“High-Resolution” NMR Spectroscopy

PHY-719: Advanced biomedical imaging methods and instrumentation

Hikari Yoshihara
Preclinical Imaging Center
ETH and University of Zurich
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Overview

>“High resolution”

> The spectrometer
- probe
- shims
- lock

> Basic NMR
- chemical shift
- scalar coupling
- spin systems

> 2-dimensional experiments
- COSY
- TOCSY
- NOESY

> Heteronuclear experiments
- INEPT & cie.
- HSQC

> Higher resolution
- HR-MAS
- Pure-shift methods

“High-resolution” NMR spectroscopy

> a.k.a. “NMR” (high-res. compared to MRS)

> Peerless tool for chemical analysis, dynamics & structure

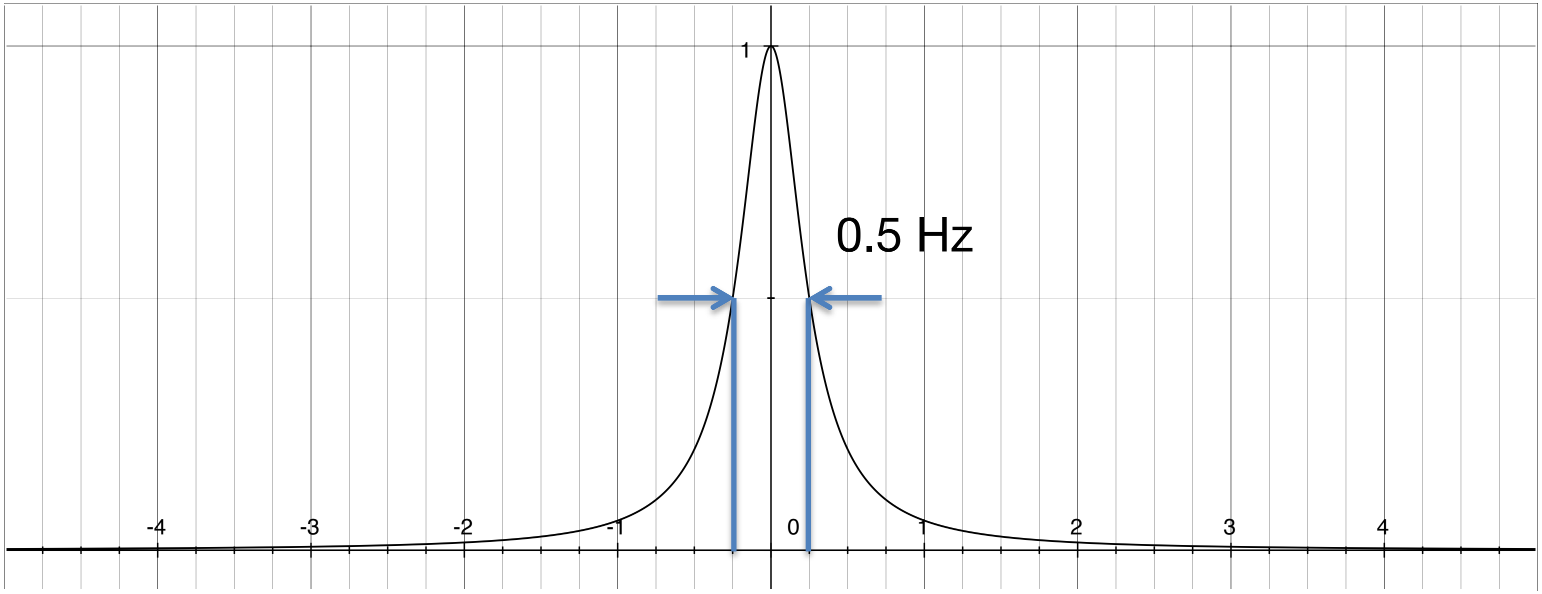
> Mainly liquid (solution) samples, but also gaseous, solid, etc.

> Sensitive to all elemental isotopes with nuclear spin angular momentum

Focus on ^{1}H, ^{13}C & small biological molecule applications

> High spectral resolution:
    < 0.5 Hz ^{1}H line width possible

Narrow spectral line: homogeneous $\mathbf{B}_0$



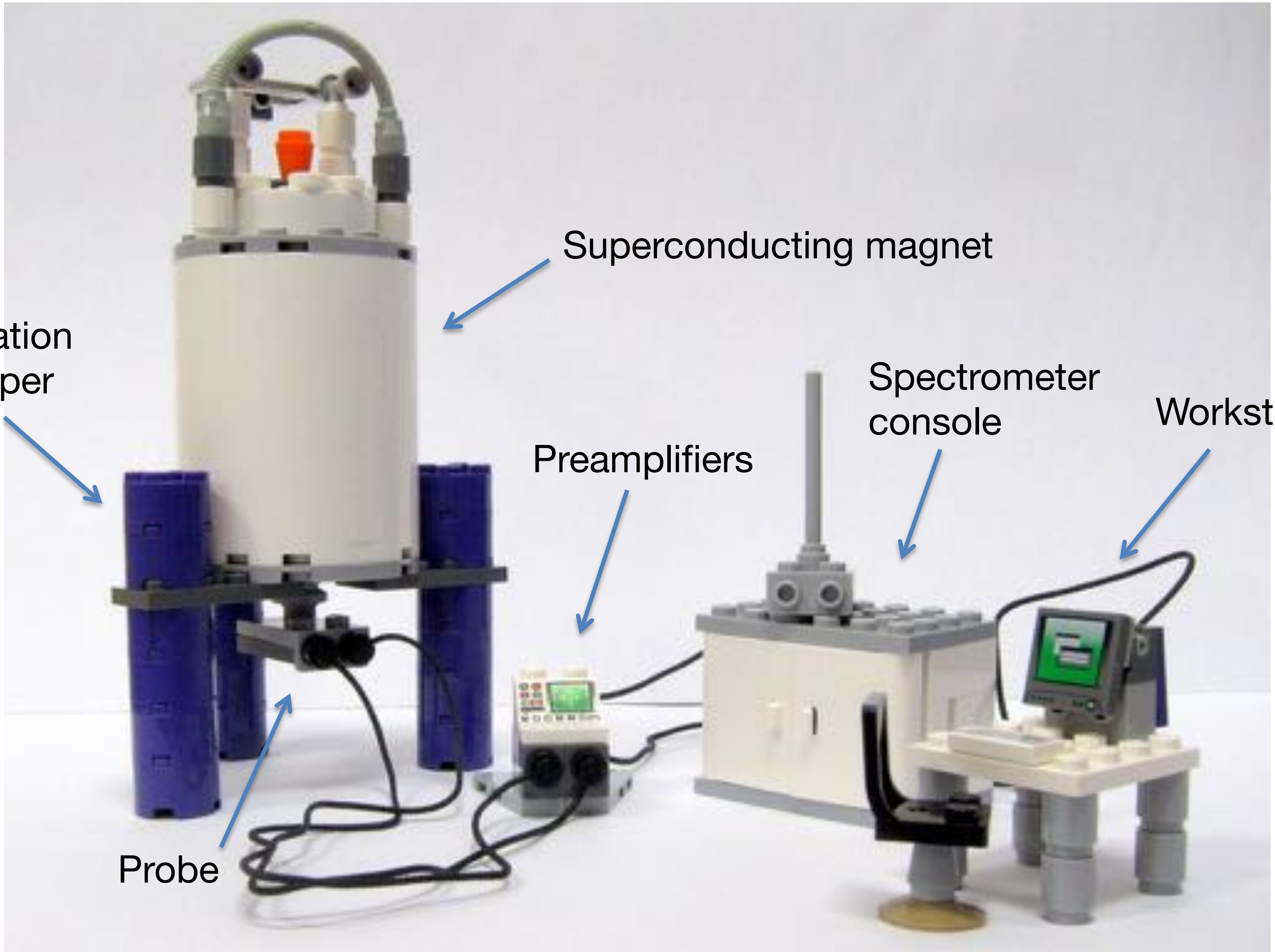
\> e.g. 0.5 Hz ^{1}H Full-width half-maximum line width @ 600 MHz (14.1T)

$\mathbf{B}_0$ homogeneity = 0.5 Hz / 600 MHz = 8.3×10^{-10}

How is this achieved?

- Superconducting magnet typically has $\mathbf{B}_0$ homogeneity of 10^{-6}
- Active (a.k.a. room-temperature) shim coils
- Avoidance of magnetic susceptibility interfaces

Overview of a typical NMR spectrometer



Superconducting magnet

Vibration damper

Spectrometer console

Workstation

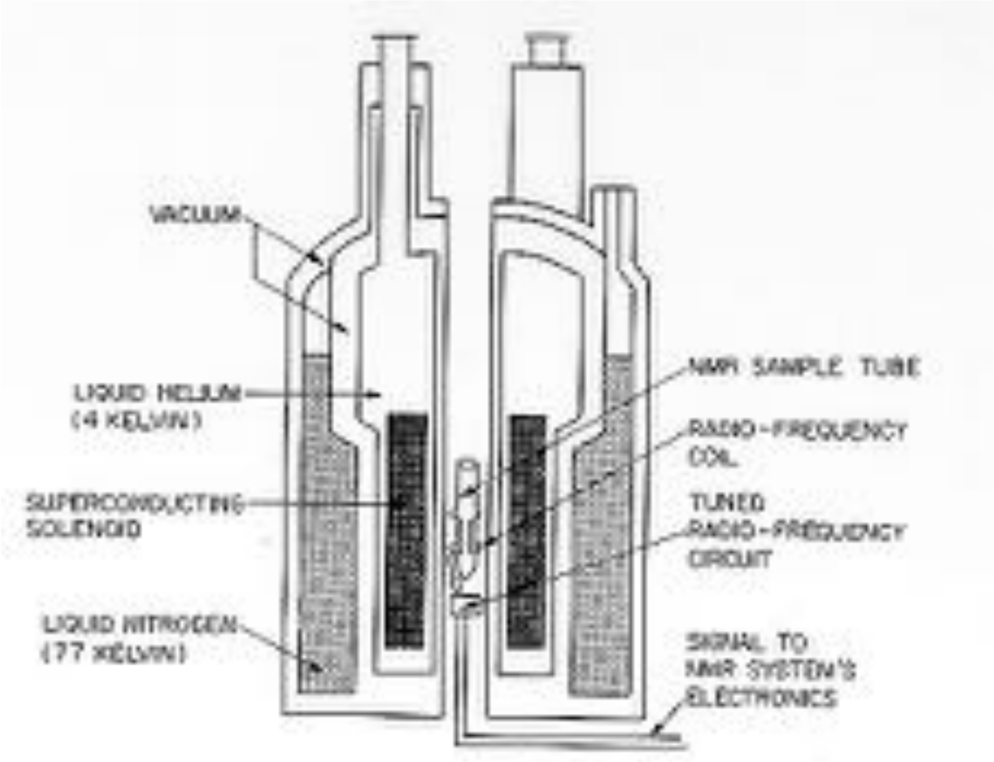
Preamplifiers

Probe

ideas.lego.com

Overview of a typical NMR spectrometer

Magnet



Magnetic field strength customarily expressed as ^1H resonance frequency

Up to 1.2 GHz (28.2T)
More Typically 300 MHz (7T) to 800 MHz (18.8T)

Overview of a typical NMR spectrometer

Preamp & console

Console houses RF electronics:
Frequency synthesizer, waveform generator, Pulse timer, phase shifter, attenuator & power amplifier for transmission (typically >2 independent channels)

Receivers with ADC & digital quadrature detection

Shim current control

Temperature control

Deuterium field lock channel

Pulsed field gradient amplifier

Sample spinning control

Preamplifiers + transcouplers (T/R switch)
Motors for automated probe tuning & matching

Overview of a typical NMR spectrometer

The probe

Probe has the RF coil(s) (and gradient coil(s) if present)

- Tuned to nuclei / frequencies of interest
- Multiple coils may be arranged concentrically
    - Coil closest to sample has greatest sensitivity

Some jargon:

“X-nuclei” – non-^{1}H isotopes with lower gyromagnetic ratio
e.g. ^{13}C, ^{15}N, ^{31}P

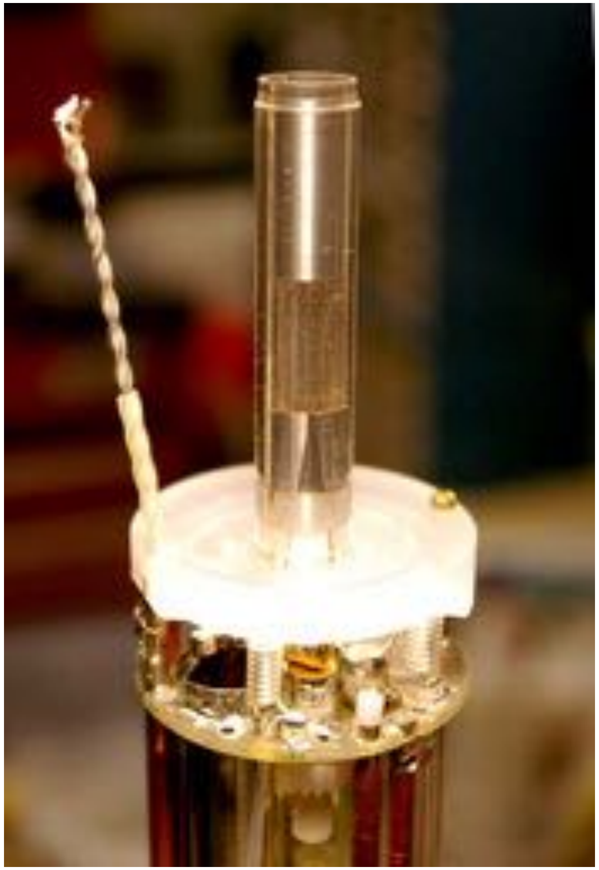
“Broadband” probe – optimized for *direct* detection of
“X”-nuclei, with a wide tuning range

“Inverse” probe – optimized for direct detection of ^{1}H (and
indirect detection of X-nuclei

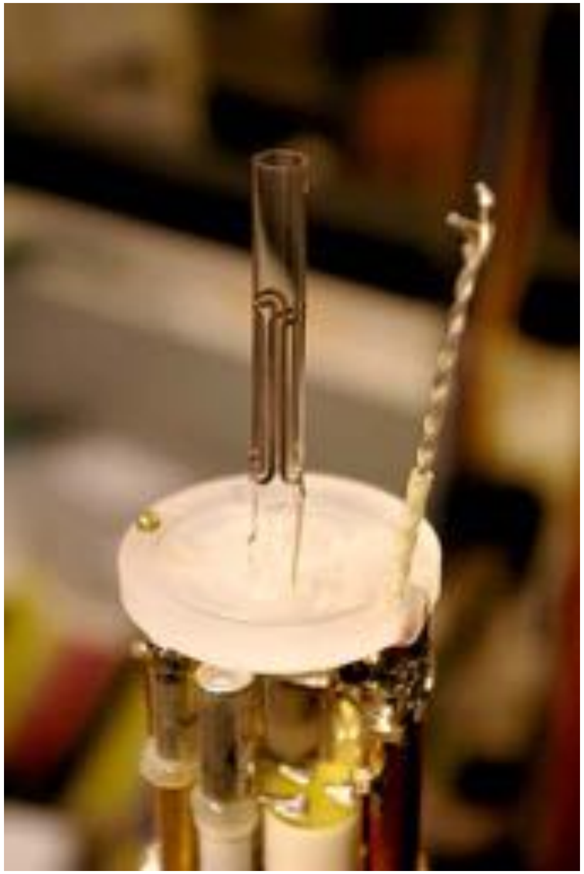
Overview of a typical NMR spectrometer

Inside a probe

Gradient coil



Outer saddle coil

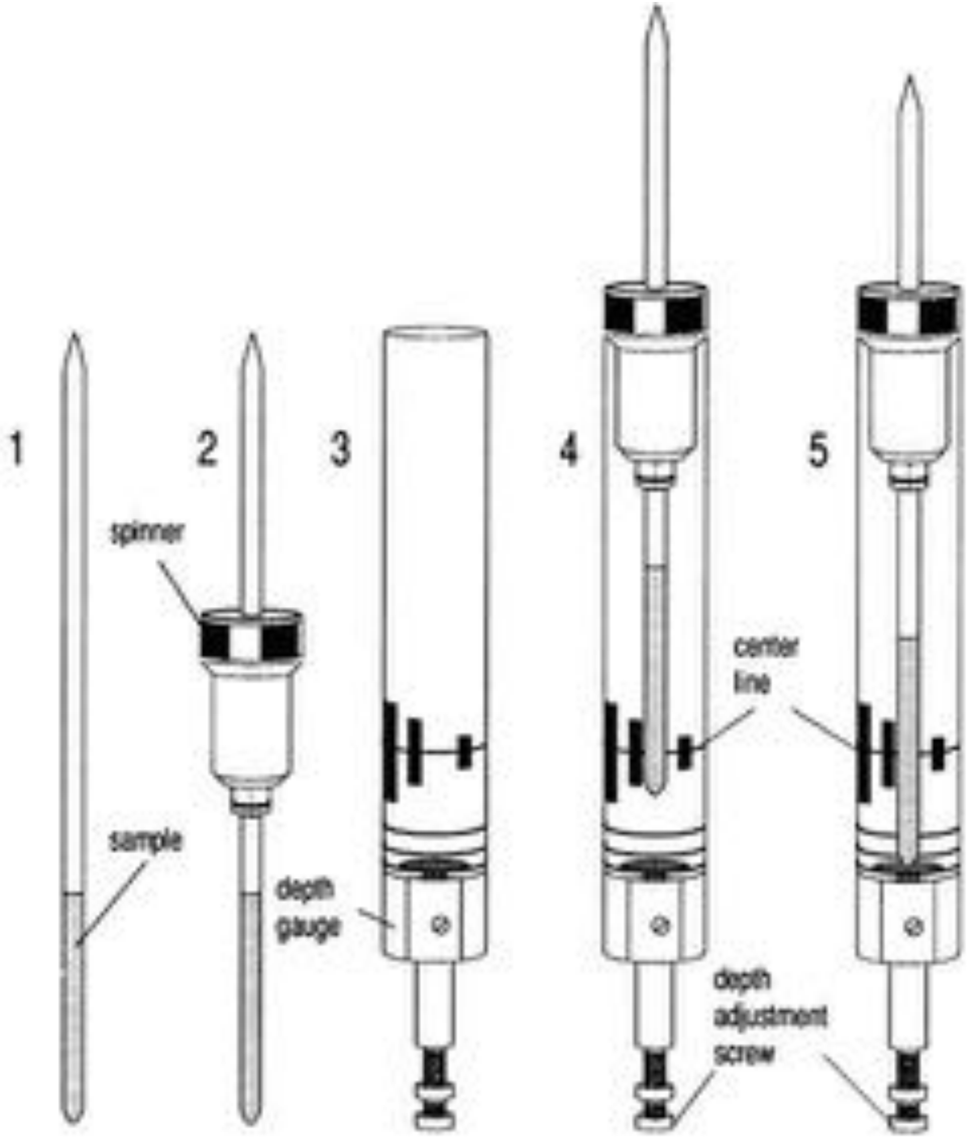


Inner Helmholtz coil

chemnmr.colorado.edu

Overview of a typical NMR spectrometer

Sample tube & spinner (Bruker)

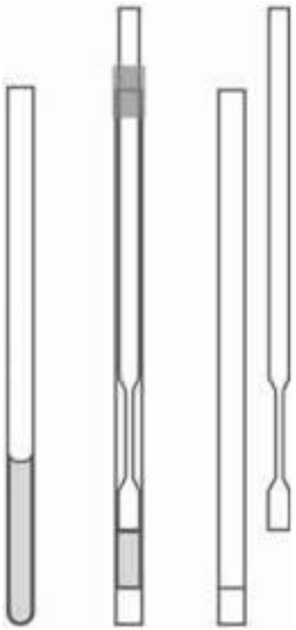


Overview of a typical NMR spectrometer

Sample tubes

- 5 mm (OD) tubes most common, but several standard sizes:
  - 10, 8, 4, 3, 2.5, 2.0, 1.7 mm

- Tubes with magnetic susceptibility-matched plugs (e.g. Shigemi) can maximize signal if sample limited



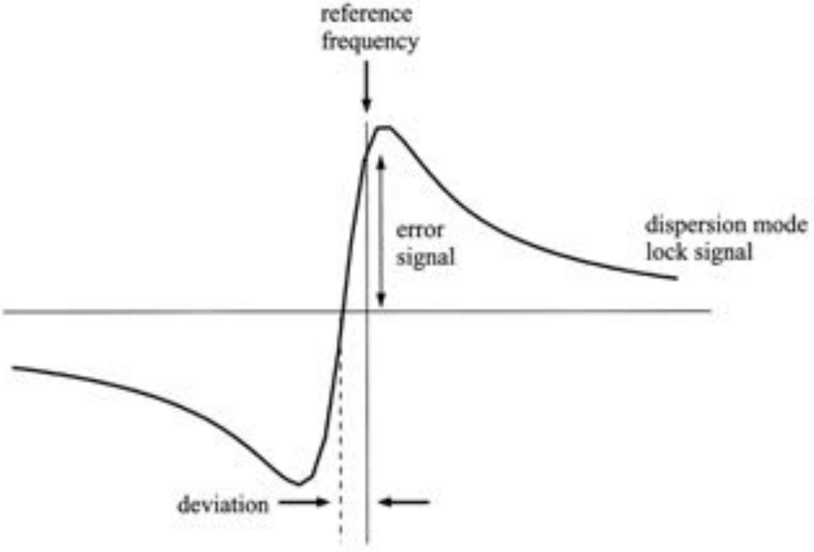
Overview of a typical NMR spectrometer

Sample solvent

- Most NMR experiments use *deuterated* solvents
    - Avoids large ^{1}H background signal (e.g. 110 M H_2O)
    - Provides reference for deuterium field lock

- Deuterium lock maintains constant field around sample to counteract magnet drift.
    - "Mini-spectrometer" monitors ^{2}H (spin $I = 1$) resonance & adjusts current to a coil to maintain $\mathbf{B}_0$

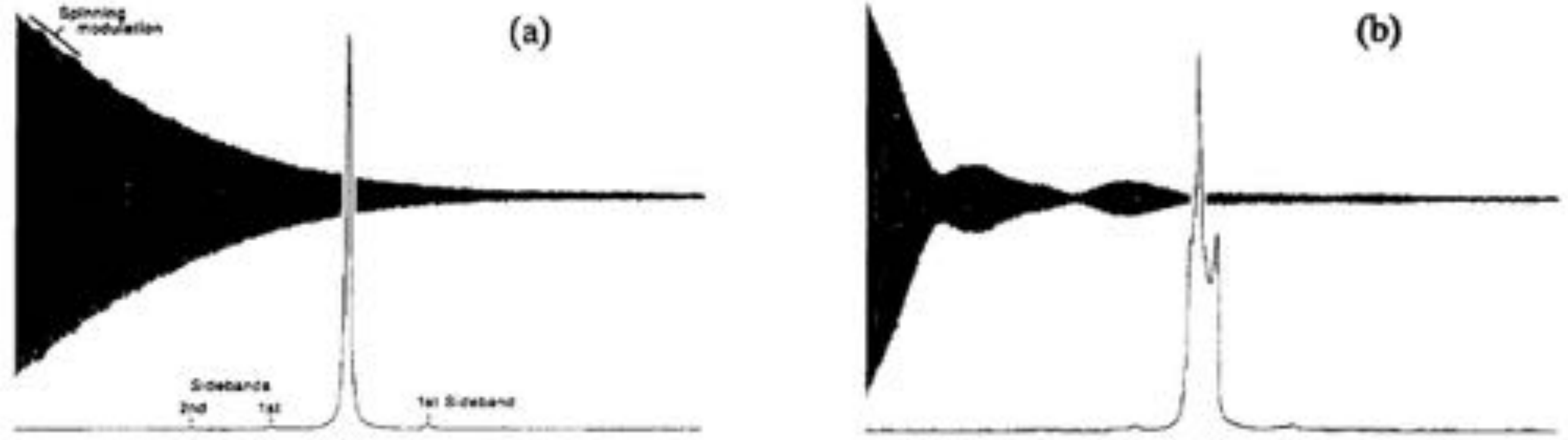
Figure 3.45. The spectrometer lock system monitors the dispersion mode signal of the solvent deuterium resonance. A shift of the resonance frequency due to drift in the static field generates an error signal that indicates the magnitude and direction of the drift, enabling a feedback system to compensate this.



