



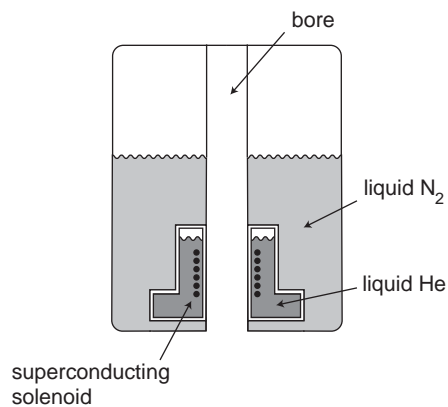
Biomedical Engineering

Magnetic Resonance Spectroscopy 2 (MRS)

Dr. Stefan Kirsch



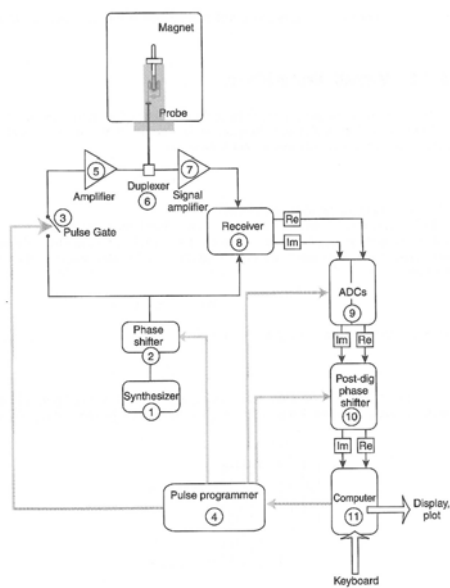
Computer Assisted Clinical Medicine
Faculty of Medicine Mannheim
University of Heidelberg
Theodor-Kutzer-Ufer 1-3
D-68167 Mannheim, Germany
Stefan.Kirsch@MedMa.Uni-Heidelberg.de
www.ma.uni-heidelberg.de/inst/cbtm/ckm/



The NMR Spectrometer II



The NMR Spectrometer III



The NMR Spectrometer IV



The Probe

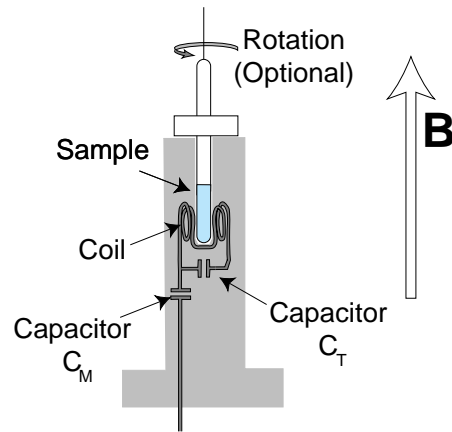
C_T = tuning capacitor

⇒ tuning the frequency of the oscillator

$$\omega_{osc} = (L \cdot C_T)^{-1/2}$$

C_M = matching capacitor

⇒ couples the external signals into the probe circuit with maximum efficiency



M. Levitt,
Spin
dynamics

The NMR Spectrometer V



The receiver section

- NMR signal oscillates at many hundreds of MHz
⇒ to fast for currents ADCs
⇒ down-convert $\Omega^0 = \omega^0 - \omega_{ref}$
- NMR signal is converted in complex signal (real and imaginary part). Why?

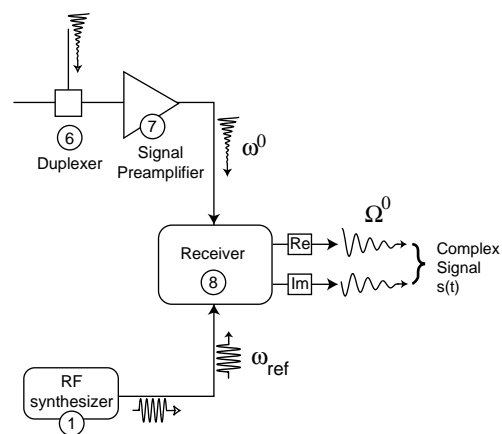
assume signal acquired $S(t) \propto \cos(\Omega^0 t) \cdot \exp(-t/T_2)$ [*]

- assume protons with $\omega_a^0 = 500.001$ MHz,
 $\omega_b^0 = 499.999$ MHz and $\omega_{ref} = 500$ MHz
⇒ $\Omega_a^0/2\pi = 1$ kHz and $\Omega_b^0/2\pi = -1$ kHz

Equation [*] does not distinguish between these frequencies

⇒ Receiver generates two output signals

$$\left. \begin{aligned} S_a(t) &\propto \cos(\Omega^0 t) \exp(-t/T_2) \\ S_b(t) &\propto \sin(\Omega^0 t) \exp(-t/T_2) \end{aligned} \right\} \Rightarrow S(t) = S_a(t) + iS_b(t) \propto \exp\left[(i\Omega^0 - \lambda)t \right] \quad ; \quad \lambda = 1/T_2$$

