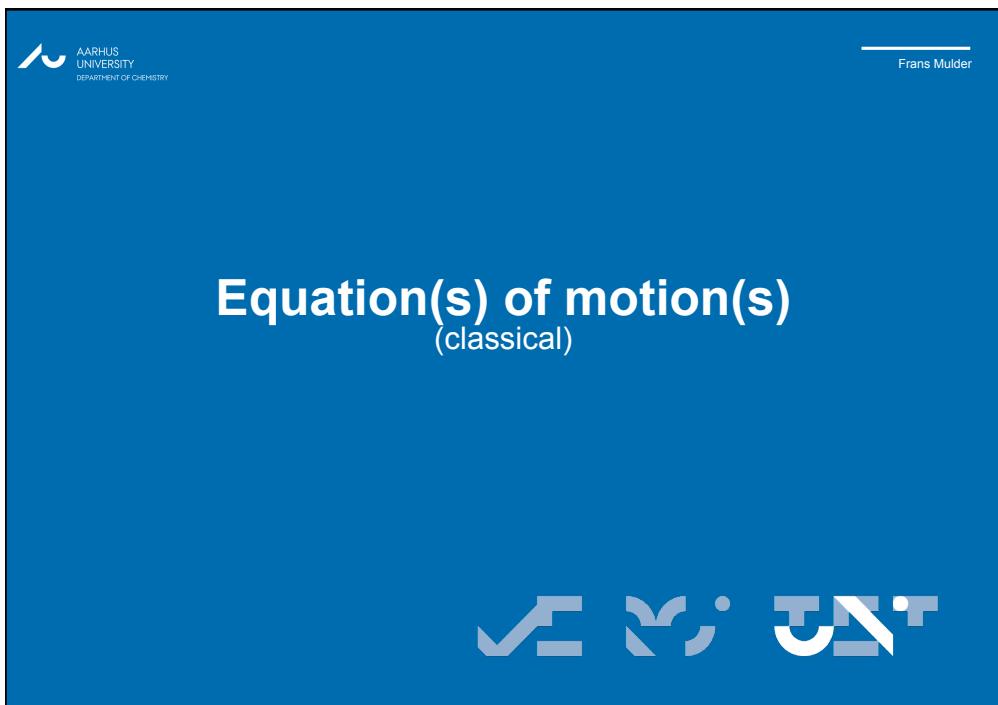
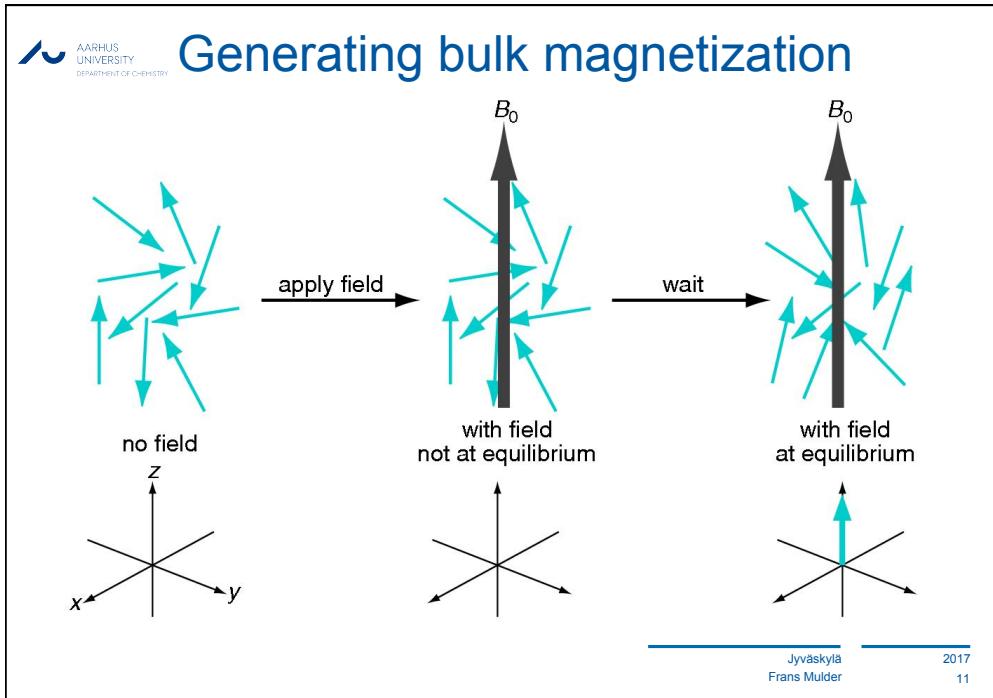
› Net bulk magnetization:  $\vec{M}_0 = \gamma N \langle I_z \rangle$

This is the magnetization we can manipulate and measure by NMR!