Overview of a typical NMR spectrometer

Dealing with $\mathbf{B}_0$ inhomogeneity: Shimming

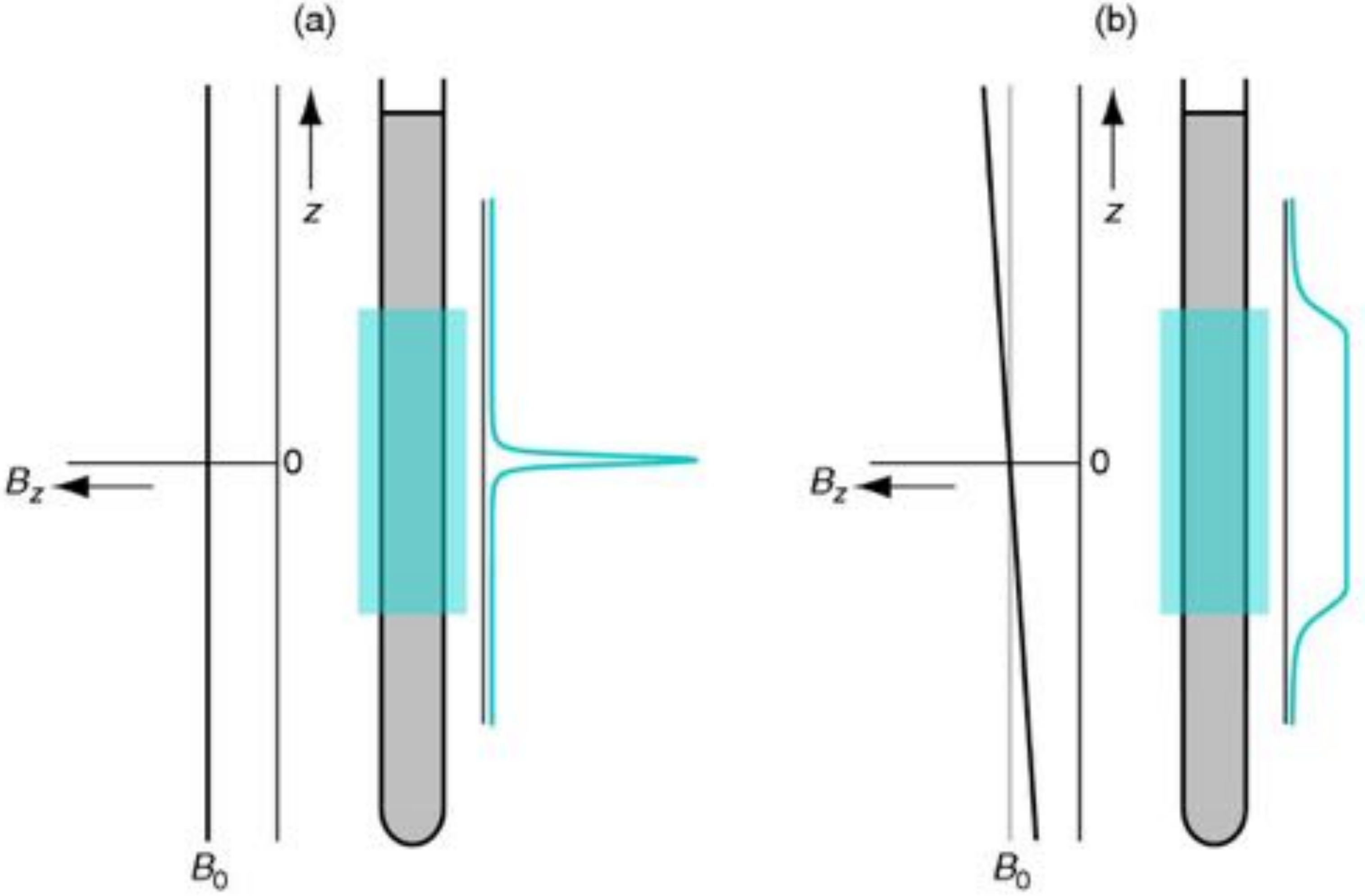
- Set of coils that adjust field spatially with spherical harmonic profile
    - Typically up to 4th order in x, y & z directions, c. 40 coils.
    - Poor $\mathbf{B}_0$ inhomogeneity correction = bad line width / shape



- (Shimming a modern Bruker instrument just involves running “topshim”)

Overview of a typical NMR spectrometer
Gradient shimming

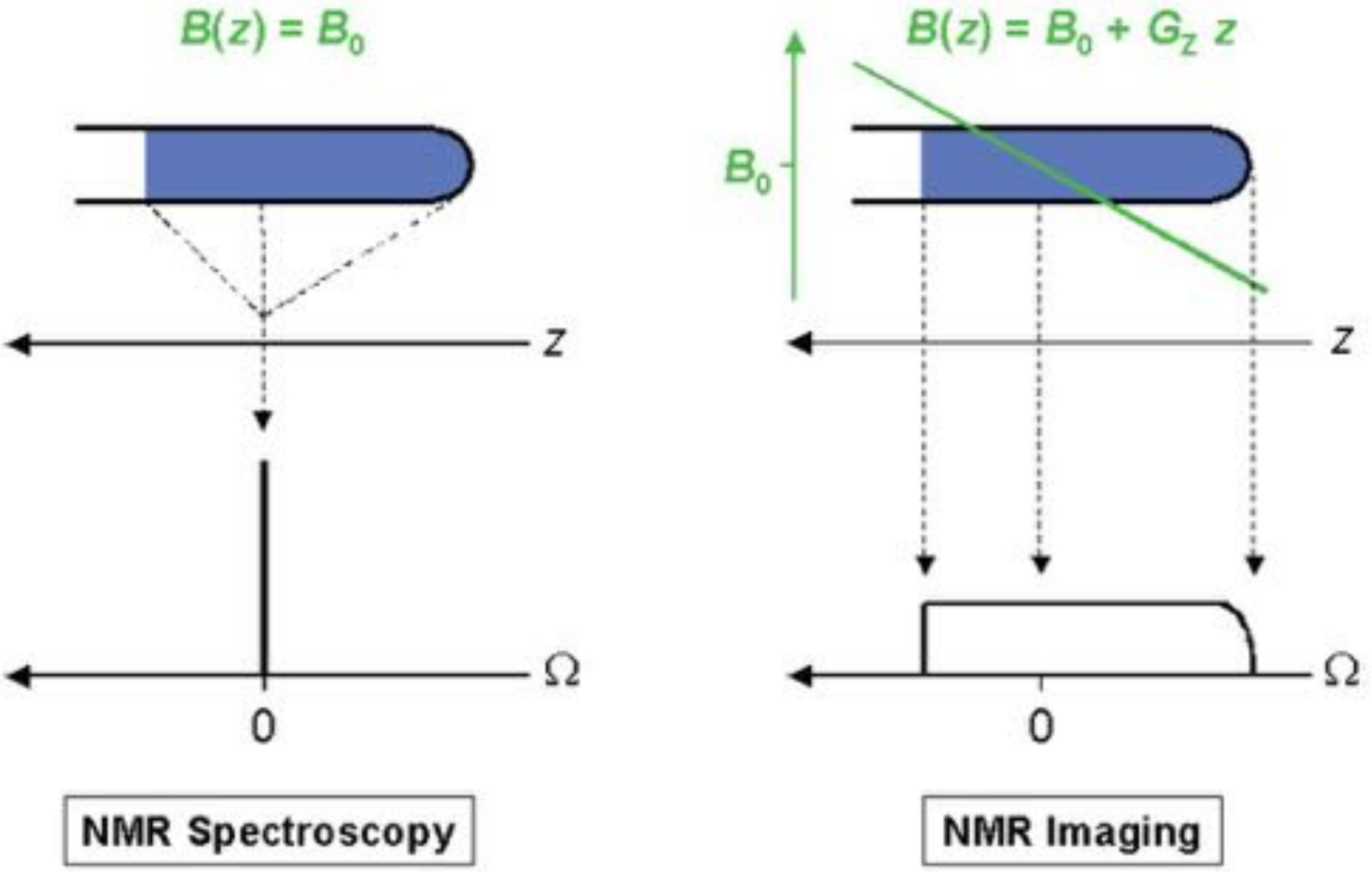
- 1D ‘imaging’ to map shim coil field



Overview of a typical NMR spectrometer

Gradient shimming

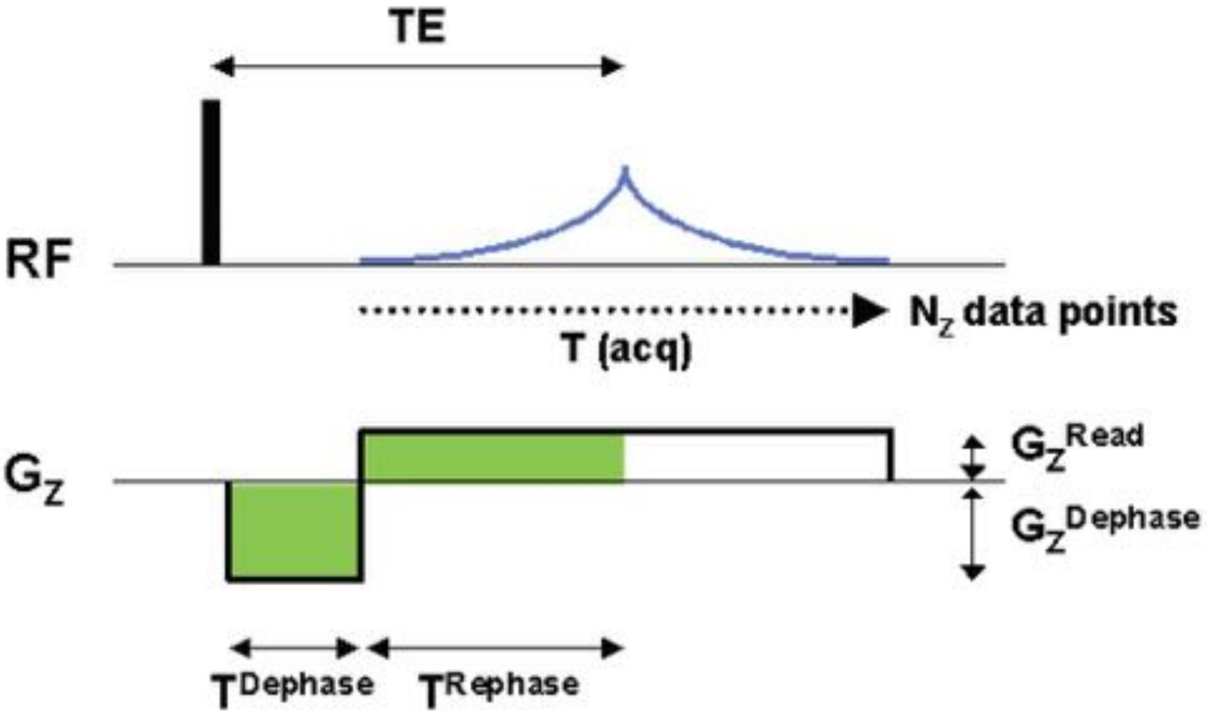
- 1D ‘imaging’ to map shim coil field



Overview of a typical NMR spectrometer

Gradient shimming

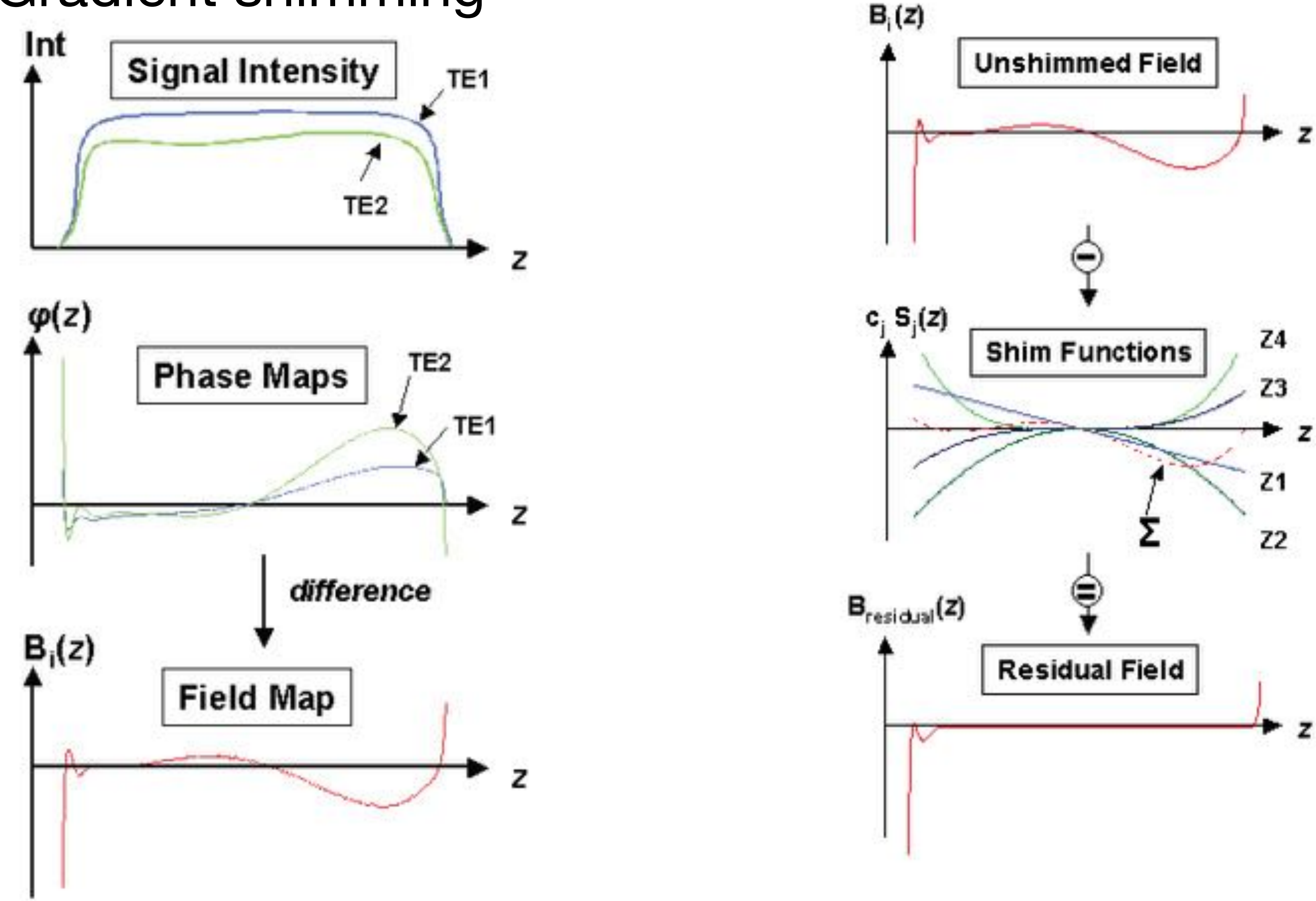
- Map field and shim coil gradients, via gradient echo with different TEs