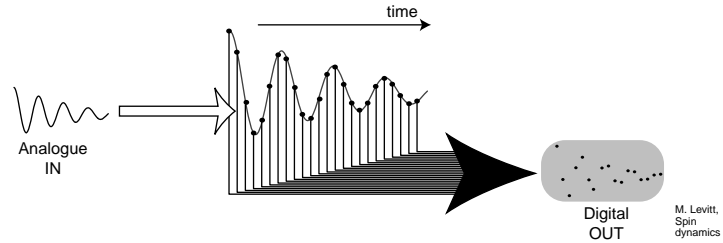


M. Levitt,
Spin
dynamics

The NMR spectrometer VI

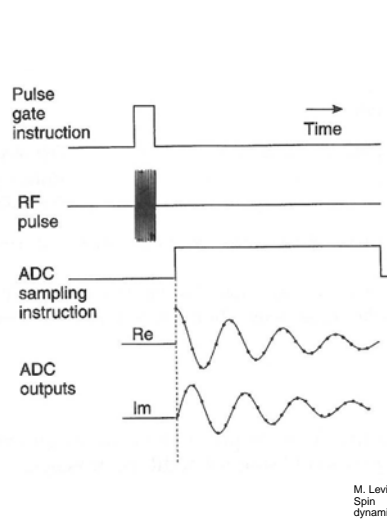


The analogue to digital conversion

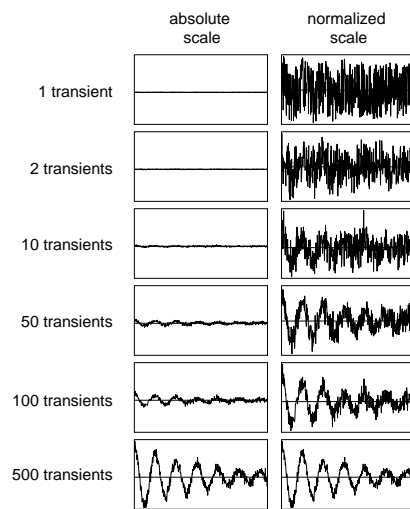


- time separation between two sampling points = *dwell time*
 - inverse of the dwell time is the [*spectral width*] / 2 (maximum range of frequencies which can be acquired)
- Why the factor 1/2 ? \Rightarrow **Nyquist-Shannon sampling theorem** R

The FID Experiment



M. Levitt,
Spin
dynamics



M. Levitt,
Spin
dynamics

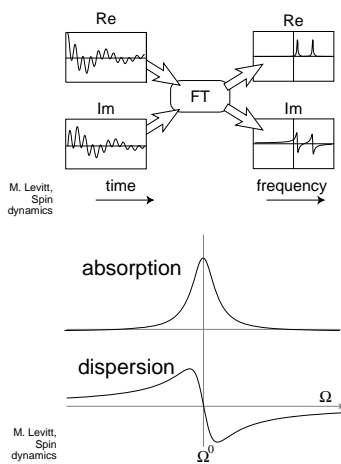
$$\text{signal / noise} \propto (N)^{1/2}$$



The Fourier Transform I

How to get from time-domain to frequency-domain?

⇒ Fourier transform



$$S(t) = \sum_t s_t(t) ; s_t(t) = a_t \exp[(i\Omega_t - \lambda_t)t]$$

$$S(\Omega) = \sum_t s_t(\Omega) ; s_t(\Omega) = ? \quad \mathbf{R}$$

$$s_t(\Omega) = a_t \cdot \frac{1}{\lambda_t + i(\Omega - \Omega_t)}$$

complex Lorentzian=L

$$\text{Re}\{L\} = \frac{\lambda_t}{\lambda_t^2 + (\Omega - \Omega_t)^2} \equiv A(\Omega, \Omega_t, \lambda_t) \quad \text{absorption Lorentzian}$$

$$\text{Im}\{L\} = -\frac{(\Omega - \Omega_t)}{\lambda_t^2 + (\Omega - \Omega_t)^2} \equiv D(\Omega, \Omega_t, \lambda_t) \quad \text{dispersion Lorentzian}$$

$$L = A + iD$$

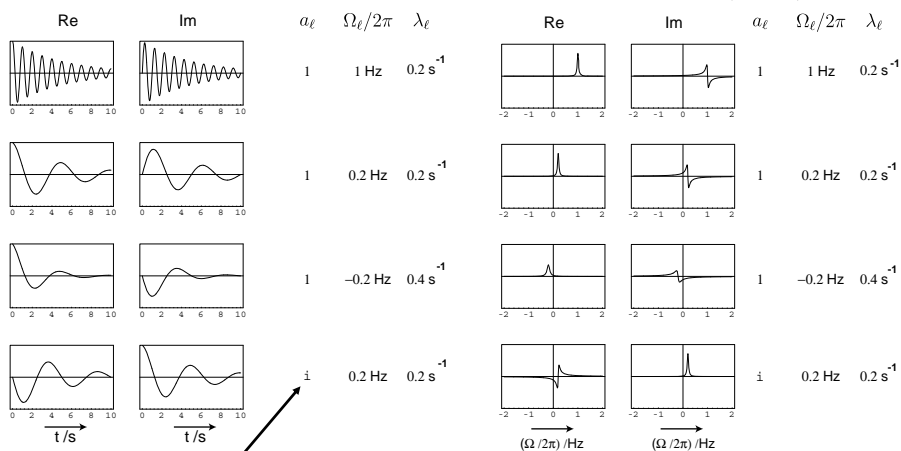
Λ is complex function A, Δ are real functions