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## The Bloch equations

$$\begin{aligned}\frac{dM_x(t)}{dt} &= \gamma M_y(t) B_z \\ \frac{dM_y(t)}{dt} &= -\gamma M_x(t) B_z \\ \frac{dM_z(t)}{dt} &= 0\end{aligned}$$



› Solution:

$$M_x(t) = M_0 \cos(\gamma B_0 t) \quad M_y(t) = -M_0 \sin(\gamma B_0 t)$$

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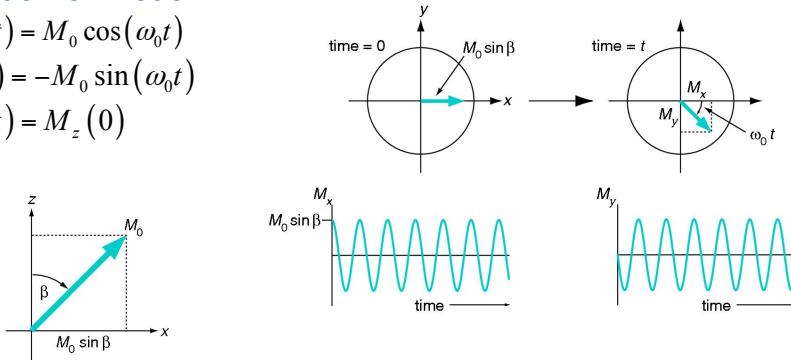
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## NMR = precession (around $B_0$ )

› Rotation around  $B_0$  with Larmor frequency!

› Equation of motion:

$$\begin{aligned}M_x(t) &= M_0 \cos(\omega_0 t) \\ M_y(t) &= -M_0 \sin(\omega_0 t) \\ M_z(t) &= M_z(0)\end{aligned}$$



› We will measure the oscillation along the x- and y-axes

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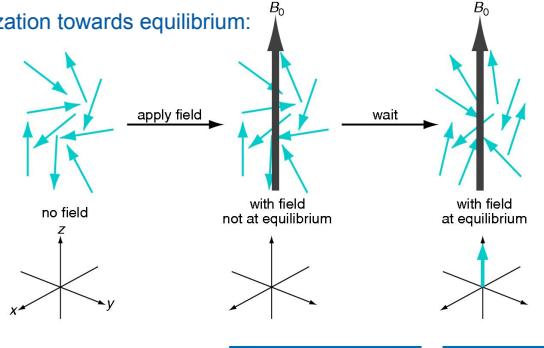
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## Relaxation (ad hoc)

› Relaxation needed to account for the return to equilibrium of the bulk magnetization

›  $T_1$  relaxation:  $\frac{dM_z(t)}{dt} = -\frac{1}{T_1}(M_z(t) - M_0)$

Longitudinal relaxation returns magnetization towards equilibrium:



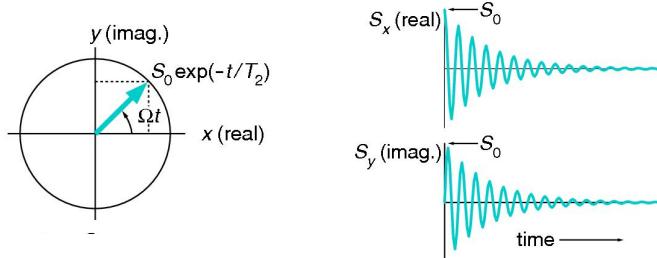
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## Relaxation (ad hoc)

›  $T_2$  relaxation:  $\frac{dM_x(t)}{dt} = -\frac{1}{T_2}M_x(t)$  and  $\frac{dM_y(t)}{dt} = -\frac{1}{T_2}M_y(t)$

$T_2$  relaxation causes transverse magnetization to decay:



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## Bloch equations incl. relaxation

› Bloch equations in 'laboratory frame'.