“Gradient Shimming: Principles and Practical Aspects”, Bruker Biospin

Overview of a typical NMR spectrometer

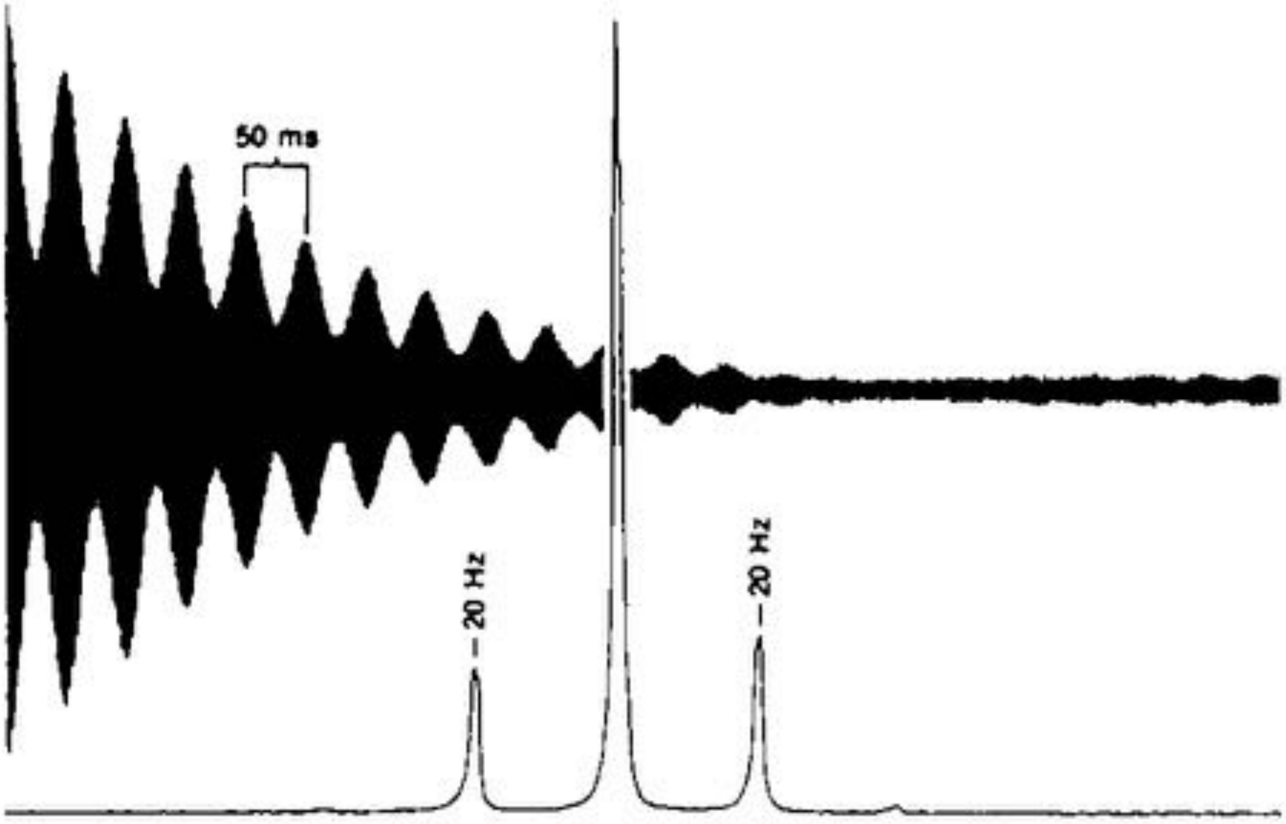
Gradient shimming



“Gradient Shimming: Principles and Practical Aspects”, Bruker Biospin

Overview of a typical NMR spectrometer

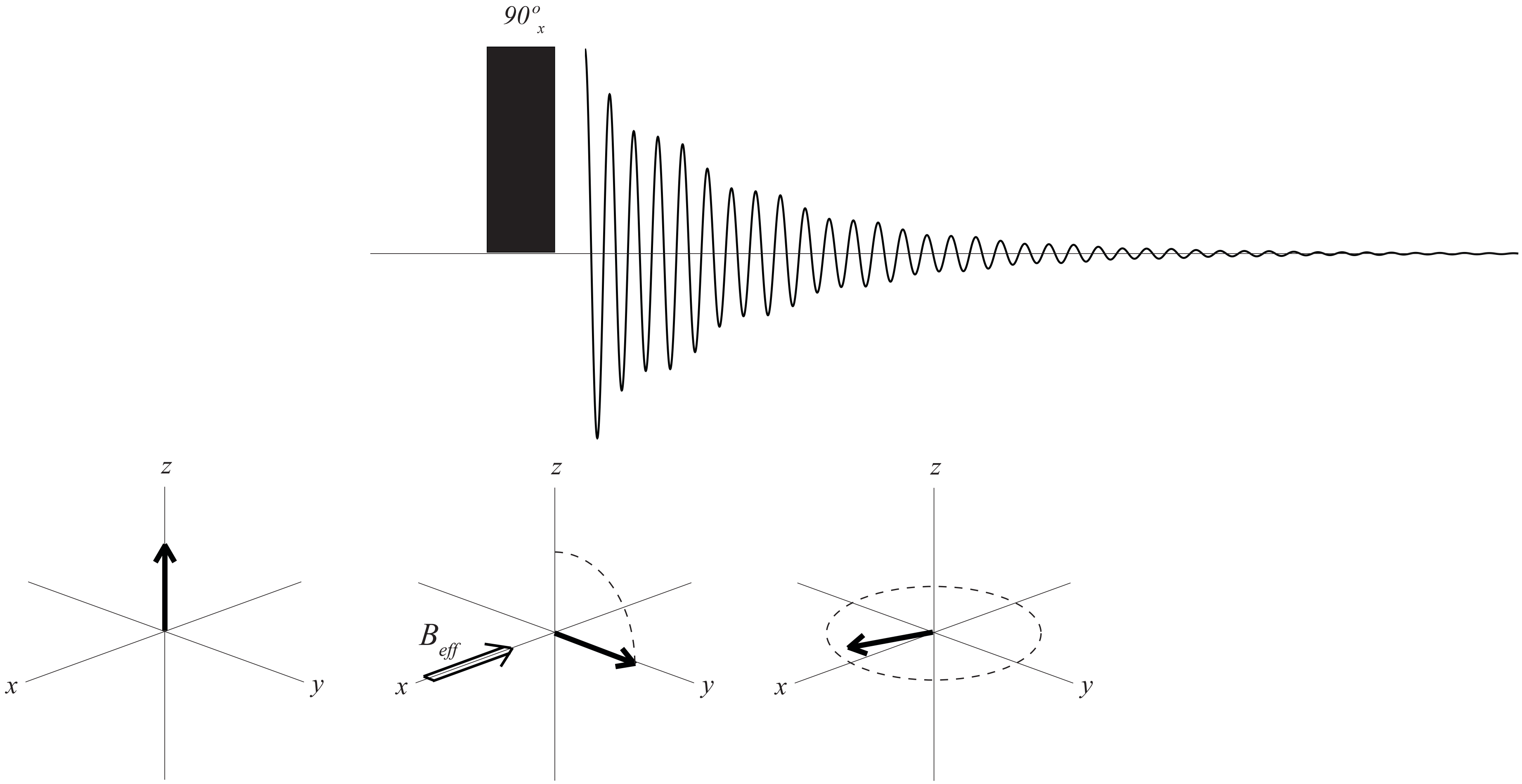
- Sample spinning is often used to average out inhomogeneities in the xy plane – modulated in FID as “spinning sidebands”



Chmurny, G. N. & Hoult, D. I. (1990), The Ancient and Honourable Art of Shimming. Concepts Magn. Reson., 2: 131–149. doi:10.1002/cmr.1820020303

The basic NMR experiment

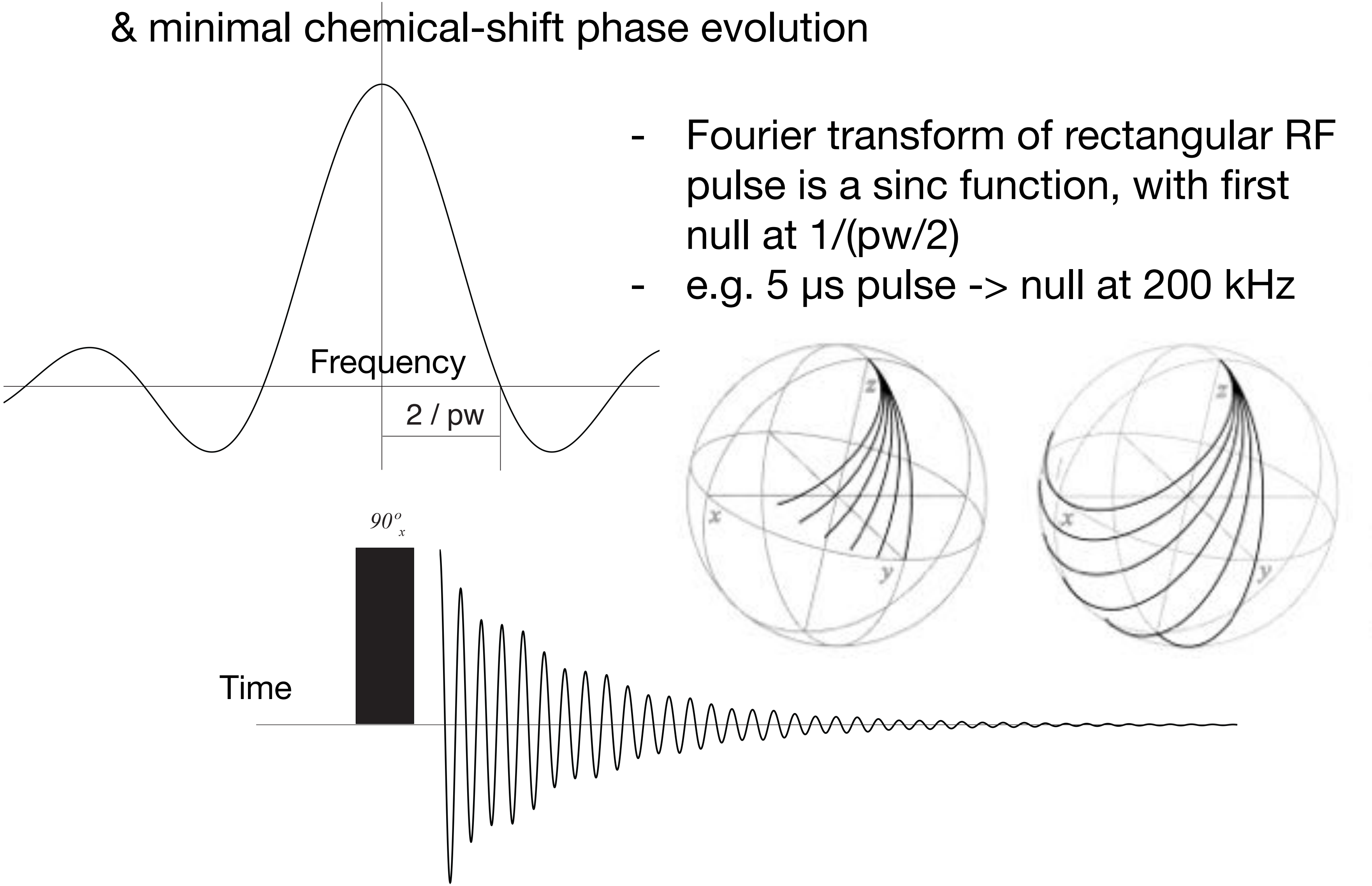
- “Pulse and acquire”
  - High-power microsecond-length rectangular RF pulse; acquire free induction decay (FID)



The basic NMR experiment

- “Pulse and acquire”
  - Short (μs) pulse: broadband excitation ~uniform across spectrum & minimal chemical-shift phase evolution

- Fourier transform of rectangular RF pulse is a sinc function, with first null at 1/(pw/2)
- e.g. 5 μs pulse -> null at 200 kHz



Chemical information from 1D NMR

- Signal directly proportional to the number of spins
  - spectral peak integral for quantitation or relative number of equivalent atoms in a molecule

- Chemical shift
  - resonant frequency influenced by chemical environment of spins, providing structural info

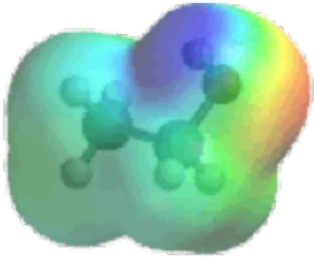
- *J*-coupling a.k.a Scalar coupling:
  - interactions of spins through chemical bonds
    – information on neighboring atoms

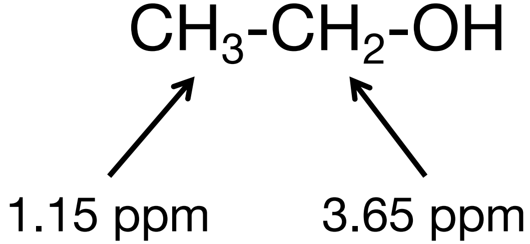
Chemical shift - δ

(Spectral resonance peak frequency – reference frequency)
/ reference frequency

Units in parts per million (ppm), standard reference: tetramethylsilane (TMS)

\> The single most important spectral parameter, the chemical shift is a function of the electronic environment of the nucleus. The surrounding electrons create a local field in opposition of the static magnetic field – “shielding” – stronger with higher electron density. Inductive effects of more electronegative substituents, e.g. oxygen, nitrogen & halogens result in “deshielding” and a higher nuclear resonant frequency.





$$CH_3\text{-}CH_2\text{-}OH$$

Chemical shift - δ

Typical range of ^{1}H chemical shifts

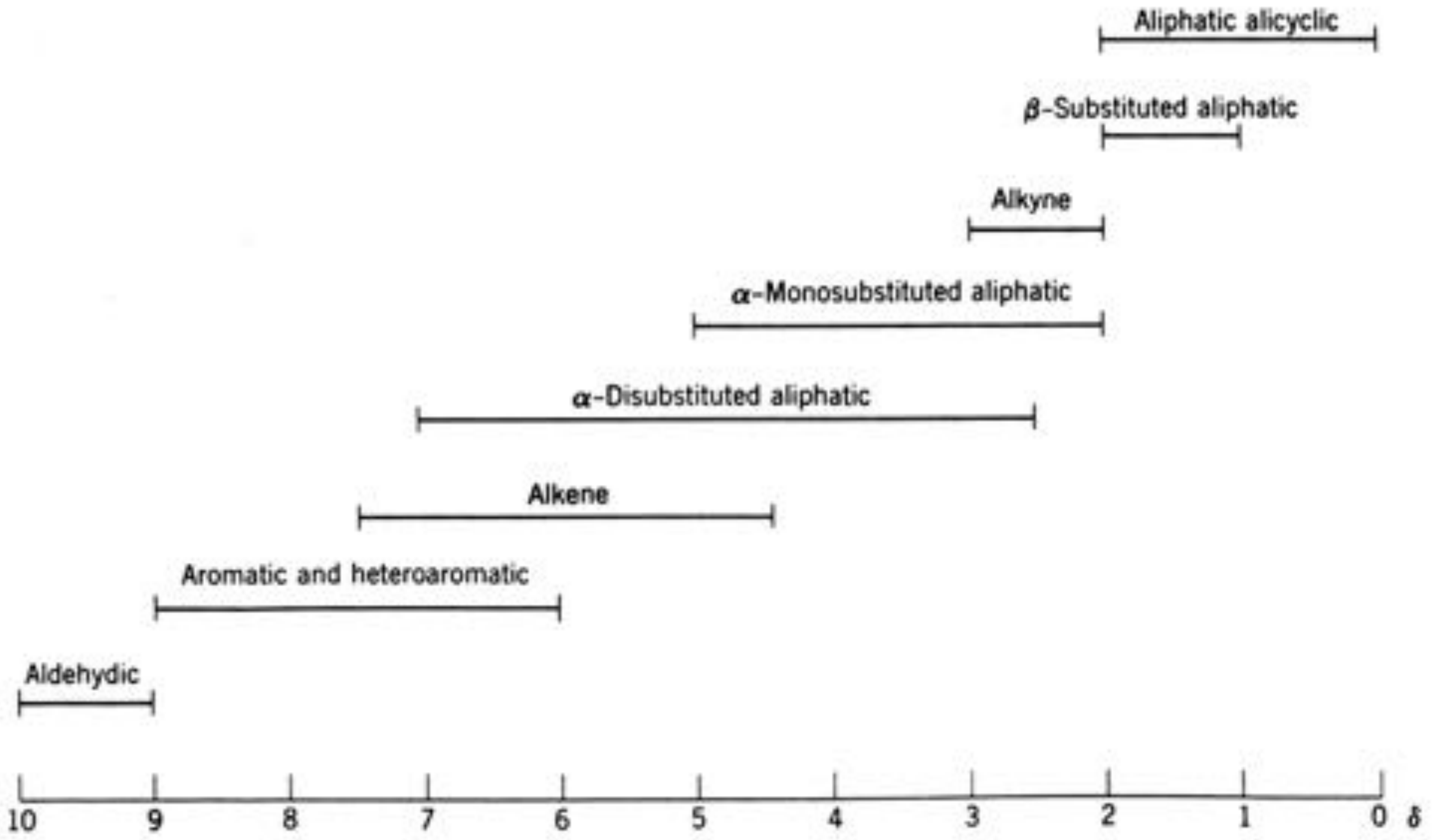
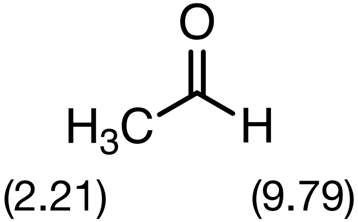


FIGURE 4.20. General regions of chemical shifts.

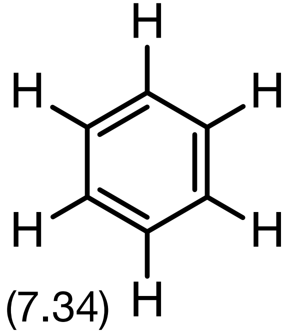
Chemical shift - δ

> Conjugated systems ("double bonds") can produce stronger local fields with higher shielding and deshielding.
> - this is the reason for aldehydic, aromatic & olefinic ^1H chemical shifts

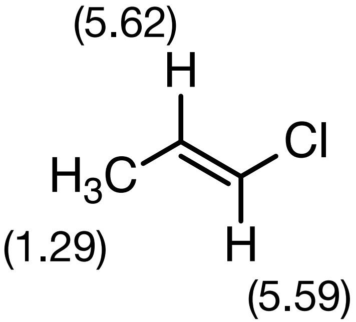
acetaldehyde



benzene



trans-1-chloropropene



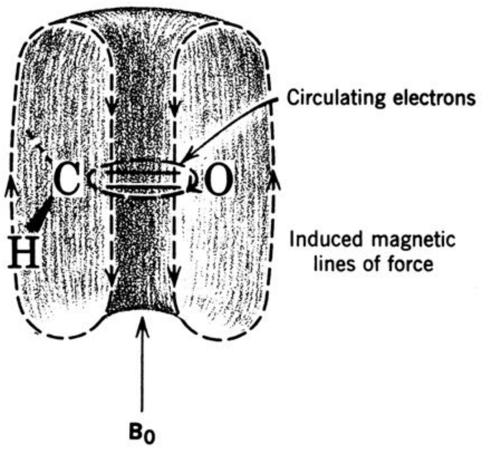


FIGURE 4.16. Deshielding of aldehydic protons.

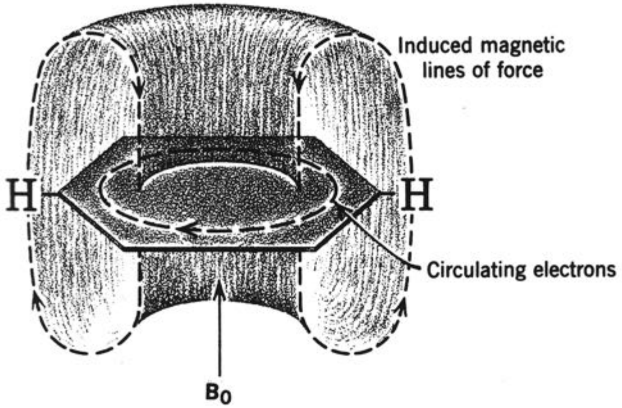
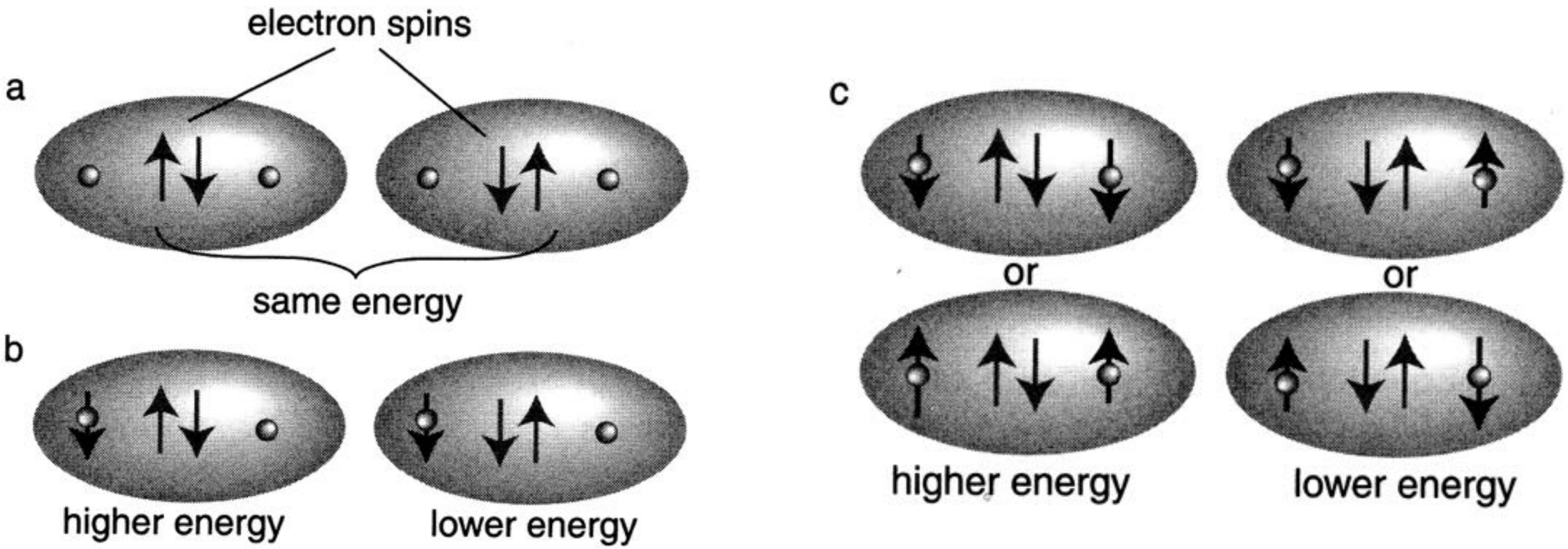


FIGURE 4.17. Ring current effects in benzene.

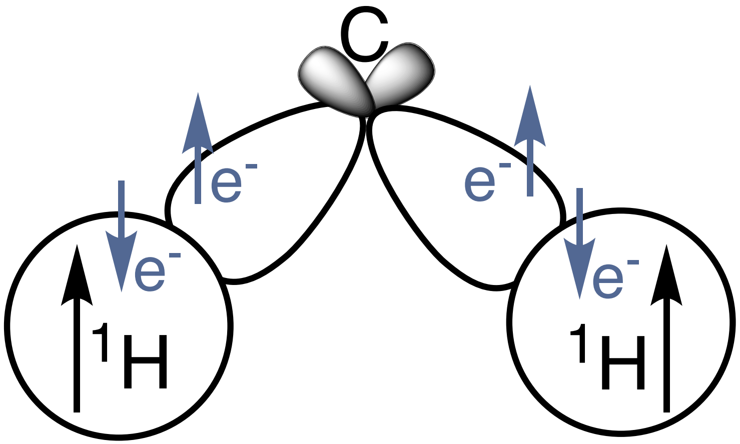
J-coupling (a.k.a. scalar coupling)

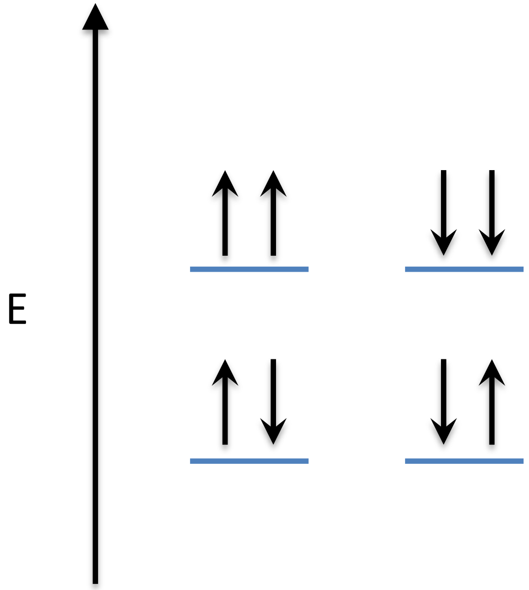
- Energy level of a spin depends on state of neighboring nuclear spins
- *J*-coupling between nuclear spins through electrons in chemical bonds (typically 1 to 3 bonds, homonuclear & heteronuclear)
  - constant – not $\mathbf{B}_0$ dependent – expressed in Hz
- Multiplicity of coupling indicates number of neighbouring spins
  Multiple interactions with different nuclei can result in very complicated splitting patterns.