The Fourier Transform II

$$s_t(t) = a_t \exp[(i\Omega_t - \lambda_t)t]$$

$$s_t(\Omega) = a_t \cdot \frac{1}{\lambda_t + i(\Omega - \Omega_t)}$$



Remember: $\exp(ix) = \cos(x) + i \sin(x)$

M. Levitt, Spin dynamics

Spectral Phase Shifts



- In general the amplitudes a_i are complex
 - ⇒ real & imaginary components of the spectrum are a mixture of absorption and dispersion Lorentzians.
 - ⇒ complex number calculus: $a_i = |a_i| \exp(i\phi_i)$
- using the identities:

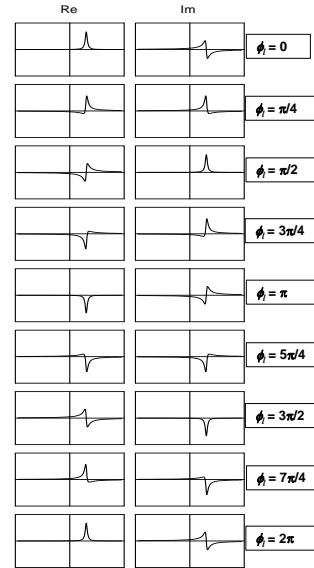
$$\operatorname{Re}\{ab\} = \operatorname{Re}\{a\} \operatorname{Re}\{b\} - \operatorname{Im}\{a\} \operatorname{Im}\{b\}$$

$$\operatorname{Im}\{ab\} = \operatorname{Re}\{a\} \operatorname{Im}\{b\} + \operatorname{Im}\{a\} \operatorname{Re}\{b\}$$

the real and the complex components of the signal $s(\Omega_i) = a_i \Lambda$ can be written as

$$\operatorname{Re}\{a_i L(\Omega, \Omega_i, \lambda_i)\} = |a_i| A(\Omega, \Omega_i, \lambda_i) \cos \phi_i - |a_i| D(\Omega, \Omega_i, \lambda_i) \sin \phi_i$$

$$\operatorname{Im}\{a_i L(\Omega, \Omega_i, \lambda_i)\} = |a_i| D(\Omega, \Omega_i, \lambda_i) \cos \phi_i + |a_i| A(\Omega, \Omega_i, \lambda_i) \sin \phi_i$$

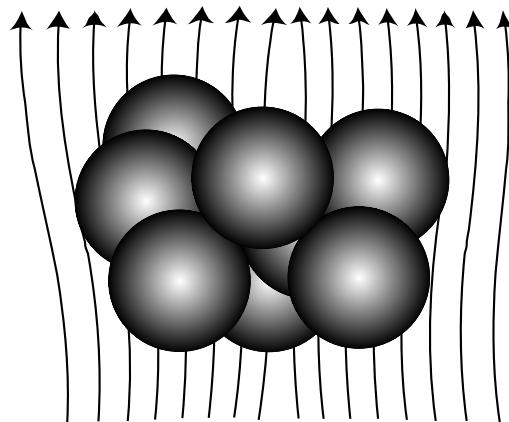


M. Levitt,
Spin
dynamics

The Chemical Shift I



- The magnetic field may be very uniform at macroscopic scale
- On microscopic scale the magnetic field has always has some inhomogeneities



M. Levitt, Spin dynamics

The Chemical Shift II



- Electrons around a nucleus or in a molecule circulate in an external field B_0

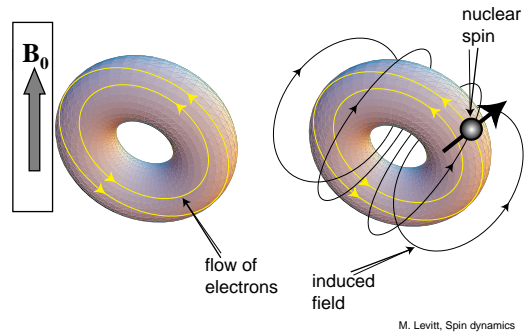
⇒ **induced secondary field**

- The secondary field shields or deshields the external field B_0
- The resonance frequency of a spin j is given by

$$\omega_{0,j} = -\gamma_j B_0 (1 + \delta)$$

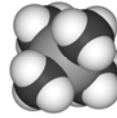
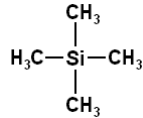
with:

$$\delta [\text{ppm}] = \frac{(v_{\text{sample}} - v_{\text{ref}}) [\text{Hz}]}{v_0 [\text{MHz}]}$$