Equations:

$$\frac{dM_x(t)}{dt} = \gamma M_y(t) B_0 - \frac{1}{T_2} M_x(t)$$

$$\frac{dM_y(t)}{dt} = -\gamma M_x(t) B_0 - \frac{1}{T_2} M_y(t)$$

$$\frac{dM_z(t)}{dt} = -\frac{1}{T_1} (M_z(t) - M_0)$$

Solutions:

$$M_x(t) = M_0 \cos(\omega_0 t) e^{\frac{-t}{T_2}}$$

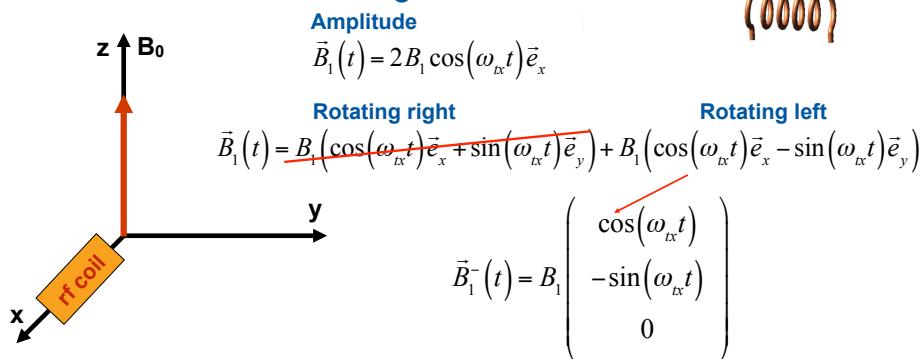
$$M_y(t) = M_0 \sin(\omega_0 t) e^{\frac{-t}{T_2}}$$

$$M_z(t) = M_0 \left(1 - e^{\frac{-t}{T_1}}\right)$$

## $B_0$ is not enough to do NMR

Need to bring magnetization into the  $xy$ -plane

Solution: Use an oscillating rf field

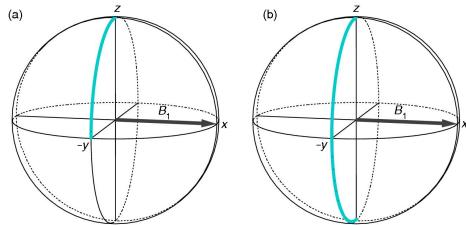


## On-resonance pulse

› On-resonance means that transmitter frequency is the same as Larmor frequency:  $\Omega = \omega_0 - \omega_{tx} = -\gamma\Delta B = 0$



› Effective field along x-axis!!!

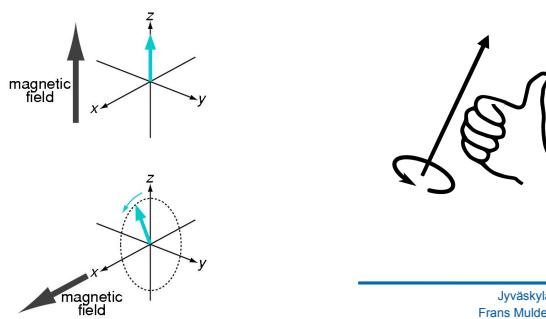


› Magnetization will rotate in the zy-plane with frequency:  $|\gamma|B_1 = \omega_1$

› Flip angle:  $\alpha = \gamma B_1 \tau_P$

## Right hand rule for rotation

An on-resonance radio frequency (rf) field of amplitude  $\omega_1 = -\gamma B_1$  turns the magnetization around itself with  $v_1 = (1/2\pi)\omega_1$  Hz. The direction is positive, i.e., if you place the thumb of your right hand in the direction of the rf-field vector the fingers point in the direction of the rotation.



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### Video of RF irradiation, as seen in the 'laboratory' and 'rotating' frame

The diagram illustrates two coordinate frames for NMR:

- Stationary frame (Laboratory frame):** A black 3D coordinate system with axes labeled **z**, **y**, and **-x**. A vertical red arrow labeled **B<sub>0</sub>** points along the **z**-axis.
- Rotating frame (On-resonance):** A black 3D coordinate system with axes labeled **z**, **y**, and **-x**. A vertical green arrow labeled **B<sub>0</sub>** points along the **z**-axis.