J-coupling example: 2-bond system

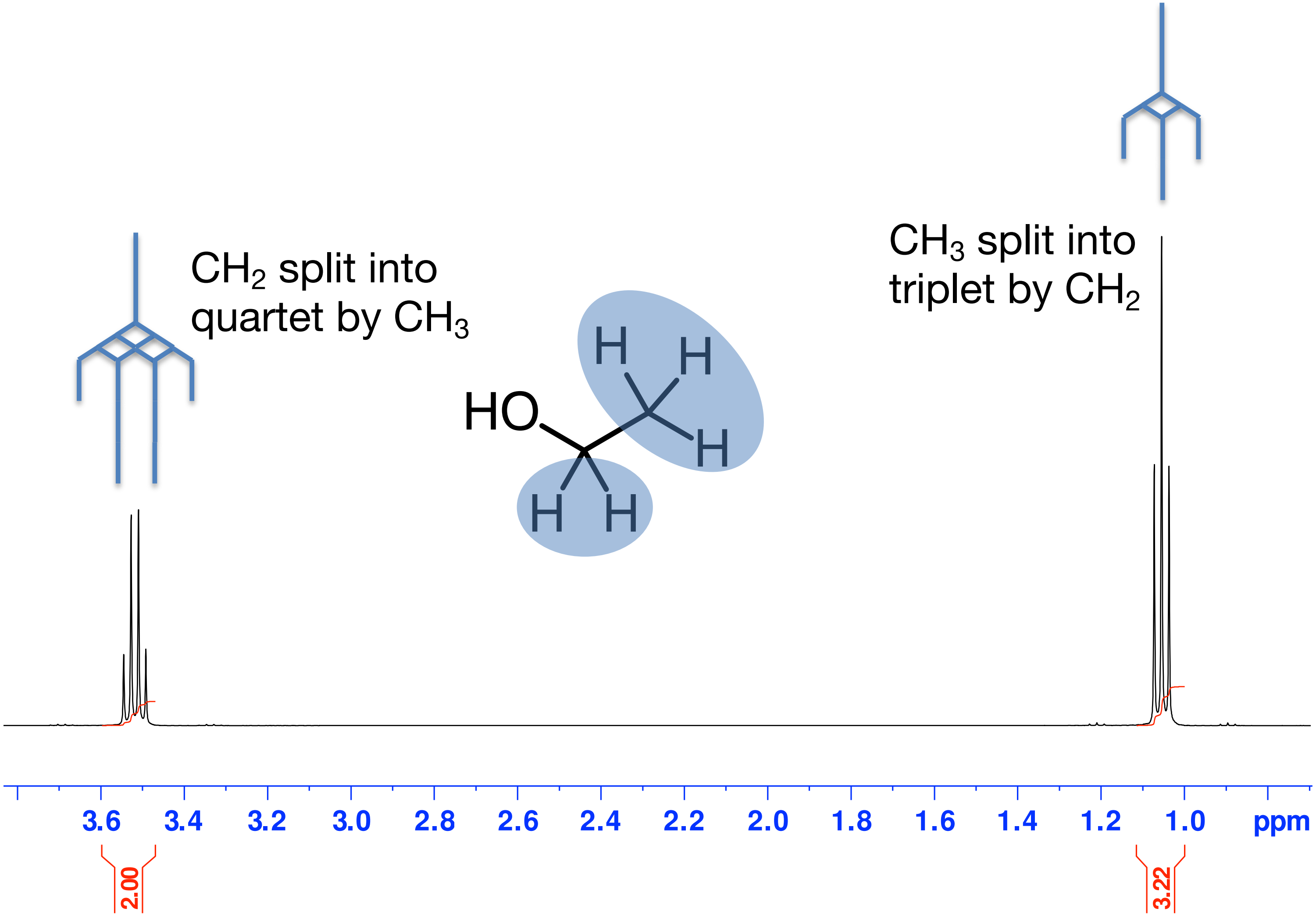
- 2-bond system: methylene ($-CH_2-$)
- (electron spins in covalent bond in opposite states – Pauli exclusion principle)
- four possible ^1H spin states, at two energy levels





J-coupling

- Simple example: Ethanol   Quartet & Triplet (*J* = 7.1 Hz vicinal 3 bond)
  multiplicity = number of neighbouring spins with same *J* value + 1



CH$_2$ split into quartet by CH$_3$

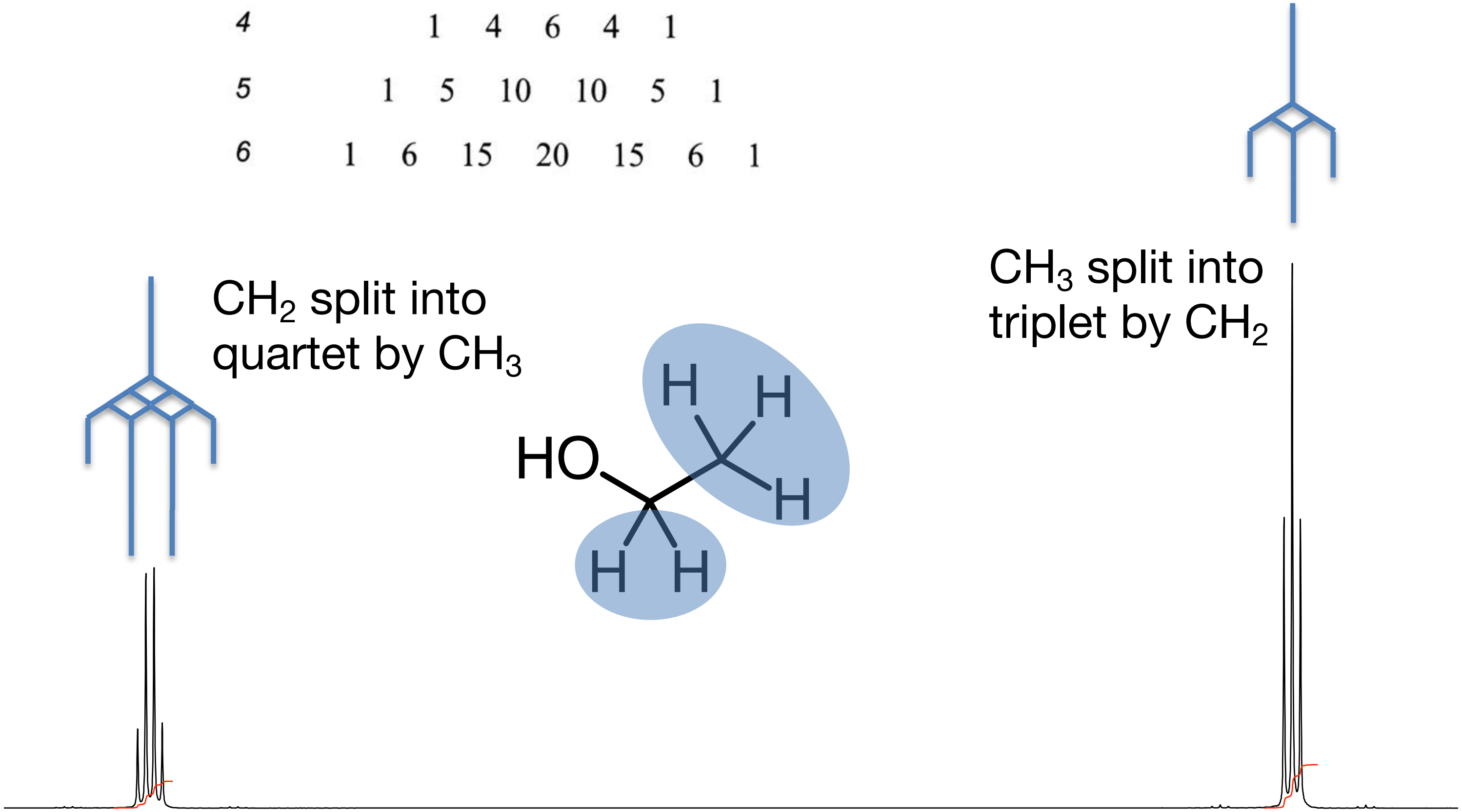
CH$_3$ split into triplet by CH$_2$

J-coupling

- Multiplet intensities follow Pascal's triangle

(a)

n	
0	1
1	1 1
2	1 2 1
3	1 3 3 1
4	1 4 6 4 1
5	1 5 10 10 5 1
6	1 6 15 20 15 6 1

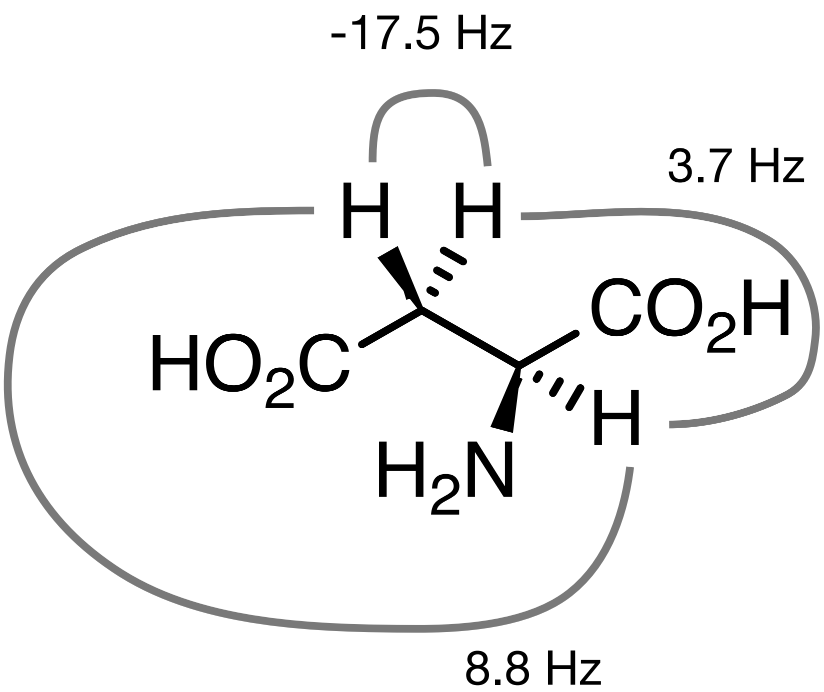


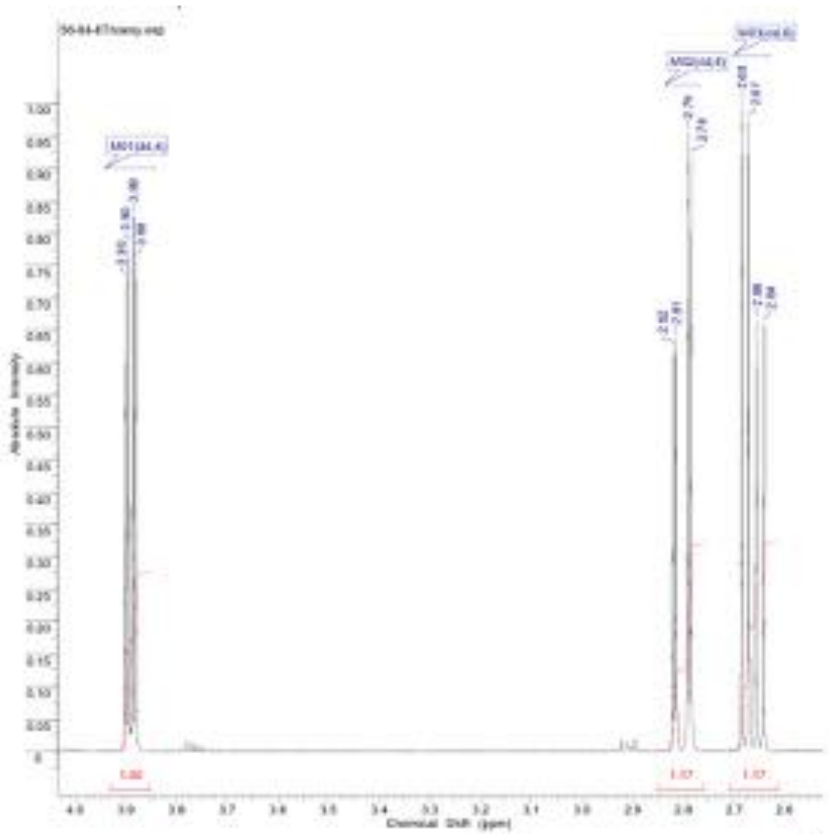
CH_2 split into quartet by CH_3

CH_3 split into triplet by CH_2

J-coupling

- A more complicated example: aspartate. The chiral centre results in the methylene (CH_2) Hs being *diastereotopic* with each in its own environment and not having the same chemical shift. They have a two-bond 17.5 Hz *geminal* coupling, and each resonates as a doublet of doublets (dd)   (“ABX” spin system)

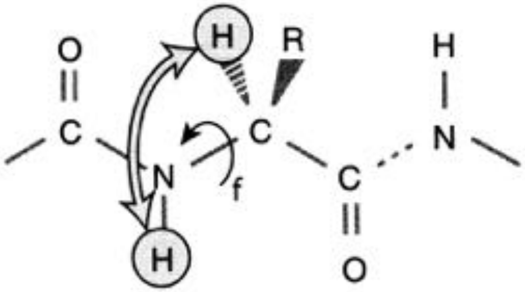


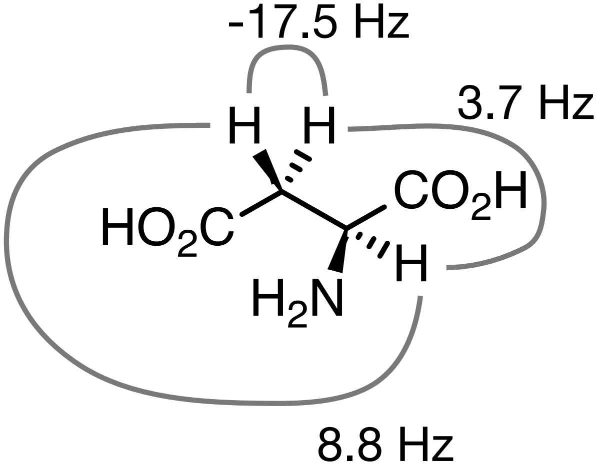


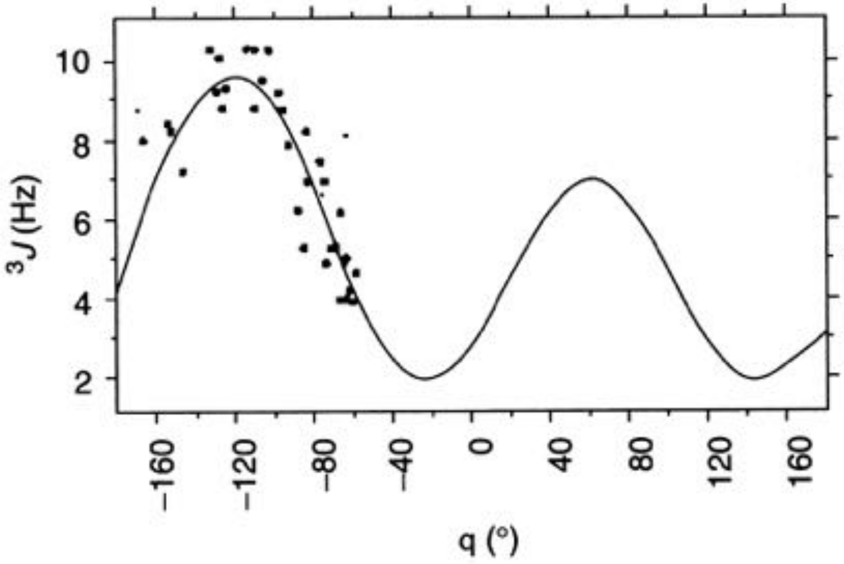
J-coupling

- The dihedral bond angle between the interacting spins has an influence on the strength of the coupling, with 180º resulting in stronger interactions, as described by the semi-empirical Karplus equation:

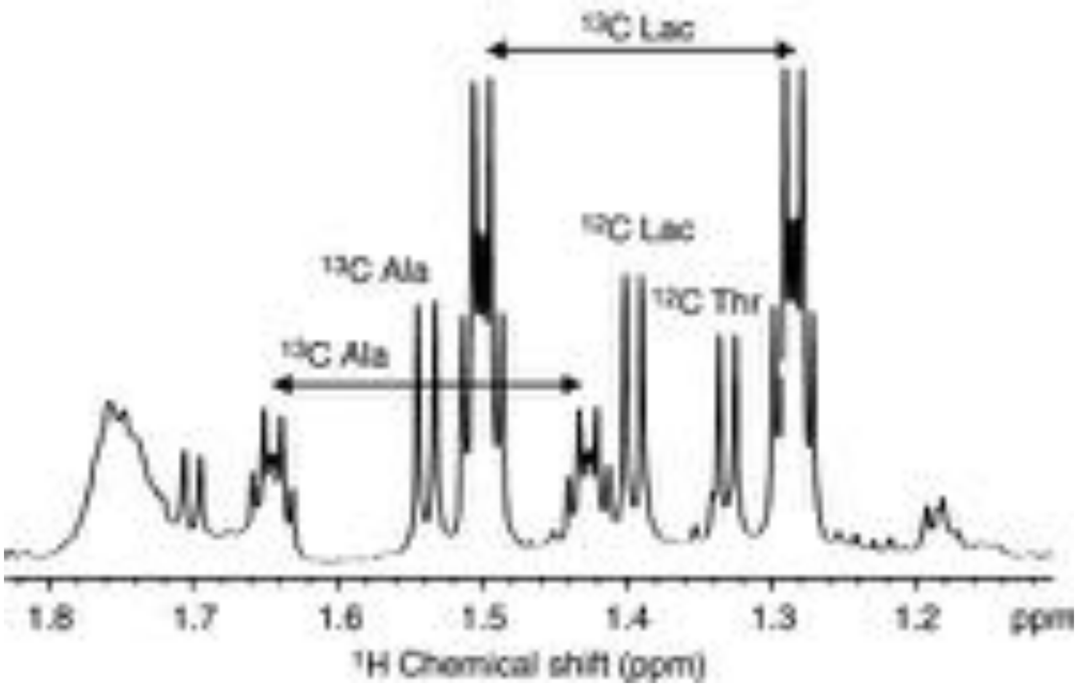
$$^{3}J = (6.4\cos^{2}\theta - 1.4\cos\theta + 1.9)\ \mathrm{Hz}$$

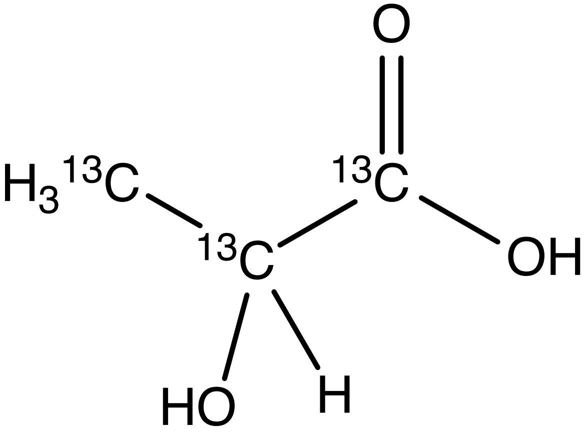






Example application: ^{13}C Isotopic enrichment in ^{1}H NMR





U-^{13}C Lactic acid

Fig. 6. 1-D NMR spectrum of rhabdomyosarcoma cell extract showing satellite peaks. Rhabdomyosarcoma cells Rh30 were grown in the presence of [U-^{13}C]-glucose for 24 h. The cells were extracted with cold 10% TCA, lyophilized, and redissolved in D_2O. The spectrum of the TCA extract was recorded at 14.1 T, 20 °C using a recycle time of 5 sec. 256 transients were co-added (22 min acquisition). The methyl region shows Lac, Ala, and Thr and the satellite peaks of Lac and Ala.

Methods in Cell Biology, Volume 84, 2008, Pages 541-588 doi: 10.1016/S0091-679X(07)84018-0

^{13}C NMR

> ^{13}C is present at ~1.1% natural abundance, and its gyromagnetic ratio is 3.977-fold lower than ^{1}H, so its sensitivity is much lower, even with 100% enrichment for ^{13}C.