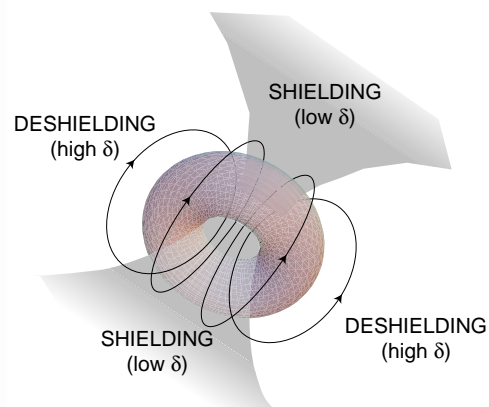
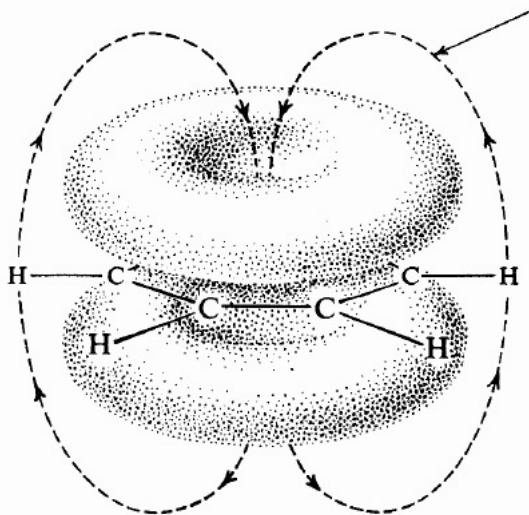


M. Levitt, Spin dynamics

- The detected frequencies (in Hz) for ^1H , ^{13}C , and ^{29}Si nuclei are usually referenced (v_{ref}) against TMS (Tetramethylsilane)

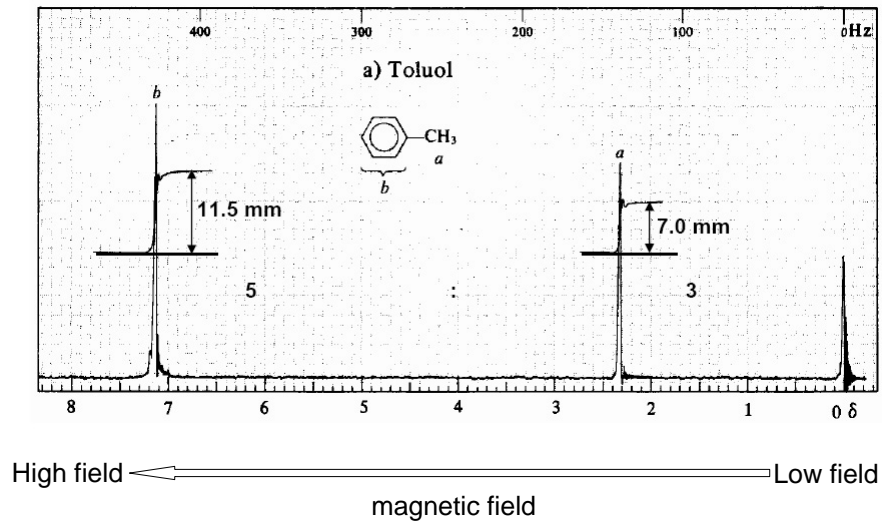


The Chemical Shift II

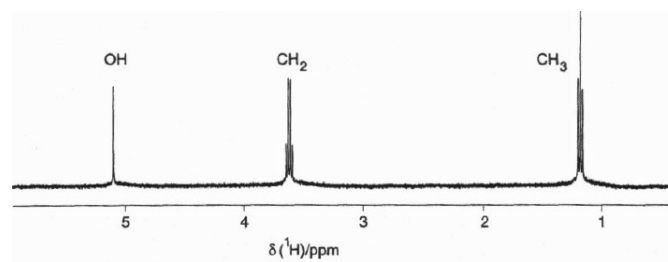
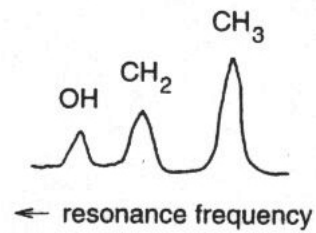
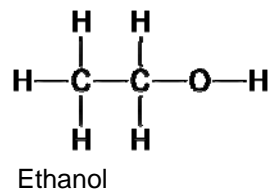