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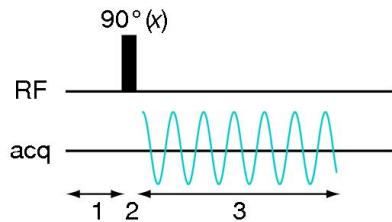
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### Single-pulse NMR experiment

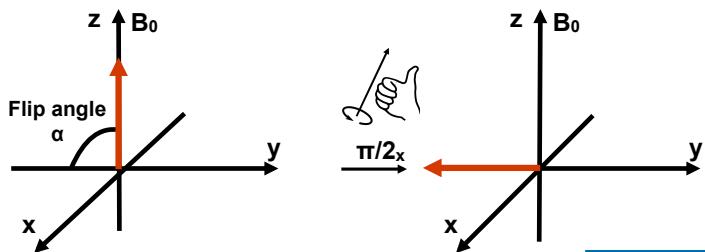
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## Single-pulse NMR experiment

› Pulse sequence:



› Rotating frame:



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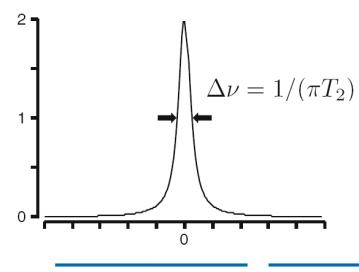
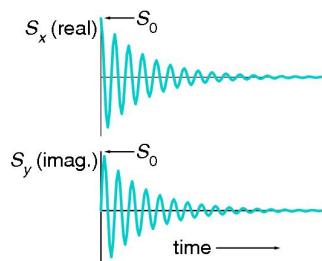
## Fourier Transformation

- Time domain (FID):

$$\begin{aligned} S(t) &= M_x(t) + iM_y(t) \\ &= M_o e^{i\omega' t} e^{-t/T_2} \end{aligned}$$

- Frequency domain (spectrum):

$$F(\omega) = \frac{T_2}{1 + T_2^2 \omega^2}$$



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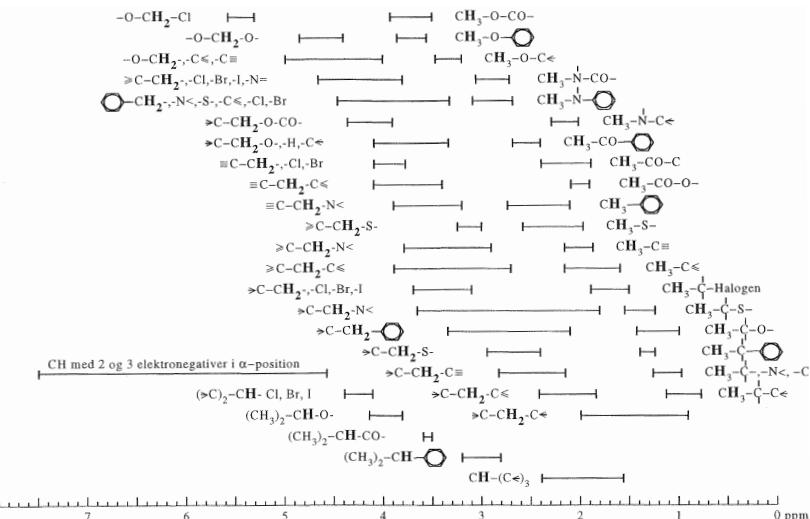
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## The chemical shift

- Factors influencing the chemical shift
- Diamagnetic response
- Electronegativity
- Inductive effect
- Conjugation and polar atoms
- Van der Waals forces
- Hydrogen bonding

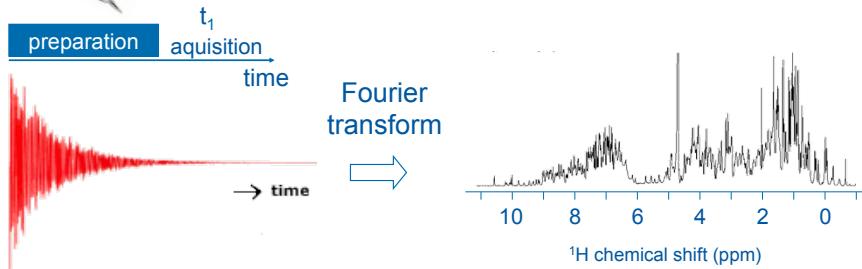
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## The chemical shift

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## Fourier-transform NMR



<http://www.nobel.se/chemistry/laureates/1991/illpres/index.html>

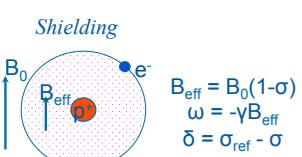
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## Chemical shifts