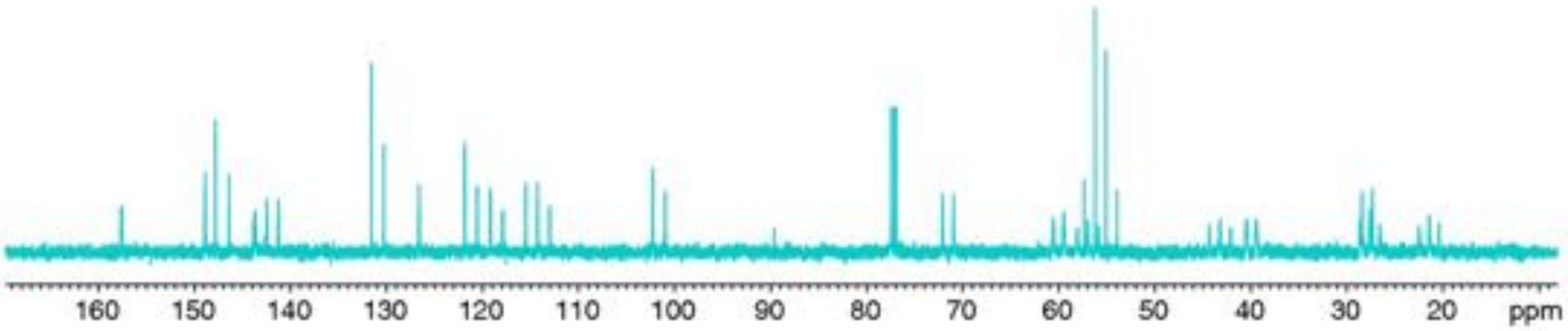
> ^{13}C NMR has excellent chemical shift dispersion (> 200 ppm) and it can be highly informative of chemical identity.

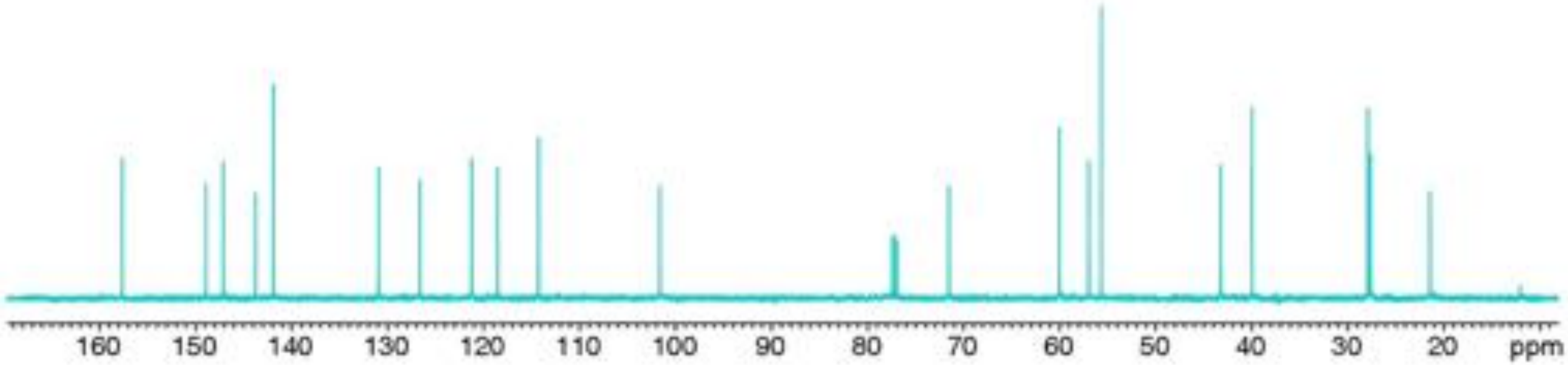
> Most carbons are bound to hydrogen in organic and biologically-derived compounds. One bond ^{13}C – ^{1}H *J* couplings are typically ~120 Hz.

- while this coupling interaction is usefully exploited (as we'll see), it does complicate ^{13}C NMR, and broadband ^{1}H-decoupled are typically acquired instead

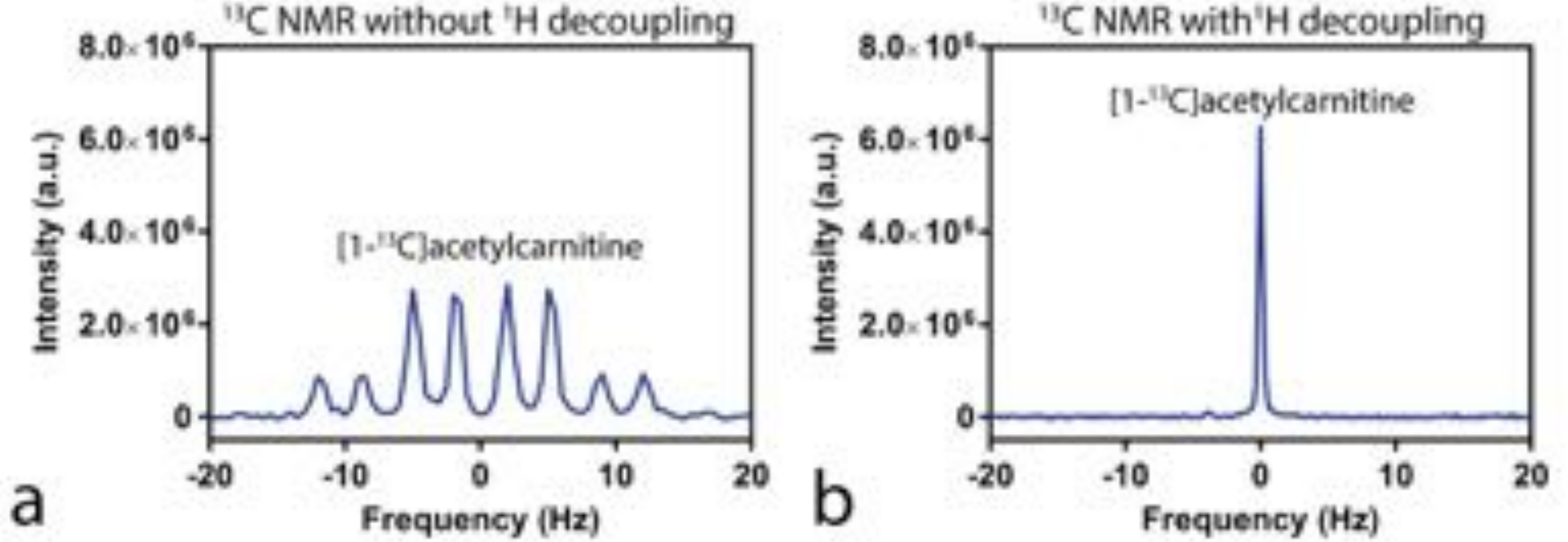
- decoupling requires rapidly inverting the population of the coupled spins during FID acquisition & is usually performed with a composite pulse train such as WALTZ-16. (also signal enhancement from NOE)

^{13}C NMR example quinine, without and with ^{1}H decoupling

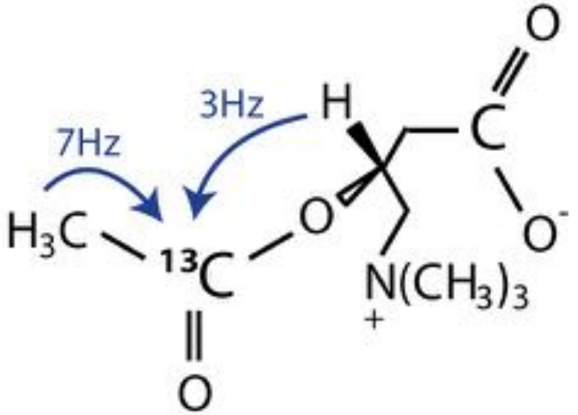




^{13}C NMR example [1-^{13}C]acetylcarnitine & ^{1}H decoupling

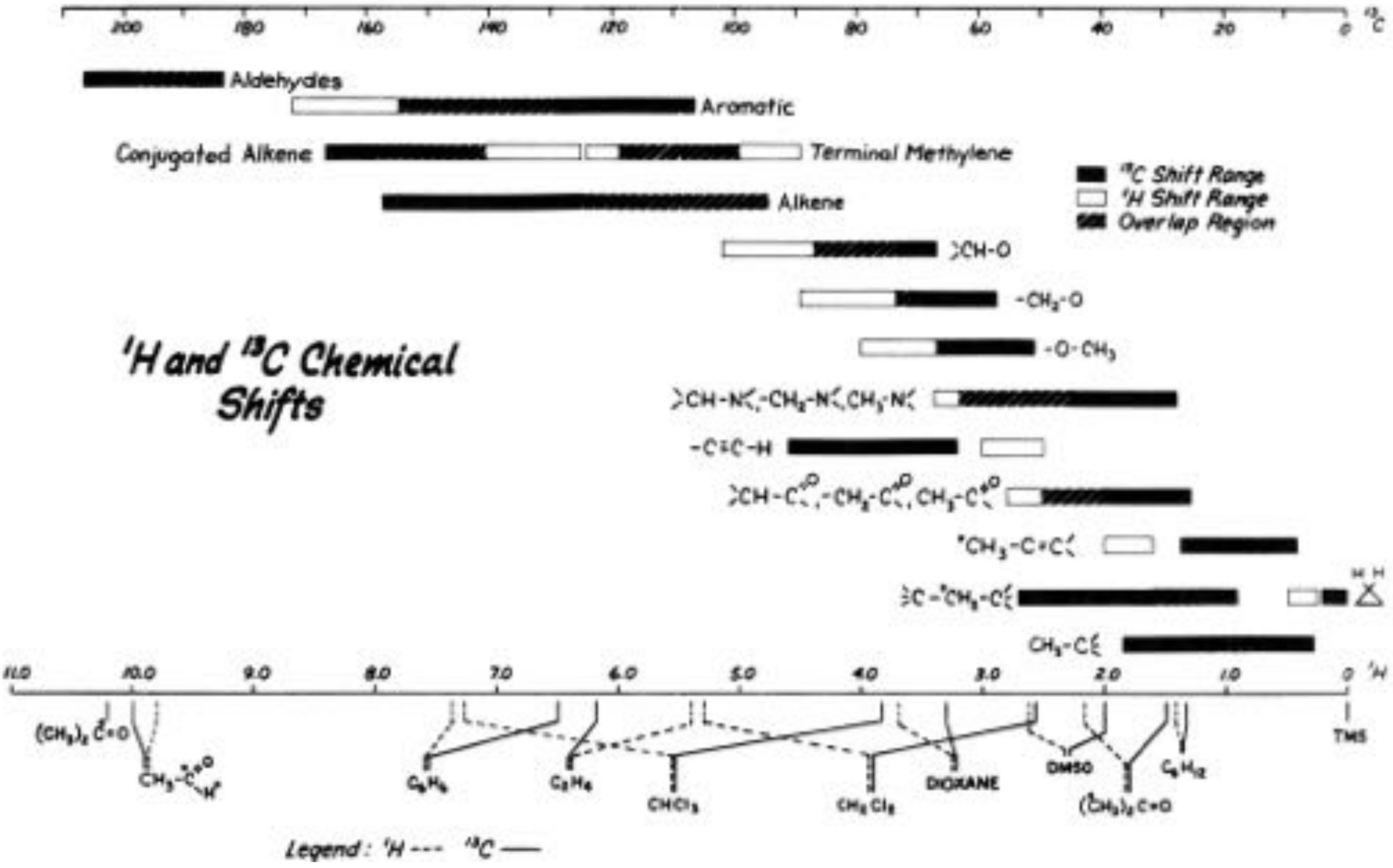


[1-^{13}C]acetylcarnitine



Courtesy of Jessica Bastiaansen

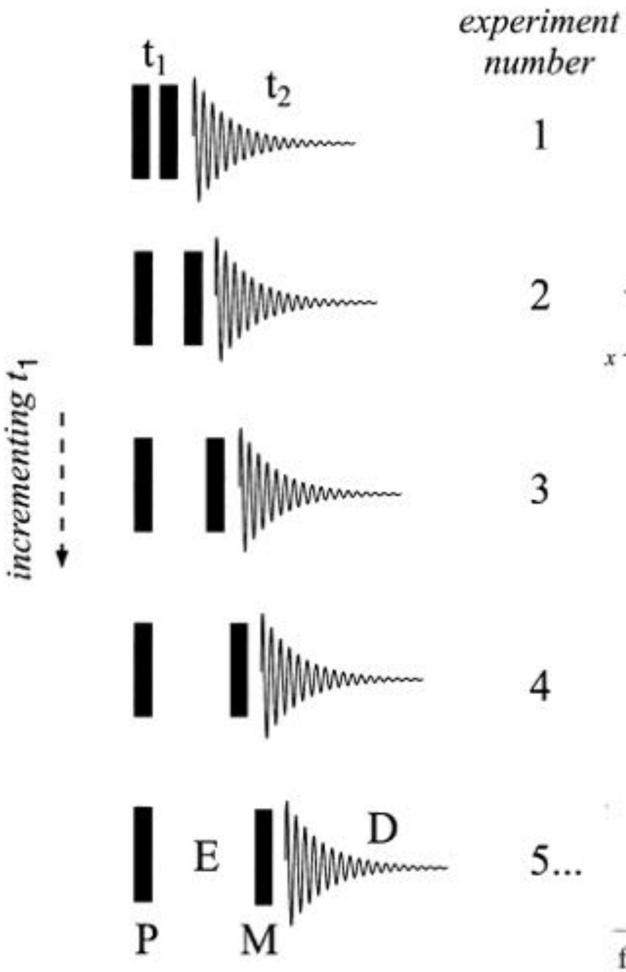
^{13}C NMR chemical shifts follow similar trends as ^{1}H

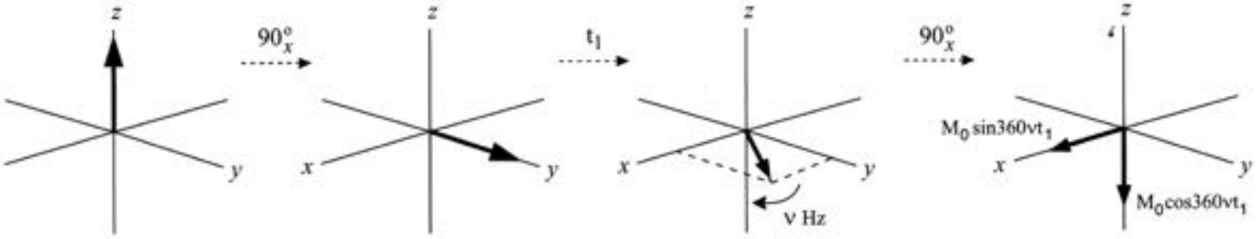


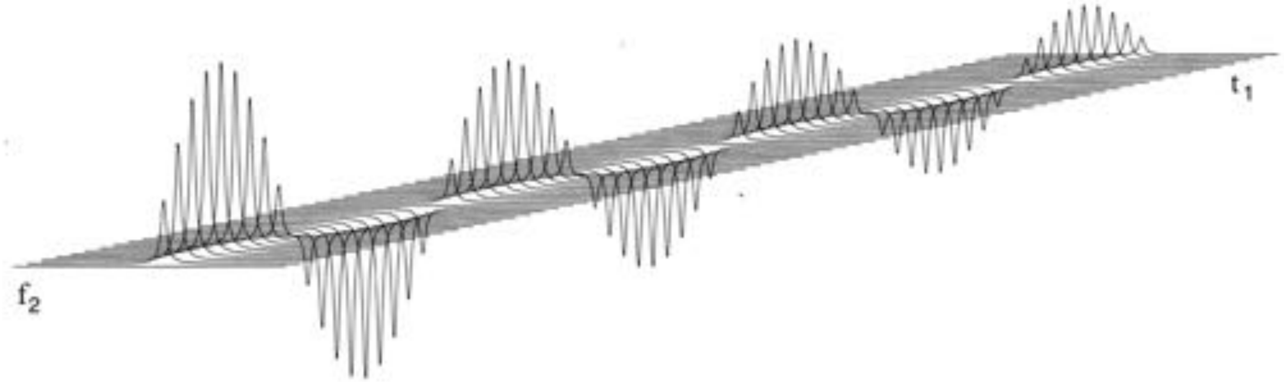
< Break? >

2D NMR

- Where does the second dimension come from?
- Consider multi-pulse experiment



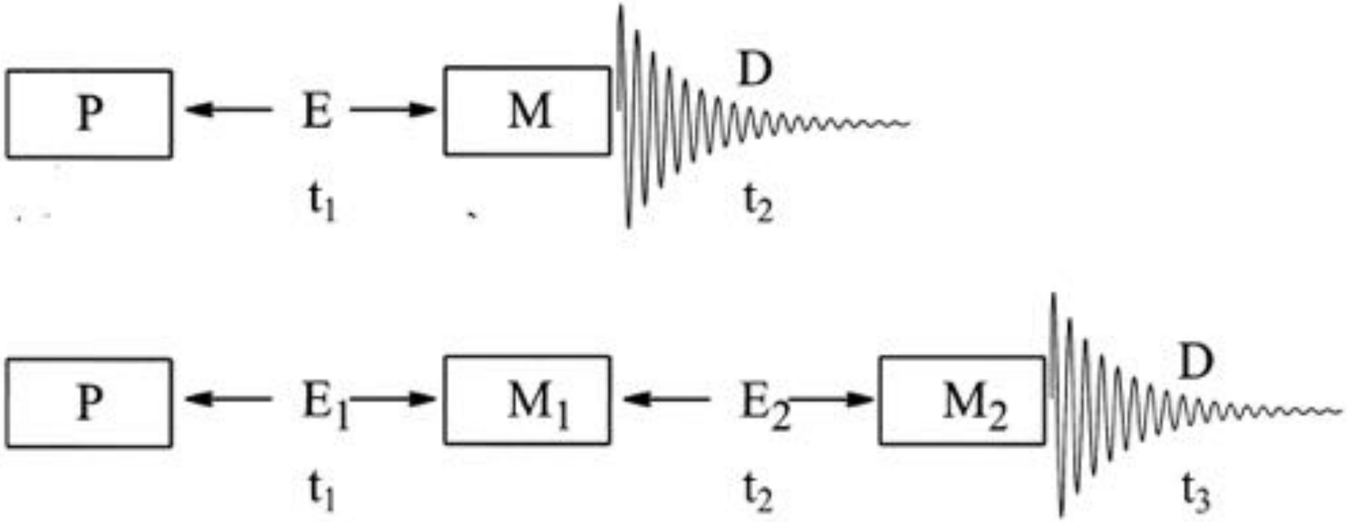




Interferogram after FT in 1st dimension

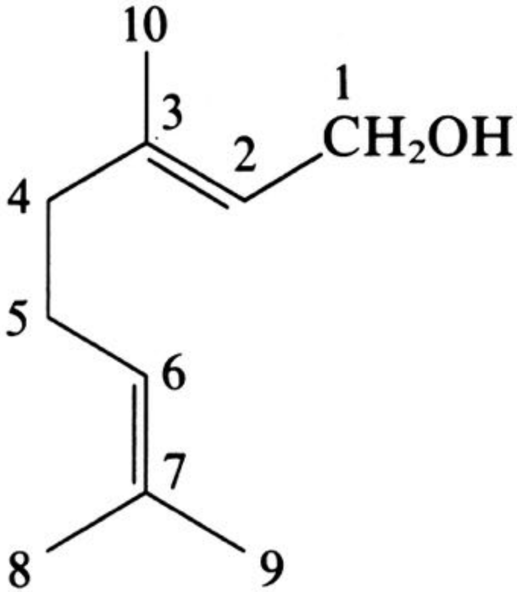
2D NMR

> This scheme with a variable evolution period can be generalized and combined with other manipulations to make 3D and higher dimensions
>
> (experiment time becomes a factor)



2D NMR – COSY correlated spectroscopy

- The double-quantum filtered variant of the COSY experiment is cleaner, singlets suppressed