M. Levitt, Spin dynamics

The Chemical Shift III



The Chemical Shift IV



J-Coupling I



- **nuclear spins in a molecule can interact via the bonding electrons**

classically: dipole-dipole interaction

nucleus 1 ↔ bonding electron(s) ↔ nucleus 2

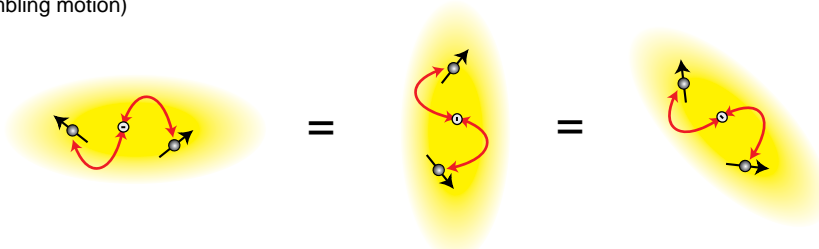
⇒ also called “*indirect dipole-dipole coupling*” or “*indirect spin-spin coupling*”

qm: there is a finite probability to find the electron(s) at the nucleus

⇒ Fermi contact interaction



- the interaction is **intramolecular** ⇒ even in a liquid no averaging (fast molecular tumbling motion)



J-Coupling II



- The J -coupling Hamiltonian is given by: $H_{jk}^{\text{full}} = 2\pi \hat{\mathbf{I}}_j \cdot \mathbf{J}_{jk} \cdot \hat{\mathbf{I}}_k$

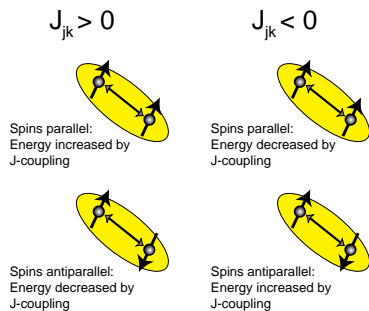
- In an isotropic liquid: $H_{jk}^{\text{iso}} = 2\pi J_{jk} \hat{\mathbf{I}}_j \cdot \hat{\mathbf{I}}_k$

↑
scalar

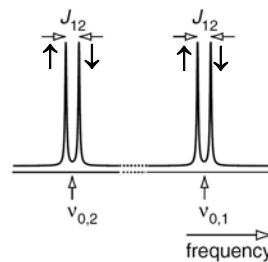
↑
tensor (3x3 matrix)

- J is quoted in Hz ⇒ Factor 2π

Consider two spins ↑↑ with freq. $\nu_{0,1}$, $\nu_{0,2}$ and J -coupling constant J_{12}



M. Levitt,
Spin
dynamics



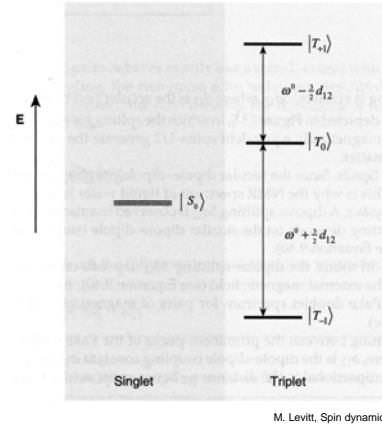
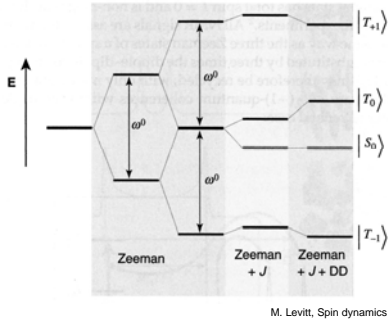
! this is a oversimplified picture ! ⇒ an exact description requires quantum mechanics

J-Coupling III



Pairs of magnetically equivalent spins

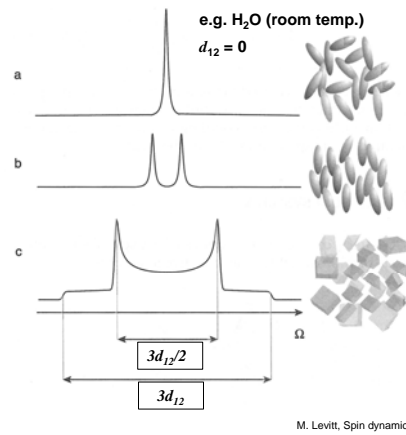
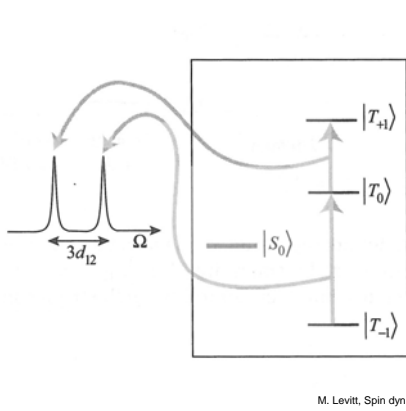
1. **magnetic equivalent** \Rightarrow (same chemical shift, spins have same couplings to all other spins)
assume: J -coupling and direct dipole-dipole coupling (denoted DD, $d_{1,2}$ interaction energy in Hz)