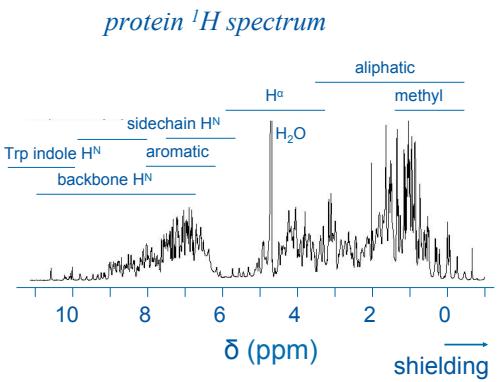
*Shielding*

$$B_{\text{eff}} = B_0(1-\sigma)$$

$$\omega = -\gamma B_{\text{eff}}$$

$$\delta = \sigma_{\text{ref}} - \sigma$$

chemical environment  
functional group  
amino acid type  
secondary structure  
e.g. torsion angles  
tertiary structure  
e.g. proximity to aromatic side chains



*protein  $^1\text{H}$  spectrum*

$\delta$  (ppm)

shielding

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The image shows two screenshots of the Bruker Almanac app. The left screenshot is for the iPhone, displaying a menu with categories: NMR, X-ray Spectroscopy, EPR, IR Spectroscopy, Mass Spectrometry, Conversion of Units, Chemical Tables, Physical Tables, and International Dialing Codes / World. The right screenshot is for the iPad, showing a detailed 'Near Infrared Band Assignment Table' with tabs for 'Second Overtone Region', 'Third Overtone Region', and 'First Overtone'. The table lists wavenumbers in cm⁻¹ from 14298 to 5862, with corresponding assignments for C-H, C=C, C≡C, and various overtone and combination bands.

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# Quantum Mechanics

-because we cannot explain J-couplings by vector model

## QM in Hilbert Space

› State of a system (state function,  $\Psi$ ):

$$|\Psi(t)\rangle = \sum_{i=1}^N c_i(t) |\Psi_i\rangle$$

N-dimensional space      Basis functions

› Operator,  $\hat{Q}$ , transform a state function into another state function:  $\hat{Q}|\Psi(t)\rangle = |\Psi'(t)\rangle$

Matrix representation of operator:  $Q_{ij} = \langle \Psi_i | \hat{Q} | \Psi_j \rangle$

› Time evolution of a state function (time-dependent Schrödinger equation):

$$i\hbar \frac{d}{dt} |\Psi(t)\rangle = \hat{H} |\Psi(t)\rangle$$

Hamilton operator is the energy operator!

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## The Density operator



## Density Operator

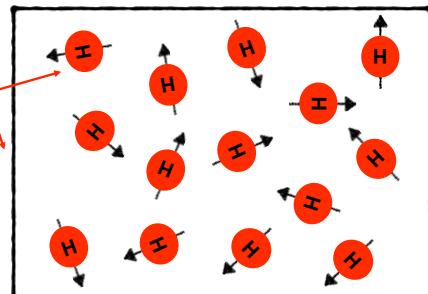
› In statistical quantum mechanics we describe system by a density operator:

$$\hat{\rho} = |\Psi\rangle\langle\Psi|$$

Density operator contains information about the ensemble average in mixed states

$|\Psi\rangle$

Wavefunction contains information about a single or pure states

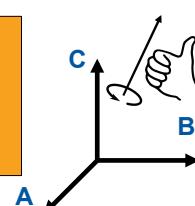


## Summary for density operator

› Product operators allow an easy way to evaluate the effects of different elements of an NMR pulse sequence, provided that the Hamiltonians (also operators) are time independent

› Solution: If **B** and **C** belong to a common operator subspace, use this subspace to determine  $\mathbf{A} = i[\mathbf{C}, \mathbf{B}]$

$$\hat{I}_c \xrightarrow{i_{\hat{B}}(\beta)} \begin{cases} \hat{I}_c & \text{if } [\hat{I}_c, \hat{I}_B] = 0 \\ \hat{I}_c \cos(\beta) + i[\hat{I}_c, \hat{I}_B] \sin(\beta) & \text{otherwise} \end{cases}$$



and the right-hand rule.