Geraniol

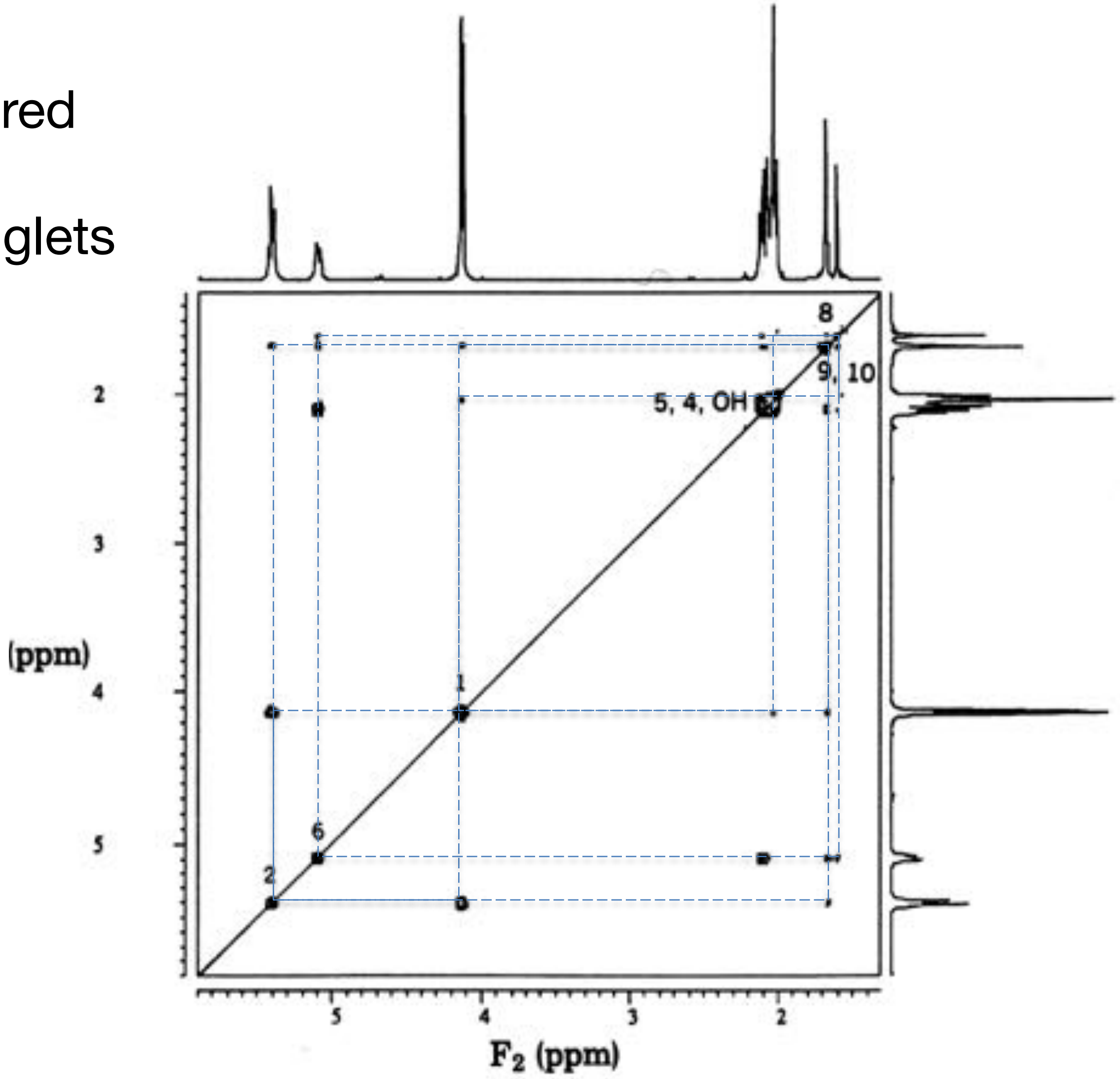
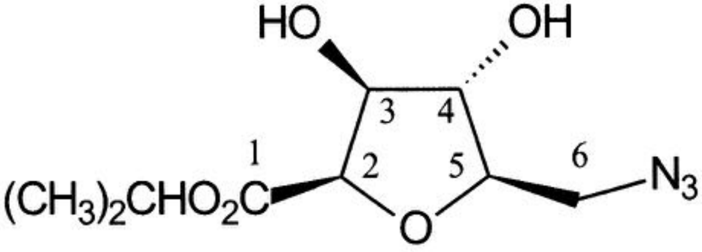


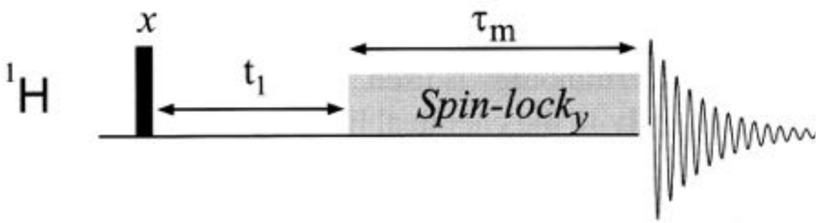
FIGURE 6.9. The DQFCOSY spectrum of geraniol, in $CDCl_3$ at 500 MHz.

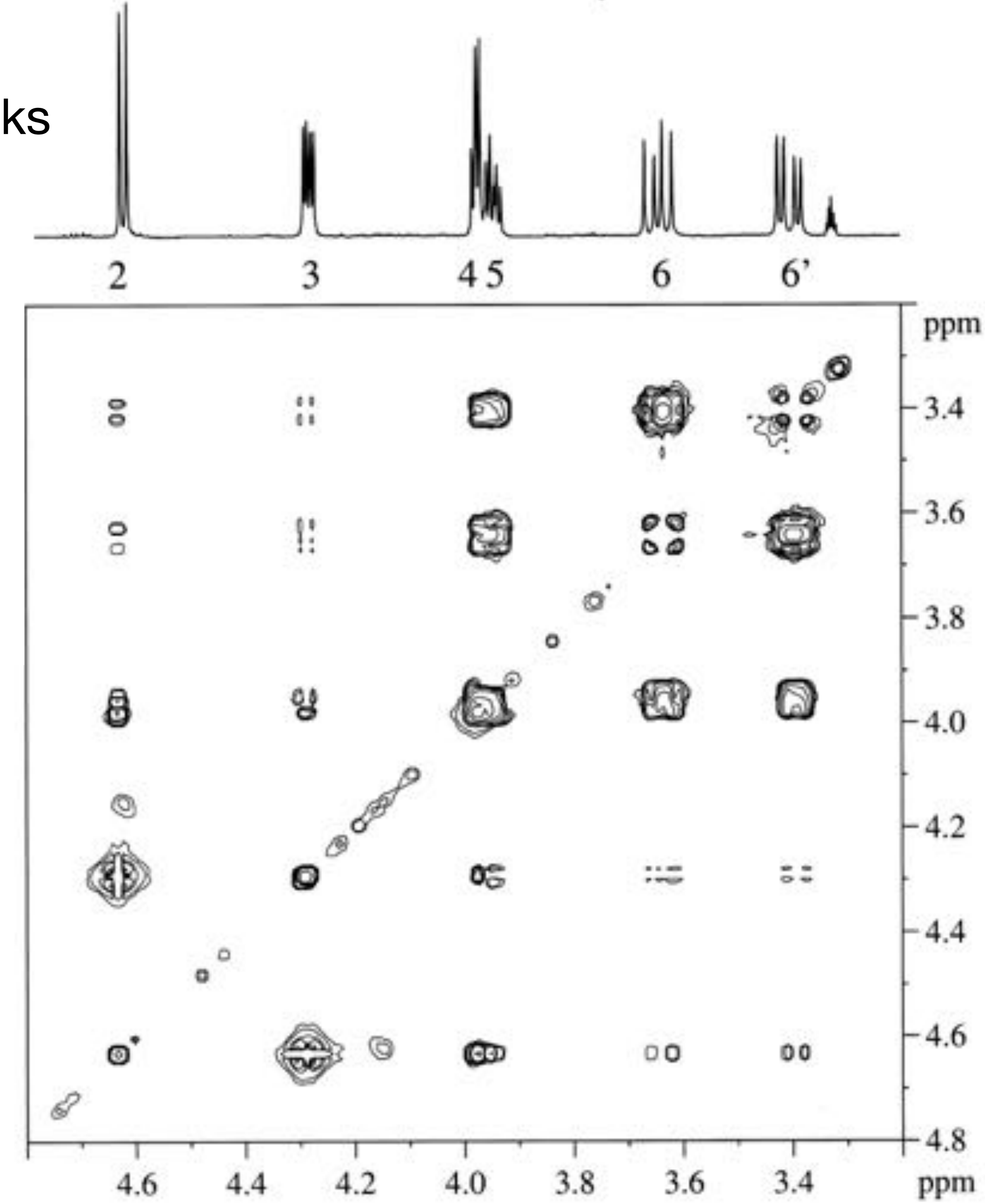
2D NMR – TOCSY total correlated spectroscopy

- The TOCSY shows crosspeaks for an entire spin system



5.1

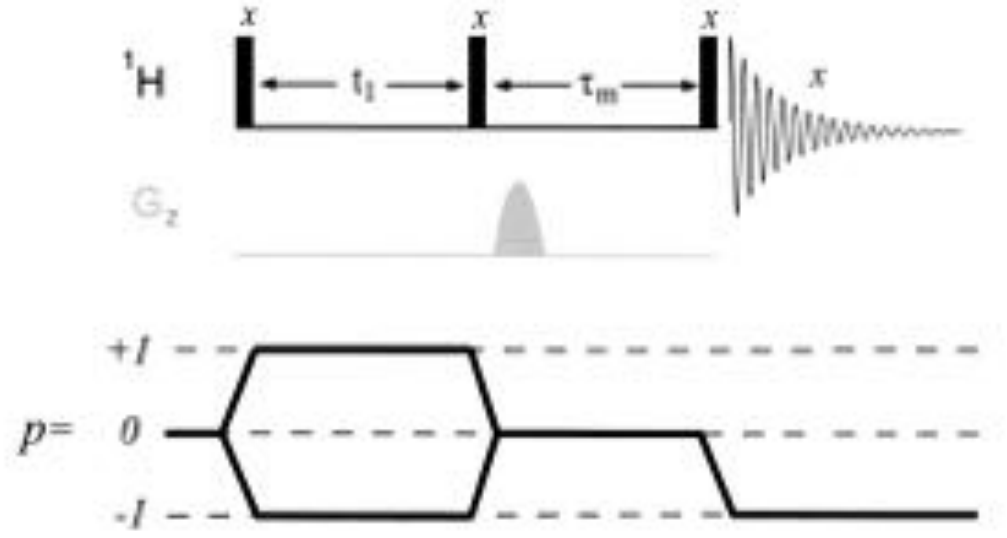


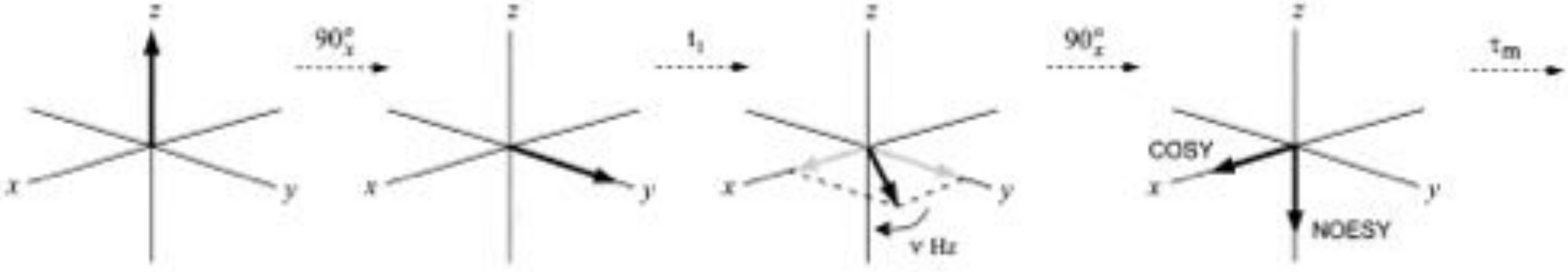


2D NMR – NOESY nuclear Overhauser effect spectroscopy

- NOESY measures through-space interactions between spins

Figure 8.34. The 2D NOESY sequence and the associated coherence transfer pathway. The optional use of a pulsed field gradient (shown greyed) during the mixing time is described in the text.





2D NMR – NOESY nuclear Overhauser effect spectroscopy

> NOE cross relaxation of dipolar-coupled spins depends on the correlation time, τ_c. The effect disappears in the intermediate regime between large and small molecules (~1000 Da, depending on field strength)

(> An alternative is the ROESY – Rotating frame Overhauser Effect)

Positive NOE

Negative NOE

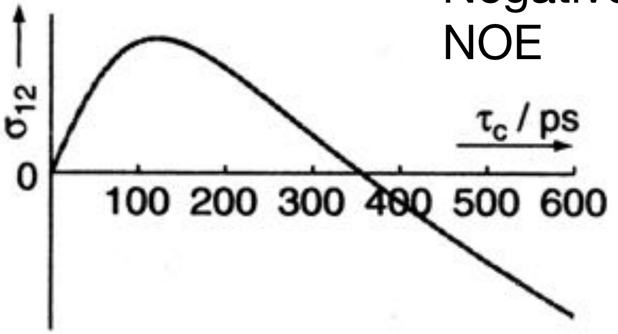
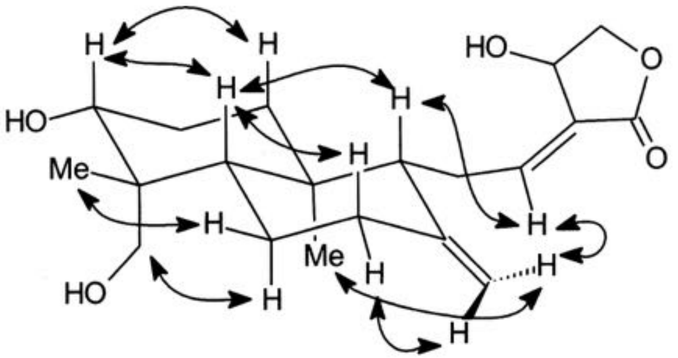
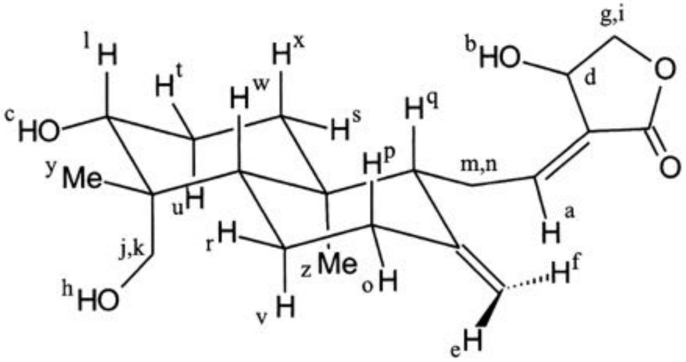


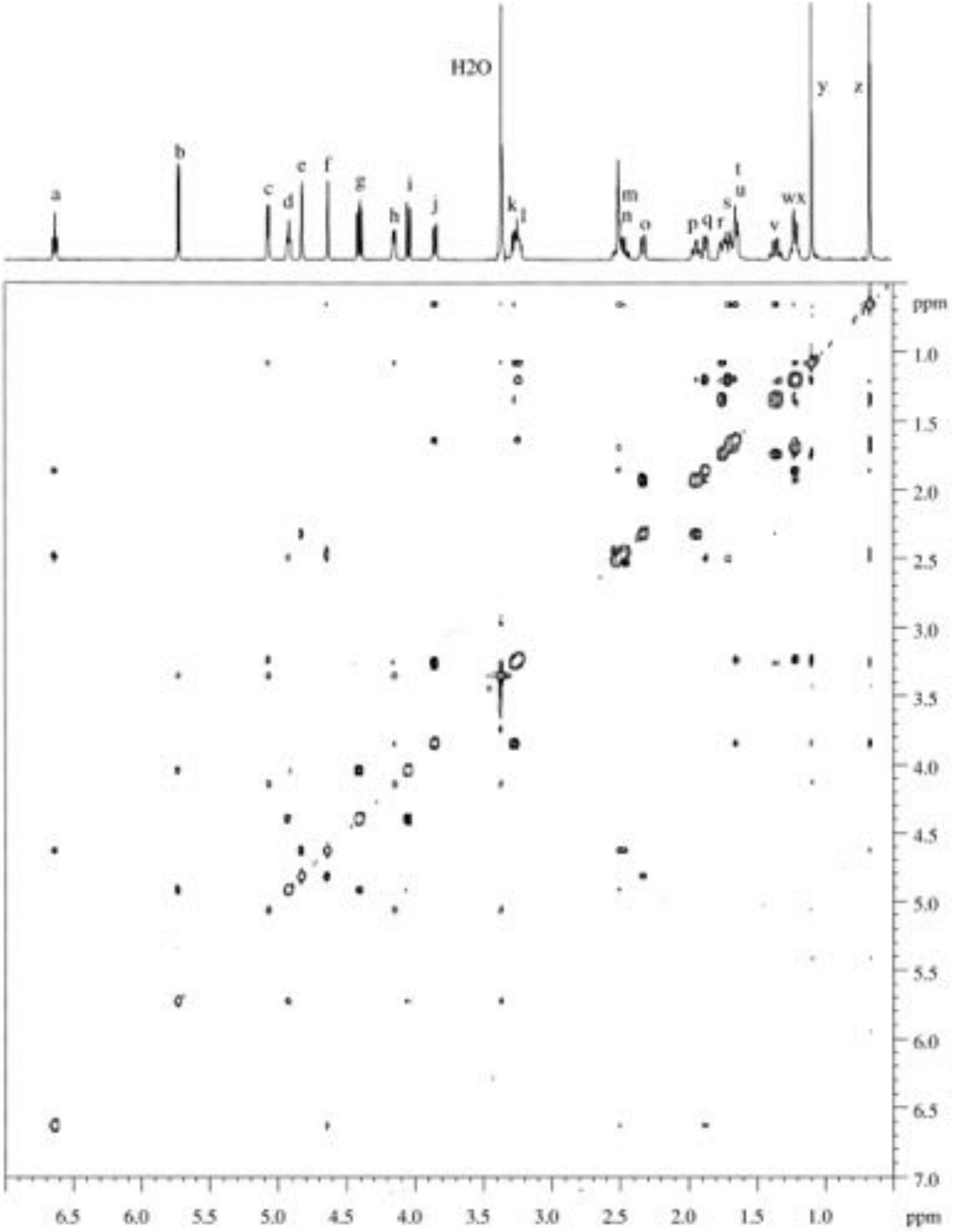
Fig. 9.19 Illustration of how the cross-relaxation rate constant, σ_{12}, changes sign from positive to negative as the correlation time is increased. The graph is computed for two protons with a Larmor frequency of 500 MHz, so the cross-over point is at $\tau_c \approx 360$ ps.

2D NMR – NOESY nuclear Overhauser effect spectroscopy

> NOESY example: andrographolide

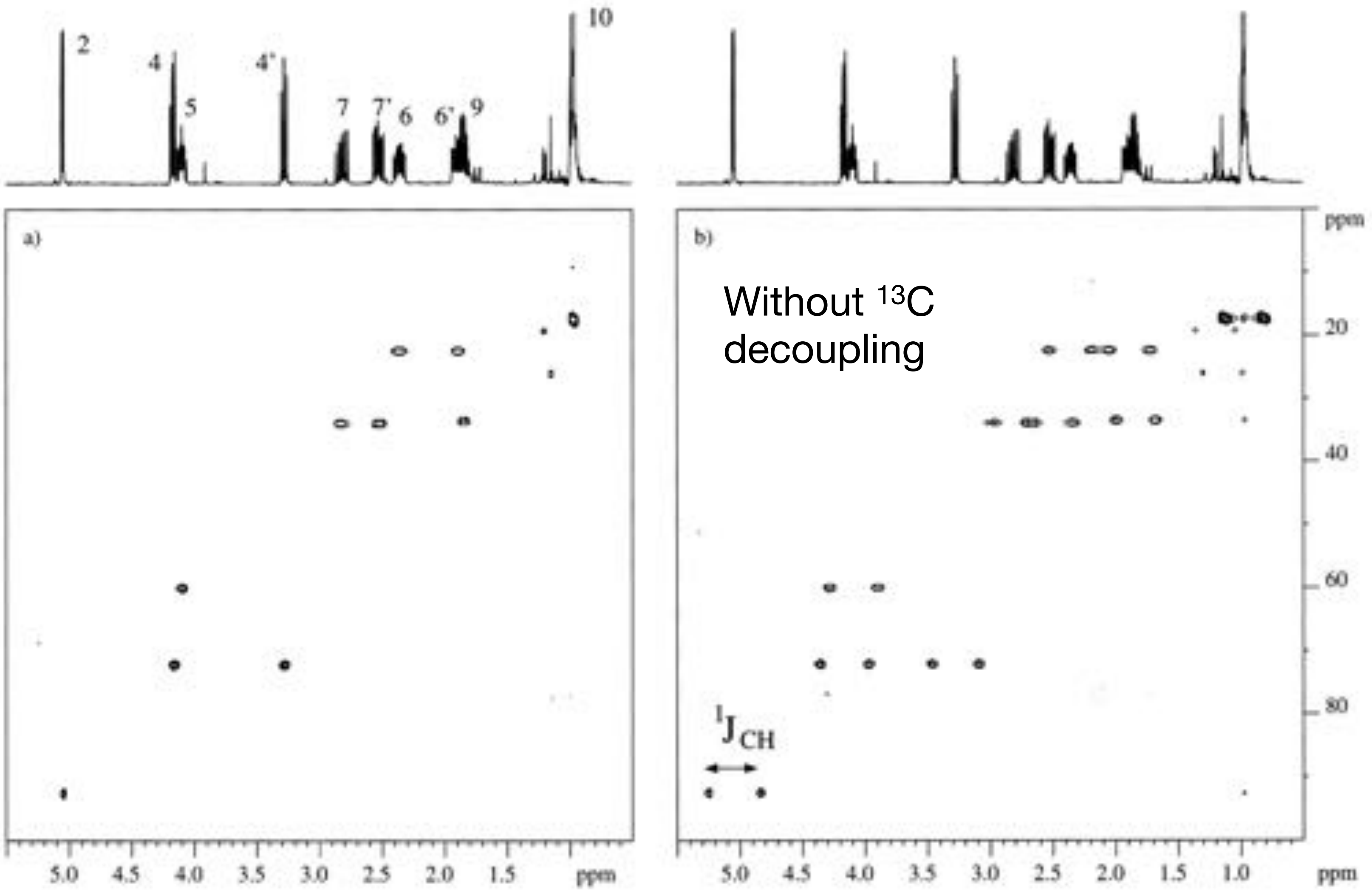






2D NMR – Heteronuclear correlated spectroscopy and indirect detection of ^{13}C (or ^{15}N, etc.)