J-Coupling IV



Pairs of magnetically equivalent spins



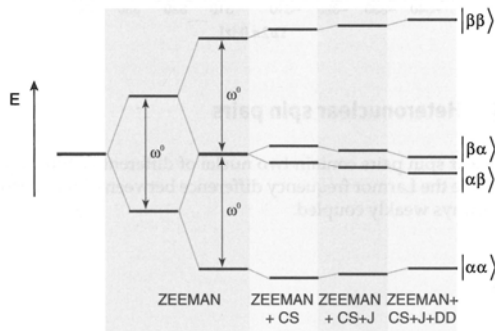
J-Coupling V



Pairs of magnetically *non-equivalent* spins

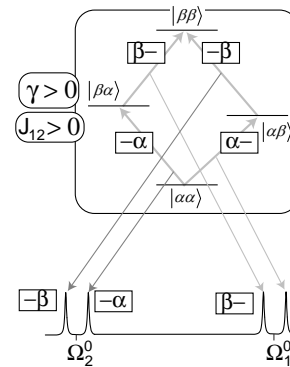
1. magnetically *non-equivalent* \Rightarrow chemical shift $\Rightarrow \Omega_1^0, \Omega_2^0$

assume: J -coupling (J_{12}) and direct dipole-dipole coupling ($d_{1,2}$ interaction energy in Hz)



M. Levitt, Spin dynamics

In isotropic liquid ($d_{1,2} = 0$)



M. Levitt, Spin dynamics

J-Coupling (Examples) I



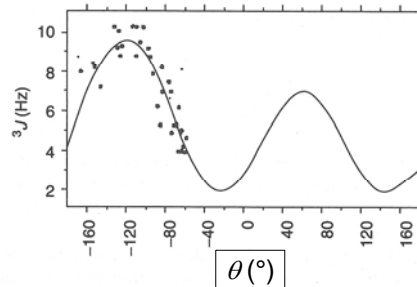
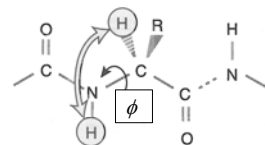
J -coupling over 3 bonds in a protein

\Rightarrow The coupling constant J depends on the torsional angle ϕ

solid line: semi-empirical Karplus equation

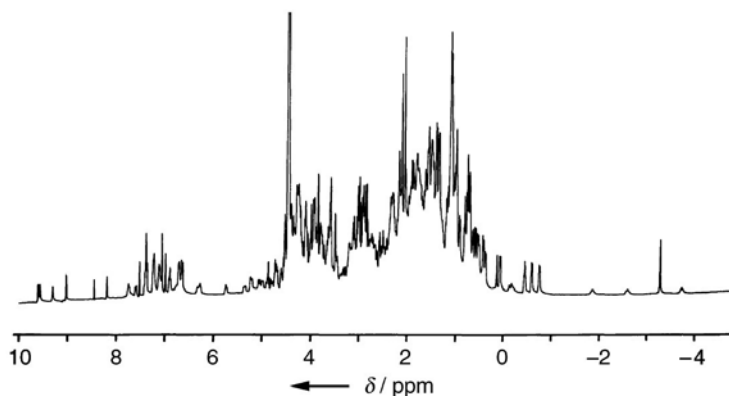
$${}^3J = (6.4 \cdot \cos^2\theta - 1.4 \cdot \cos\theta + 1.9) \text{ Hz}$$

with $\theta = \phi - \pi/3$



M. Levitt, Spin dynamics

J-Coupling (Examples) II



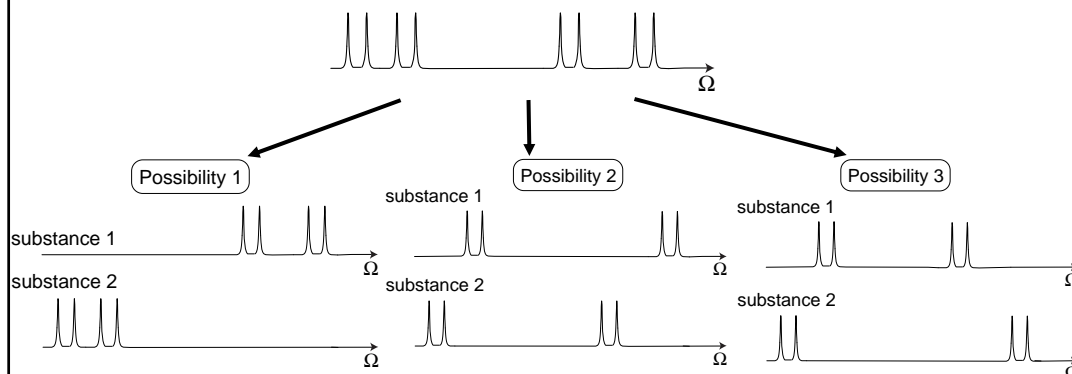
1D-¹H-NMR-spectra (360 MHz) of a protein (horse ferredoxin-c
M = 12000). K. Wüthrich, *Angew. Chem.* 2003, 115, 3462 – 3486

2D Spectroscopy I



The assignment problem

- sample: mixture of 2 compounds, each with different AX system but *identical* J-coupling
- nmr spectrum (e.g.)