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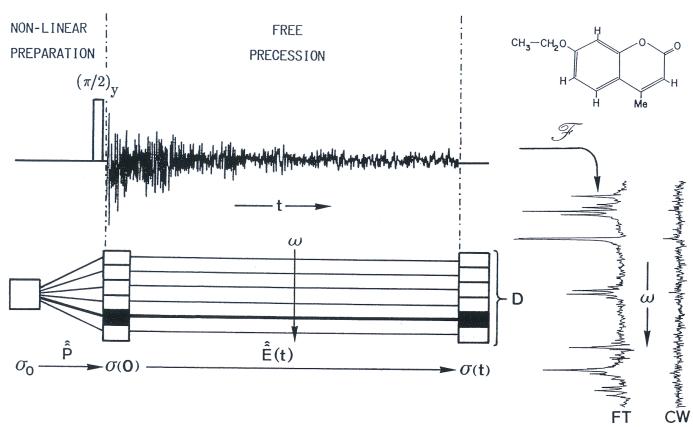
## 2D NMR Spectroscopy:

**See:** Multidimensional NMR Spectroscopy  
**Mulder & Scheek (2012)**  
**in: Encyclopedia of Biophysics**



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## 1D NMR spectroscopy



The diagram illustrates the 1D NMR experiment. It starts with a **NON-LINEAR PREPARATION** pulse of  $(\pi/2)_y$ . This is followed by **FREE PRECESSION** during which the magnetization vector  $\hat{E}(t)$  rotates in the  $\sigma_x$ - $\sigma_y$  plane. The magnetization is then **DEPHASING** ( $D$ ) along the  $\sigma_z$  axis. Finally, the signal is **FOURIER TRANSFORMED** (FT) to obtain the **CW** spectrum. A chemical structure of Caffeine is shown with its proton assignments.

Images: courtesy of Richard Ernst

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## 2D NMR spectroscopy

The diagram illustrates a 2D NMR experiment. At the top, a horizontal timeline shows two pulses separated by time  $t_1$ , followed by a second set of pulses separated by time  $t_2$ . Below this, a 'TRANSFER' section shows a diamond-shaped grid representing the evolution of magnetization. The grid is labeled with  $\omega_1$  and  $\omega_2$  along its diagonals. A central point on the grid is highlighted. The pulse sequence is labeled as follows:  $\sigma_0 \rightarrow \hat{P} \rightarrow \sigma(t_1) \rightarrow \hat{E}(t_1) \rightarrow \hat{R} \rightarrow \hat{E}(t_2) \rightarrow \sigma(t_1, t_2) \rightarrow D$ . The  $\hat{E}$  and  $\hat{R}$  blocks represent evolution and mixing periods, respectively. The final signal is detected as  $D$ .

$$S(t_1, t_2) = \eta e^{i(\omega_A t_1)} e^{i(\omega_B t_2)}$$

intensity modulation      detected FID

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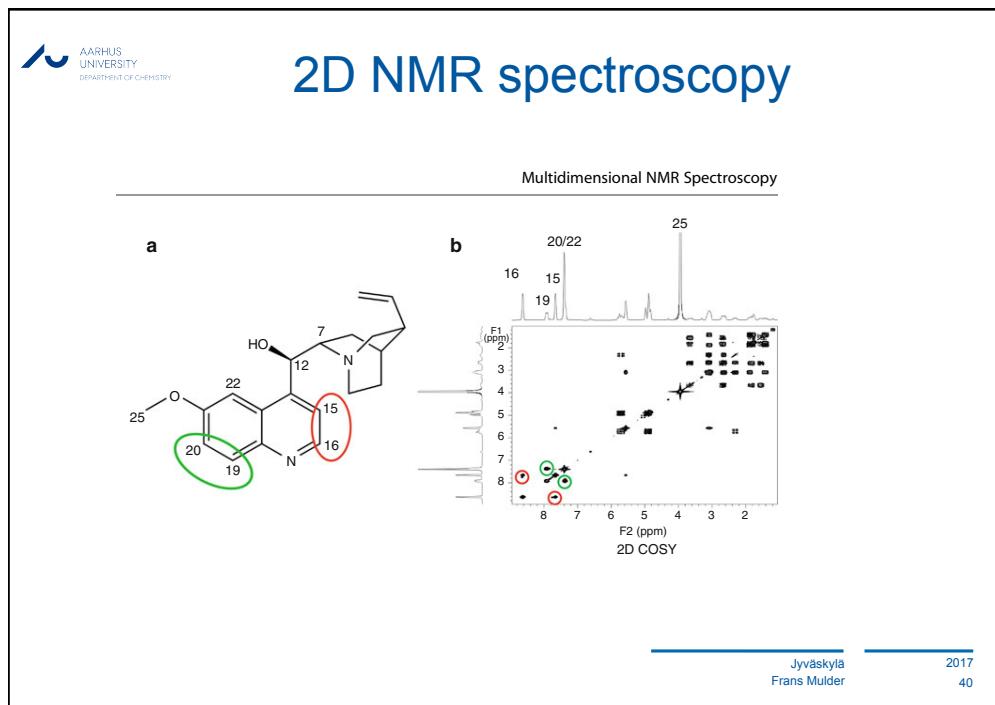
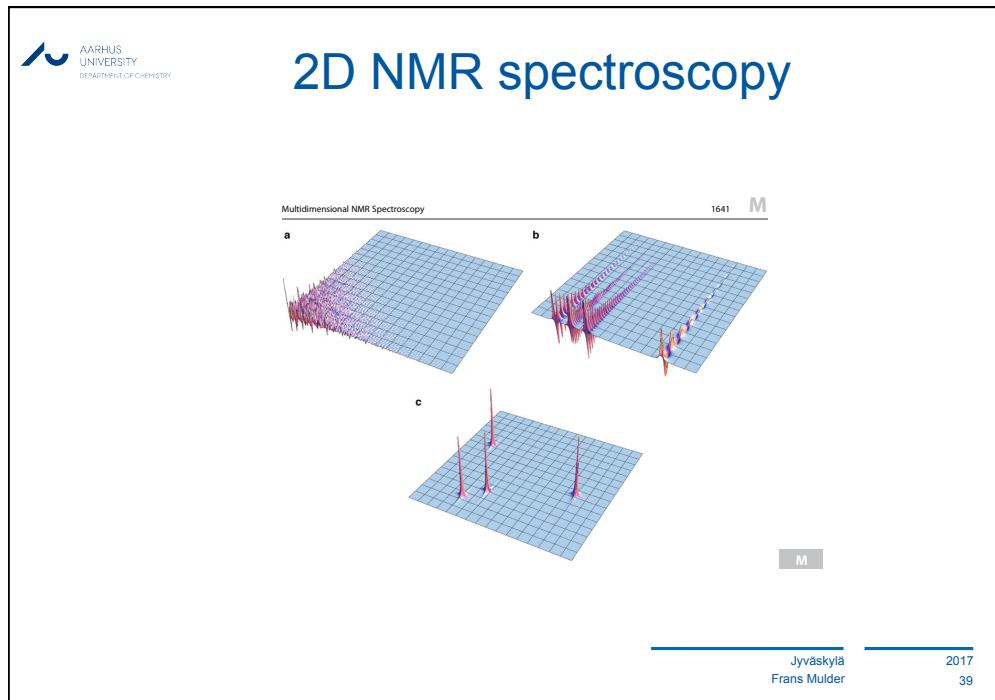
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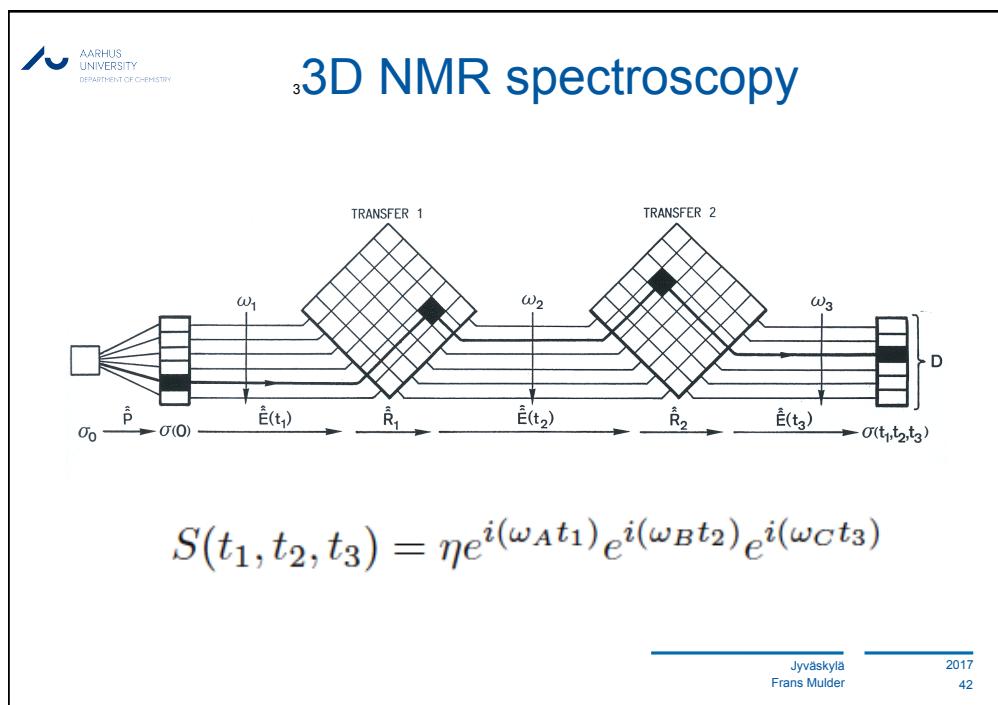
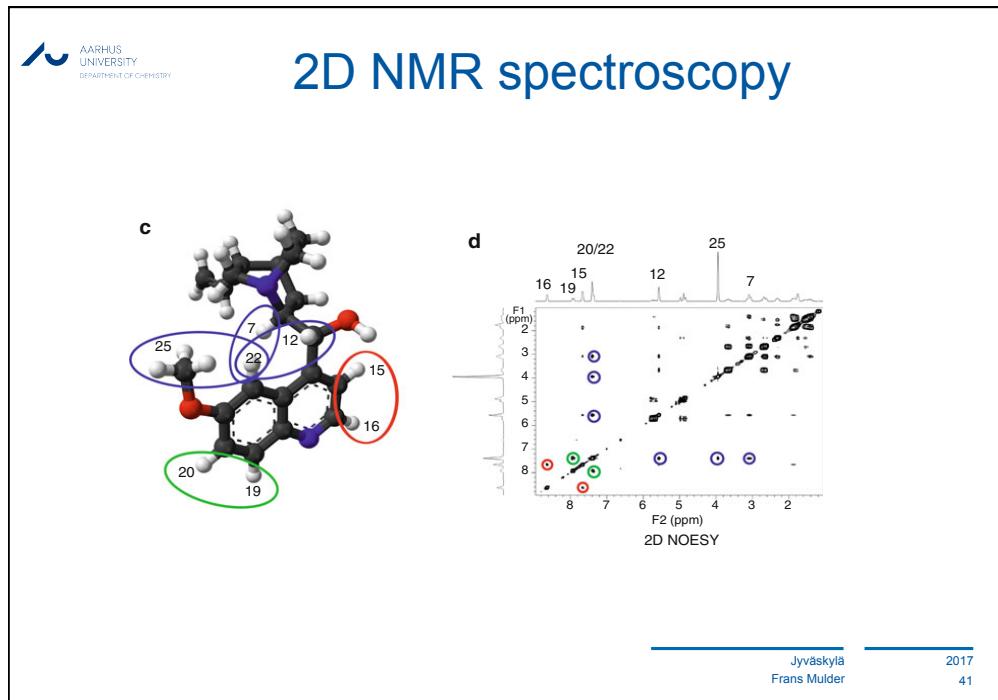
## 2D NMR spectroscopy