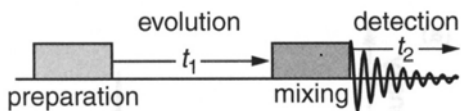
Solution ⇒ two-dimensional spectroscopy

M. Levitt, Spin dynamics

2D Spectroscopy II



The COSY (correlation spectroscopy) pulse sequence



M. Levitt, Spin dynamics



Jean Jeener

First described this 2D NMR experiment on a conference in 1971, but never published it in a journal.



Richard R. Ernst

Ernst's group used the idea and developed multidimensional NMR into powerful methods for molecular structure determination (while giving full credit to Jeener).

(Nobel Prize in chemistry 1991)

Assume two spins (**AX system**) with resonance frequency Ω_1^0 and Ω_2^0 and J -coupling J_{12}

$$S(t_1, t_2) \propto \exp[i(\Omega_1^0 \pm \pi J_{12})t_1] \exp[i(\Omega_2^0 \pm \pi J_{12})t_1] \\ \times \exp[i(\Omega_1^0 \pm \pi J_{12})t_2] \exp[i(\Omega_2^0 \pm \pi J_{12})t_2]$$

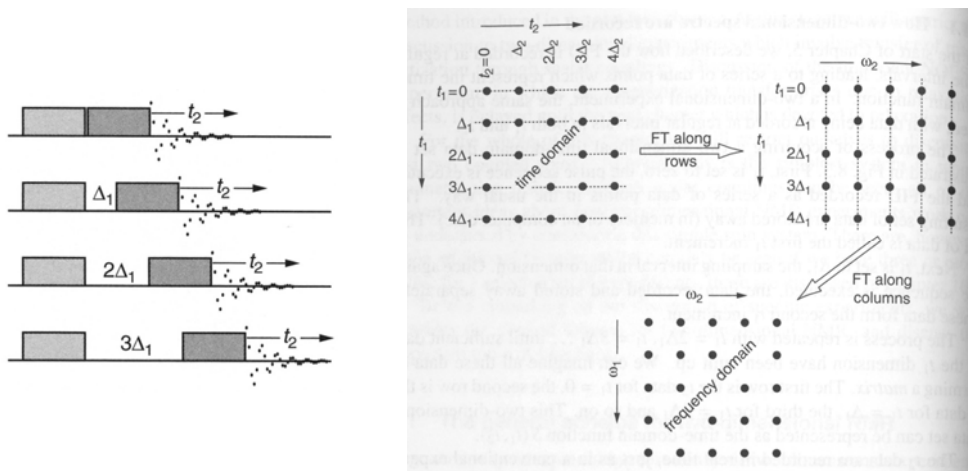
Very simplified expression. For a detailed calculation see e.g. M. H. Levitt, Spin Dynamics or J. Keeler, Understanding NMR Spectroscopy.

\Rightarrow 2D FT of $S(t_1, t_2) \Rightarrow$ **8 Peaks** in the two dimensional frequency domain $S(\Omega_1, \Omega_2)$

2D Spectroscopy III



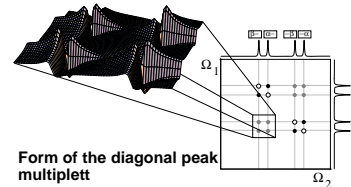
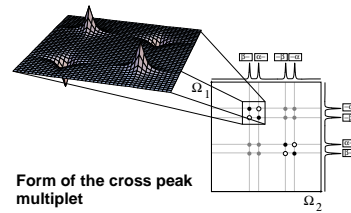
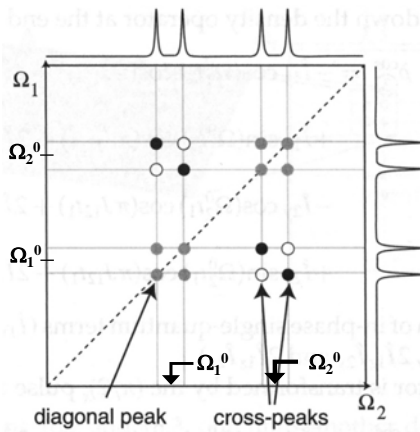
The principle of 2D spectroscopy



2D Spectroscopy IV



AX spin system: $S(t_1, t_2) \propto \exp[i(\Omega_1^0 \pm \pi J_{12})t_1] \exp[i(\Omega_2^0 \pm \pi J_{12})t_1] \Rightarrow$ 2D FT of $S(t_1, t_2)$
 $\times \exp[i(\Omega_1^0 \pm \pi J_{12})t_2] \exp[i(\Omega_2^0 \pm \pi J_{12})t_2] \Rightarrow$ **8 Peaks** in frequency domain $S(\Omega_1, \Omega_2)$



2D Spectroscopy V



COSY in multiple spin systems

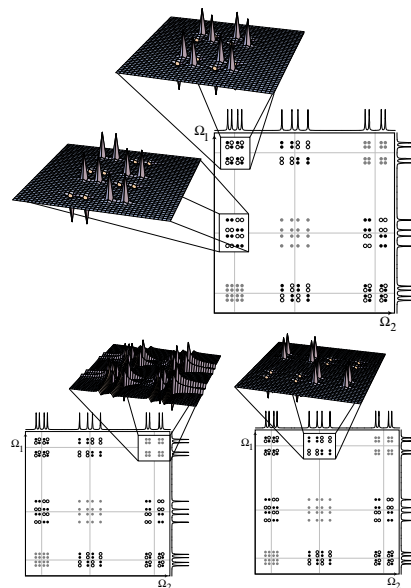
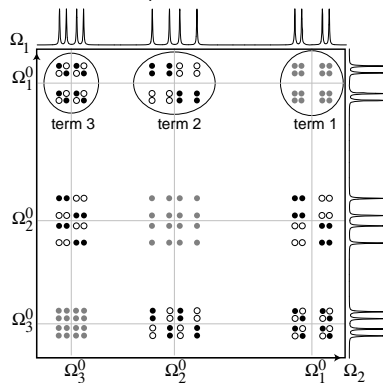
e.g. the AMX spin system:

3 spins with $\Omega_1^0, \Omega_2^0, \Omega_3^0$ and J_{12}, J_{13}

Term 1 \rightarrow diagonal peak

Term 2 \rightarrow cross peak

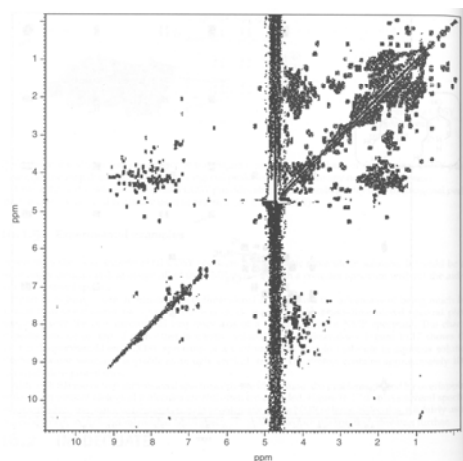
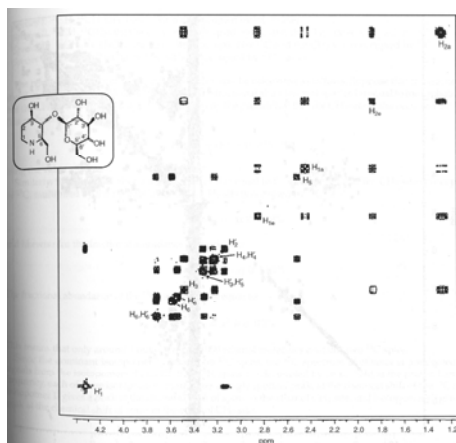
Term 3 \rightarrow cross peak



2D Spectroscopy VI



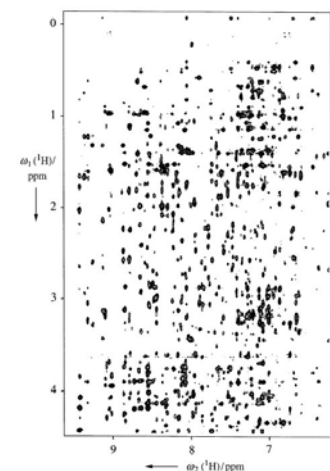
Complex COSY spectra



2D Spectroscopy VII



Structure determination of proteins

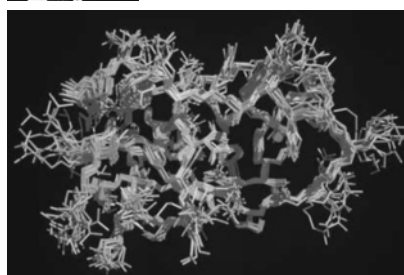


2D-[¹H, ¹H]-NOESY Spectrum of a Protein M = 15 000, K. Wüthrich, *Angew. Chem.* 2003, 115, 3462 – 3486



Kurt Wüthrich
Developed NMR spectroscopy for determining the three-dimensional structure of biological macromolecules in solution.

Nobel Prize in chemistry 2002



bovine pancreatic trypsin inhibitor, BPTI
A protein, that is used as medication administered by injection to reduce bleeding during complex surgery, such as heart and liver surgery.



Summary

- The NMR hardware: magnet, gradient system (localization), receive & transmit coils, complex receiver signal, Nyquist-Shannon sampling theorem
- The FID Experiment: multiple (N) transients $\Rightarrow s/n \propto (N)^{1/2}$
- Fourier transform: complex exponential decay \Rightarrow complex Lorentzian, spectral phase shifts
- Chemical shift: induced fields via molecular electronic currents, shielding & deshielding the external field
- J -coupling: coupling of nuclear spins via bonding electrons \Rightarrow multiplet structure spectra
- 2D spectroscopy: the COSY experiment, two time domains \Rightarrow 2D Fourier transform \Rightarrow 2D freq. domain, analysis of complex spectra (e.g. proteins)