The diagram illustrates a 2D NMR experiment. It shows four horizontal timelines representing different evolution periods. The top timeline is labeled 'PREPARATION PERIOD'. The second timeline is labeled 'EVOLUTION PERIOD' and contains a rectangular pulse followed by a wavy line representing evolution. The third timeline is labeled 'MIXING PERIOD' and contains a rectangular pulse followed by a wavy line representing evolution. The bottom timeline is labeled 'DETECTION PERIOD' and contains a rectangular pulse followed by a wavy line representing evolution. The evolution periods are labeled  $t_1$  and  $t_2$ . A dashed line labeled  $s(t_1, t_2)$  represents the resulting 2D correlation signal.

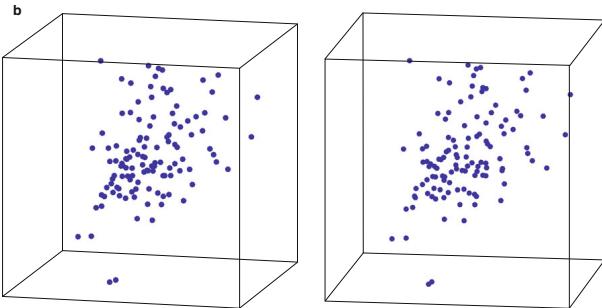
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## 3D NMR spectroscopy



$$S(t_1, t_2, t_3) = \eta e^{i(\omega_A t_1)} e^{i(\omega_B t_2)} e^{i(\omega_C t_3)}$$