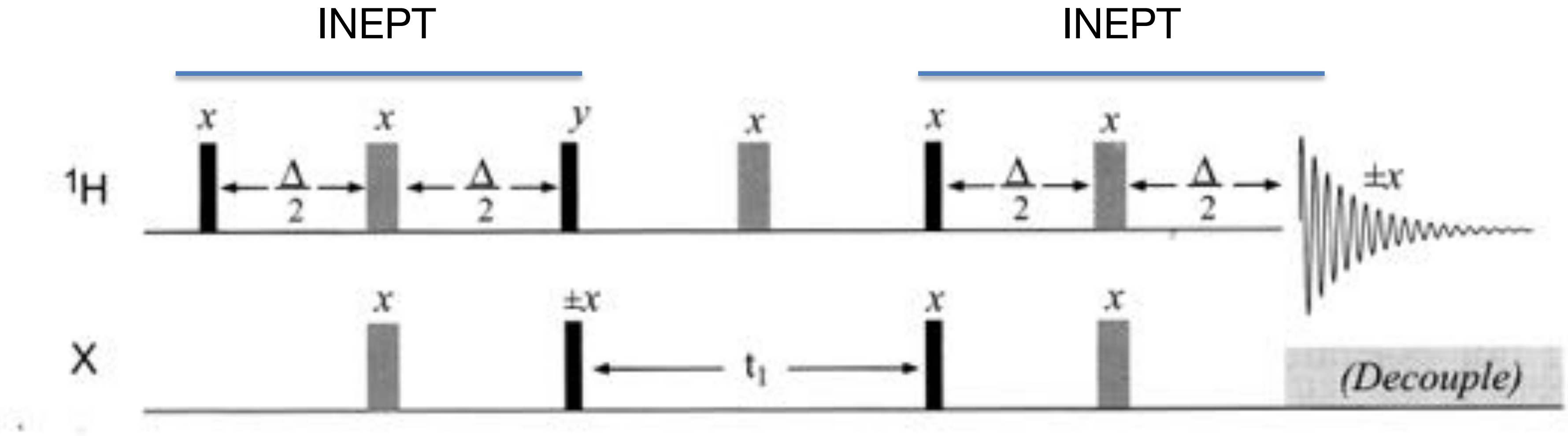
> HSQC Heteronuclear Single Quantum Correlation: 1-bond connections



Without ^{13}C decoupling

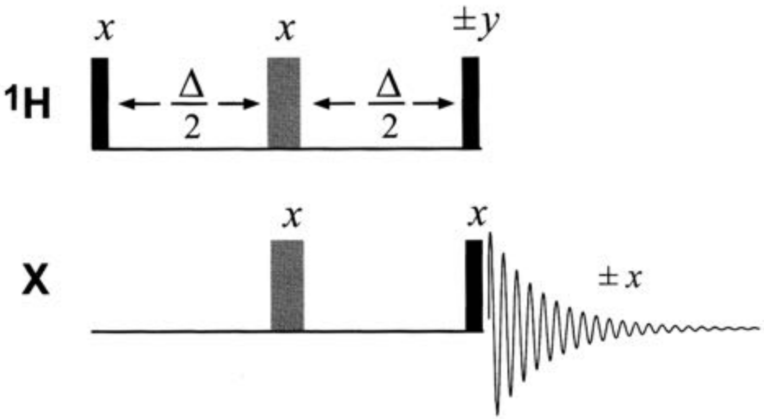
2D NMR – Heteronuclear correlated spectroscopy and indirect detection of ^{13}C (or ^{15}N, etc.)

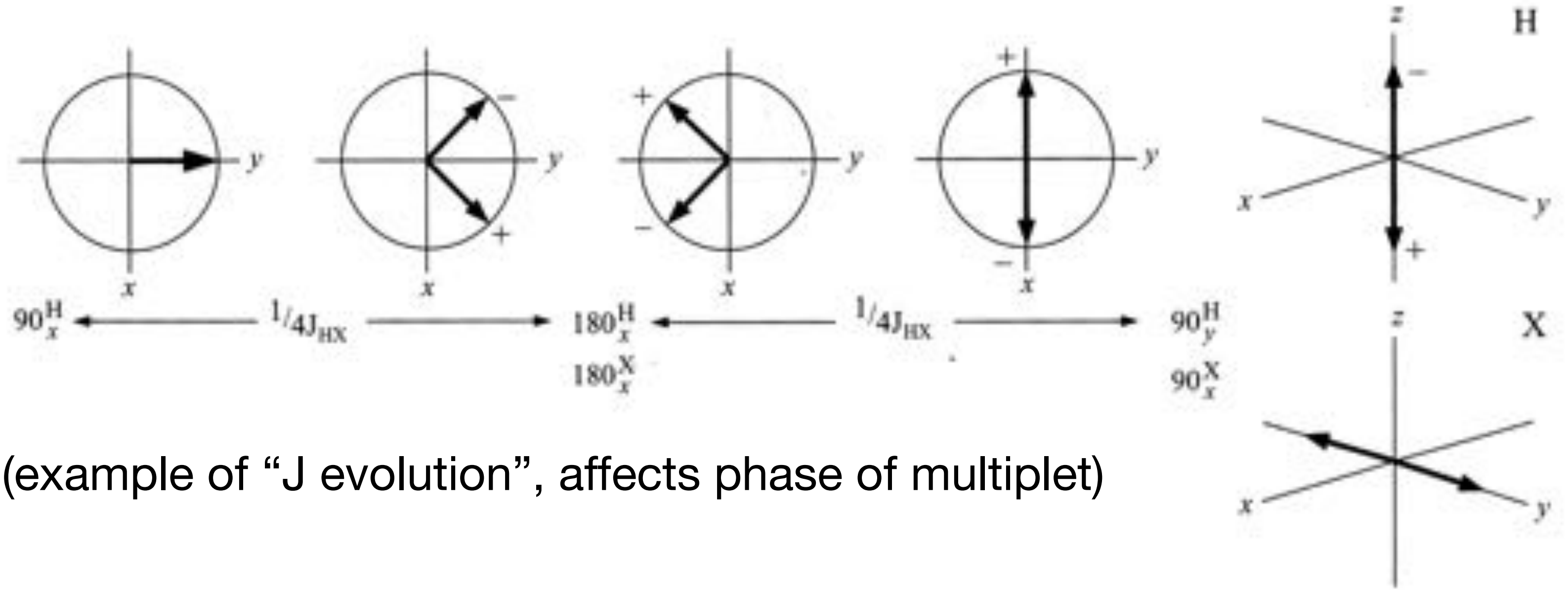
> HSQC uses INEPT blocks to transfer ^{1}H polarization to ^{13}C and back again



INEPT (Insensitive Nuclei Enhanced by Polarization Transfer)

- Transfer ^1H polarization to ^{13}C
- More useful for 2D experiments than ^{13}C direct detection (DEPT is better for that)

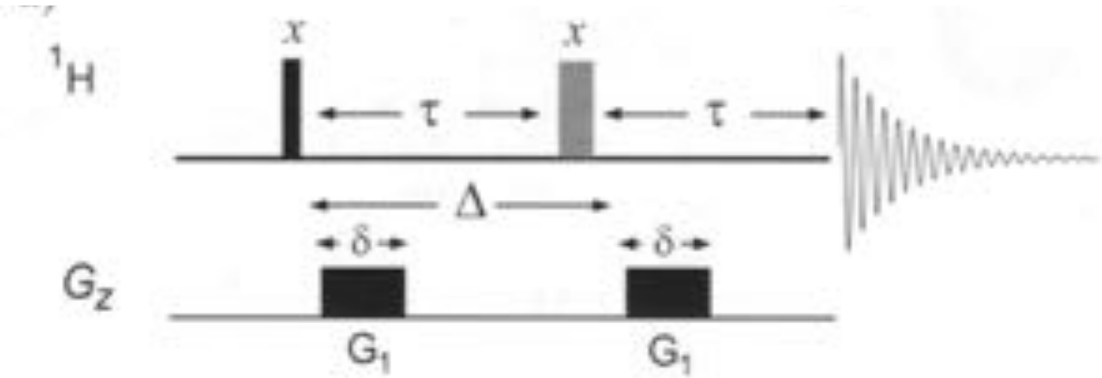


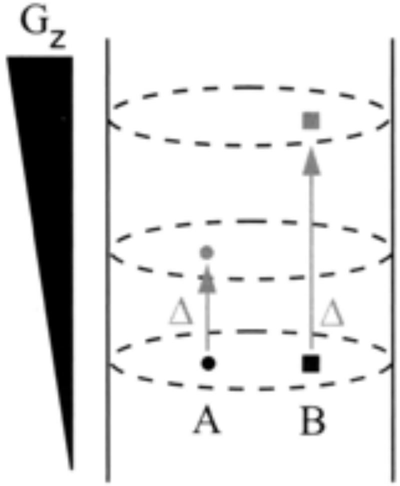


(example of “J evolution”, affects phase of multiplet)

DOSY – diffusion-ordered spectroscopy

- gradient pulses dephase and re-phase
- greater diffusion results in lower signal

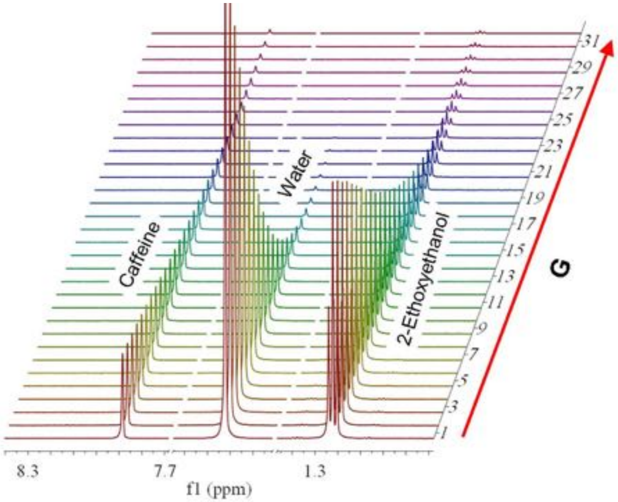




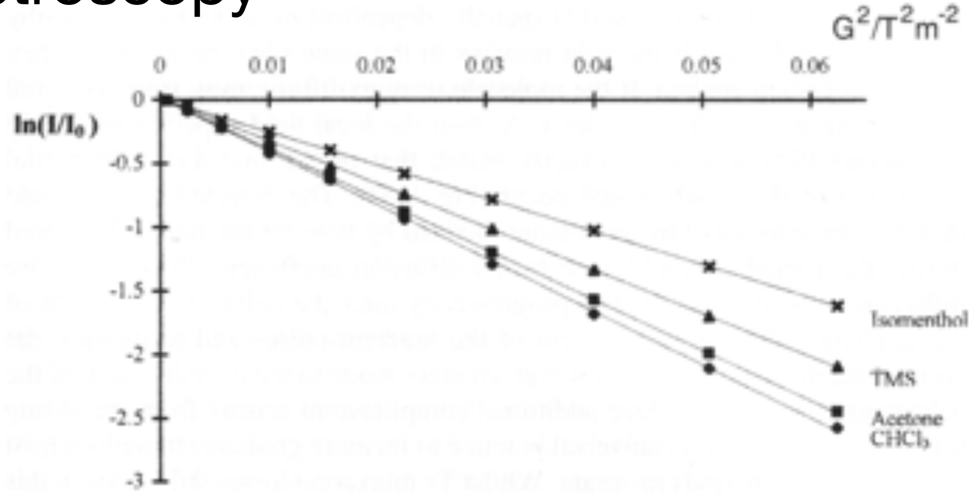
Stejskal–Tanner equation: $I = I_0 \exp\left({}^{-2\tau}/_{T_2} - (\gamma\delta G)^2\, D\left(\Delta - {}^{\delta}/_{3}\right)\right)$

D – diffusion coefficient
γ – gyromagnetic ratio of the observed nucleus
G – applied gradient strength
δ – length of the gradient pulse

DOSY – diffusion-ordered spectroscopy

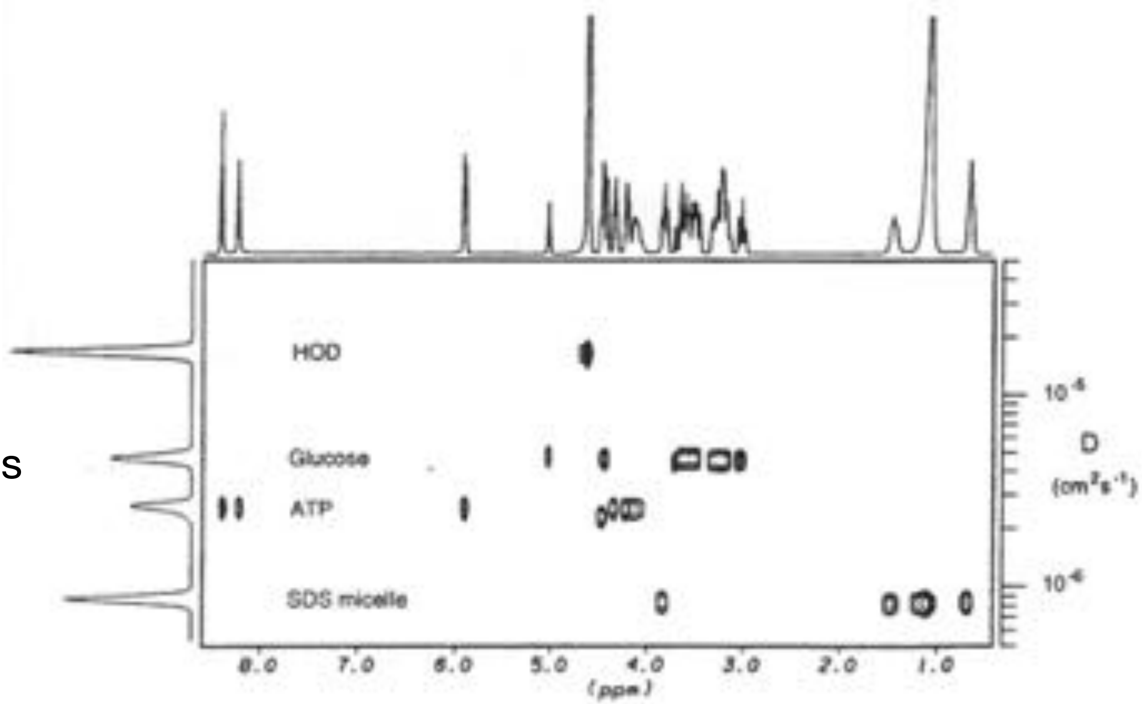


Series of spectra with increasing gradient strength



$\ln(I/I_0)$ vs. G^2 – slope ∝ to diffusion coefficient

2D DOSY can resolve overlapping spectral peaks in complex mixtures



nmr-analysis.blogspot.com

Some notes on material not covered here, but worth mentioning

> The *product operator formalism* is a useful way to describe the evolution of spin coherence, especially in multidimensional experiments

> 2D & >2D experiments can take a lot of time & can be accelerated by non-uniform sampling (NUS), which is analogous to compressed sensing

> 2D experiments require *coherence pathway selection*, which can be done with *phase-cycling* to cancel out unwanted pathways, or faster & more typically with *pulsed field gradients*

> Processing the indirect dimension with *linear prediction* and a *window function* can improve resolution and shorten the number of increments required.

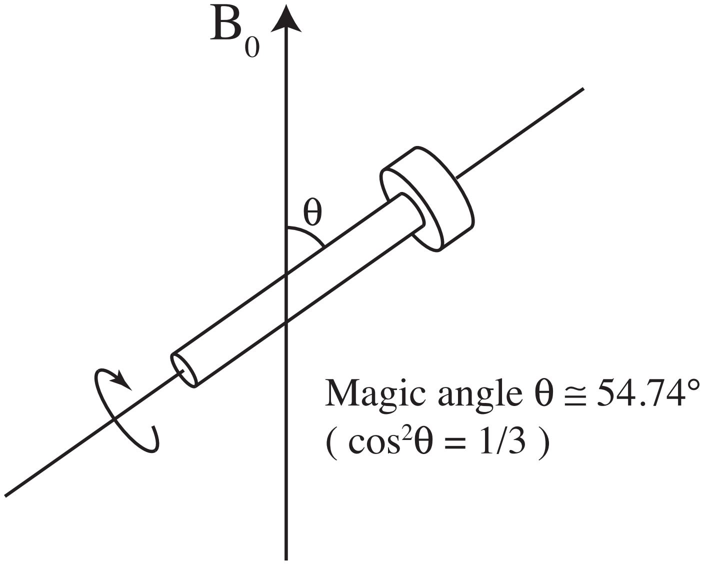
“High-resolution” NMR in (semi) solid-state samples

> Lack of molecular tumbling in solid state
> - broad resonance linewidth from dipolar coupling, CSA, magnetic susceptibility interfaces

> Rapid spinning at the “magic” angle cancels out these effects, allowing narrow linewidths in heterogeneous samples

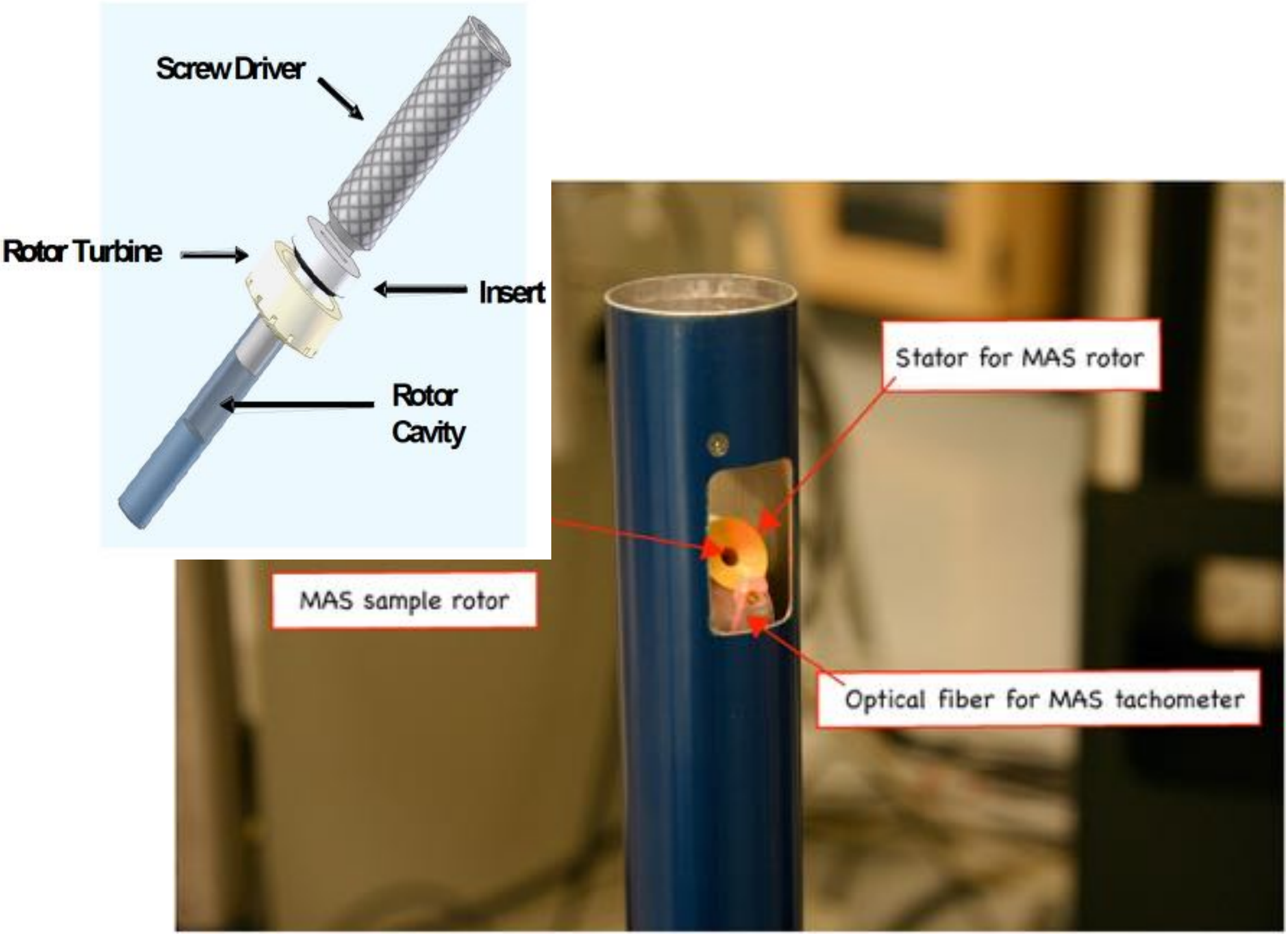
dipole – dipole coupling:

$d_{jk} = b_{jk}\ \tfrac{1}{2}\ (3\cos^2\theta_{jk} - 1)$

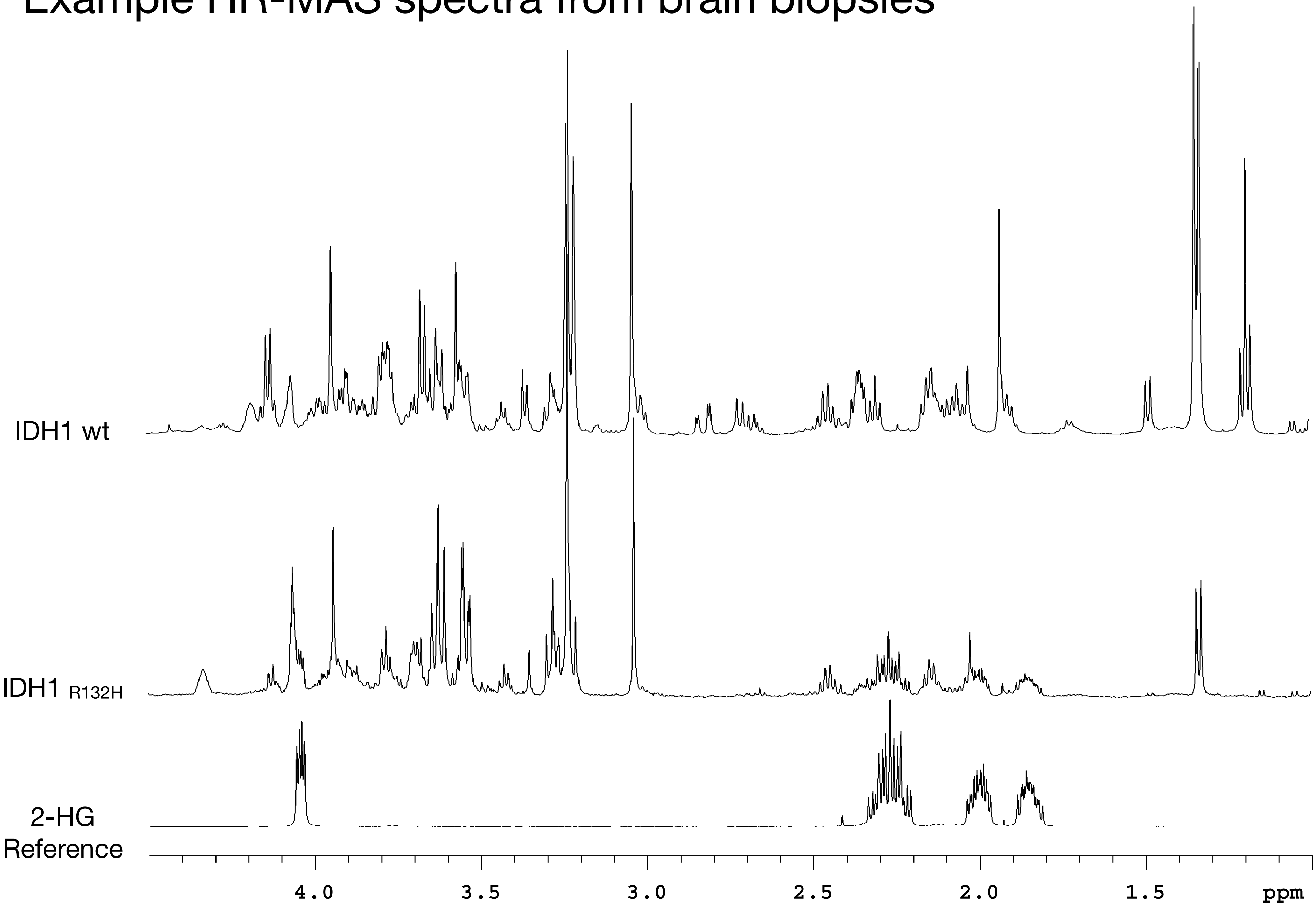


Magic angle $\theta \cong 54.74°$
($\cos^2\theta = 1/3$)

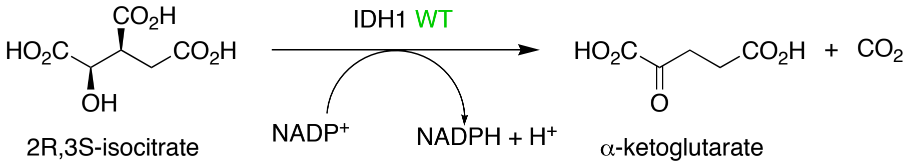
HR-MAS example: Nanoprobe

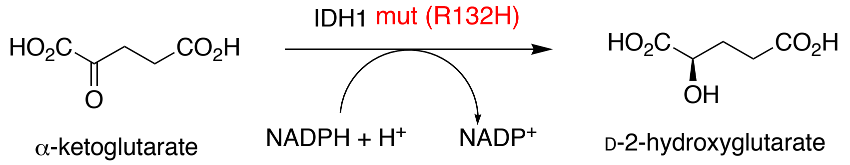


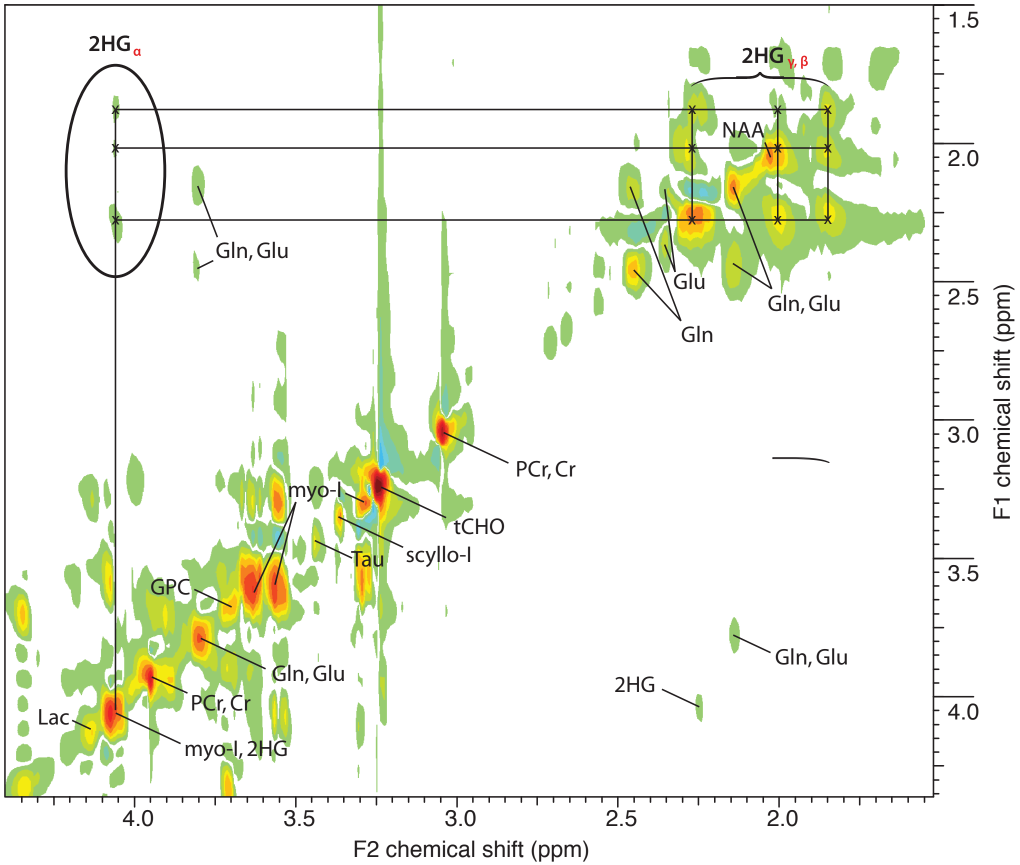
Example HR-MAS spectra from brain biopsies



2-Hydroxyglutarate in IDH1 mutant brain tumours





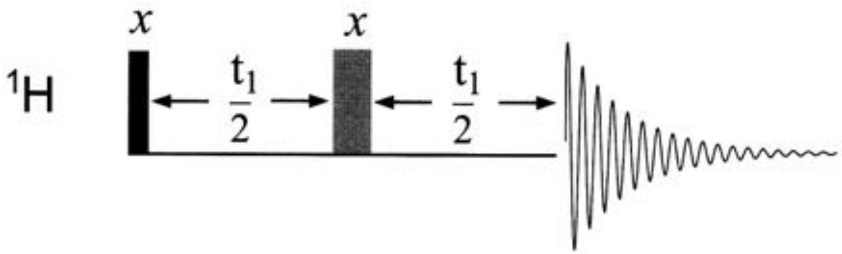


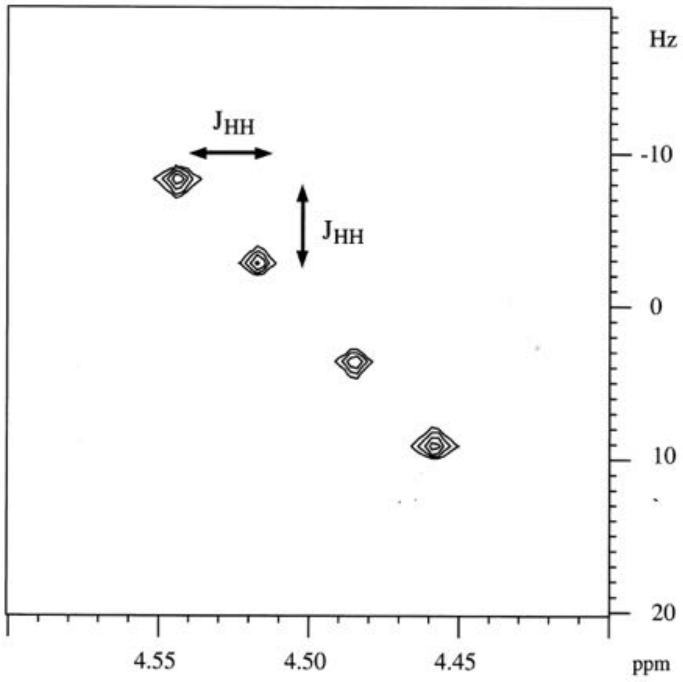
R132H mutation in isocitrate dehydrogenase-1 (IDH1) has *gain of function* & converts α-ketoglutarate to D-2-hydroxyglutarate

2-HG in HR-MAS TOCSY of biopsy
Sci. Transl. Med. **2012**, *4*, 116ra5

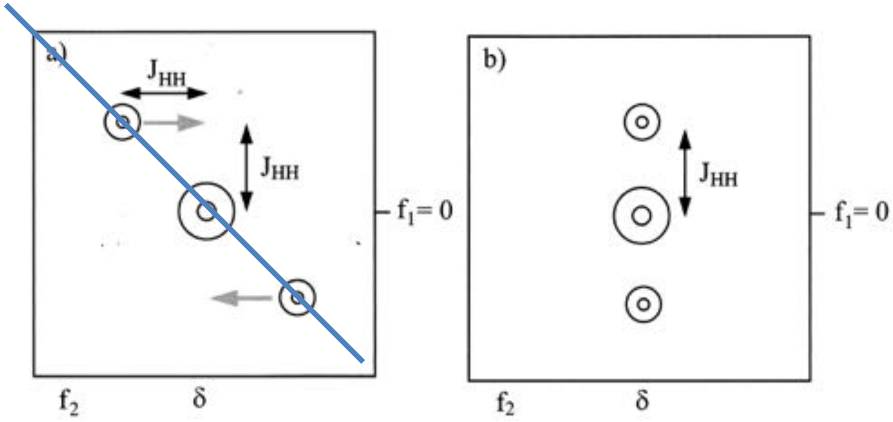
Can we get rid of all those multiplets?

- Peak multiplicity can be useful but can also interfere with other peaks
- Is it possible to effectively do broadband homonuclear decoupling?
  - *J*-resolved spectroscopy

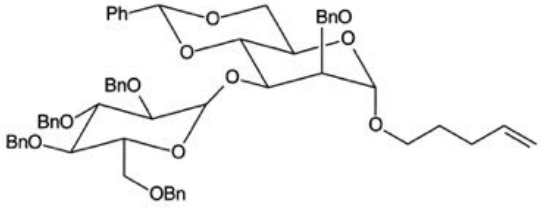


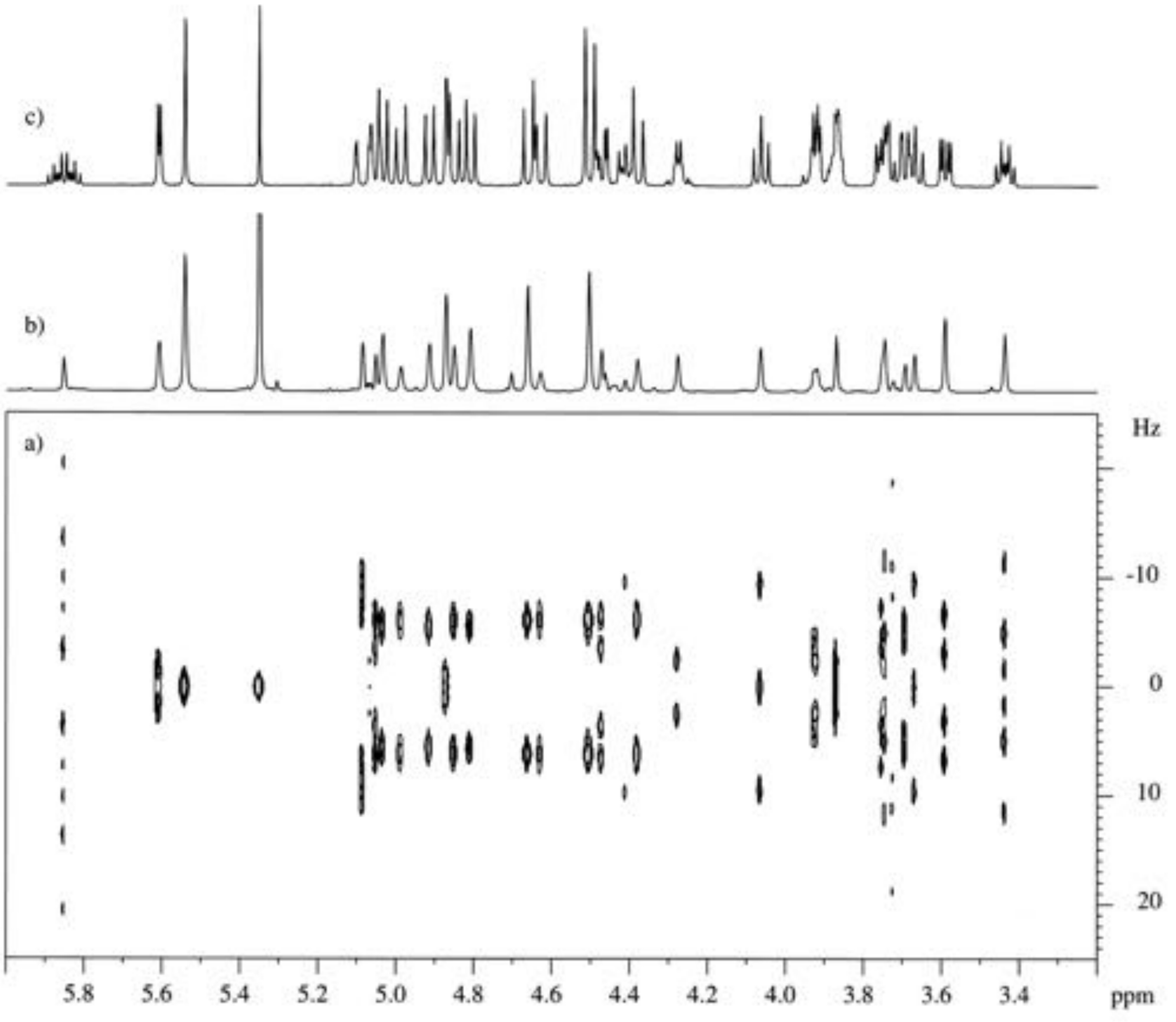


45° projection



J-resolved spectrum example

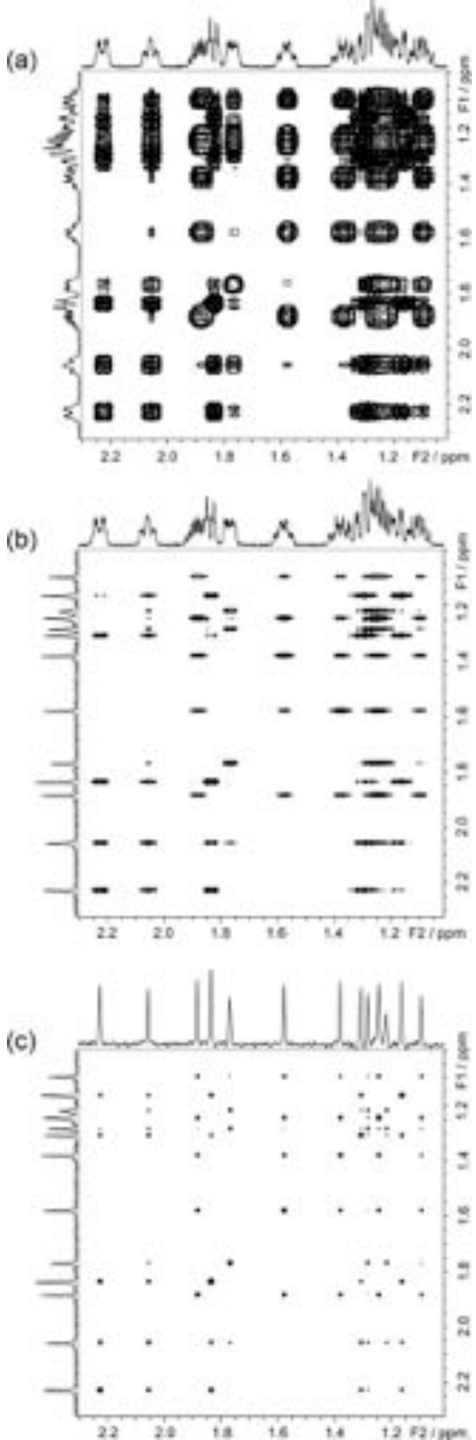




Can we get rid of all those multiplets?

> Pure-shift spectroscopy PSYCHE method

TOCSY of estradiol



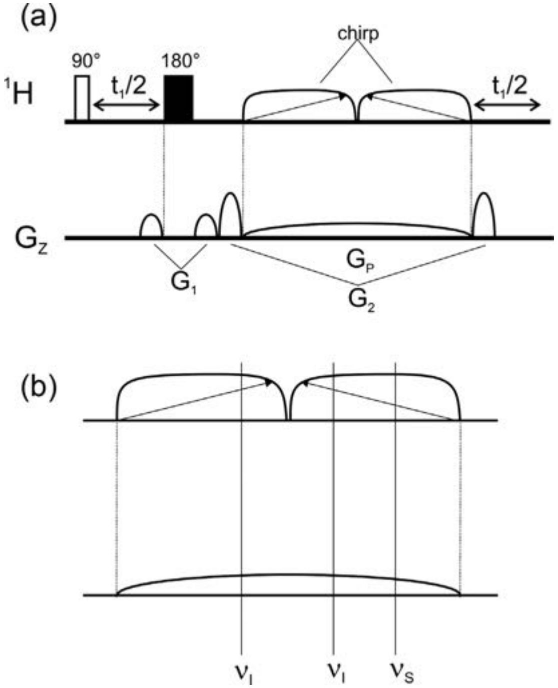
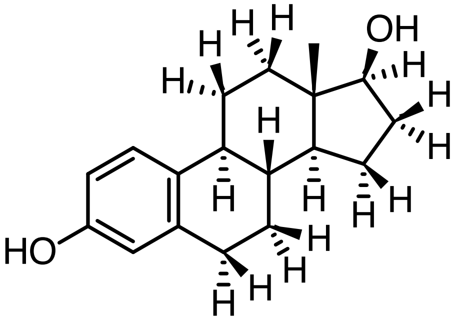


Fig. 11. Pulse sequence element of the PSYCHE method. In the middle of t_1 two swept-frequency, low power chirp pulses are applied in a symmetric fashion in the presence of a weak gradient. The two small flip angle chirp pulses act like the two small angle pulses in an anti z-COSY experiment. "Diagonal peak" signals, which experience the same chemical shift ɱ during the two pulses yield observable magnetization. On the other hand, "cross peak" signals experience a different gradient during the first chirp pulse at ɱ and the second at ɱ2, and are eliminated. White and black rectangles represent 90 ° and 180 ° pulses respectively. The chirp pulses are indicated by rectangles with round edges. Their frequency sweep in opposite directions is indicted by mirror-symmetry arrows.



estradiol

Prog. Nuc. Mag. Res. Spec. 86-87 (2015) 1–20 http://dx.doi.org/10.1016/j.pnmrs.2015.02.002

Summary

> NMR spectroscopy is a powerful, versatile method of chemical analysis

> The *chemical shift*, *relative intensity* and *multiplicity* of spectral resonances provide a spectral signature of various metabolites

> Besides creating multiplets, *J*-coupling between nuclear spins is the basis of several multidimensional NMR experiments
(and also *J* evolution)

References & additional reading

- "High-Resolution NMR Techniques in Organic Chemistry”, Timothy Claridge, Pergamon Press, 1999

- “Understanding NMR Spectroscopy, 2nd Edition”, James Keeler, Wiley, 2010

- “Spin Dynamics: Basics of Nuclear Magnetic Resonance, 2nd Edition”, Malcolm H. Levitt, Wiley, 2008

- “Spectrometric Identification of Organic Compounds, 8th Edition”, Robert M. Silverstein, Francis X. Webster, David J. Kiemle, David L. Bryce, Wiley, 2015

- *Plus many useful online tutorials*

Real NMR spectrometers at the EPFL

NMR Platform at ISIC

- >20 spectrometers
- 400 – 900 MHz
- Liquid & solid-state samples

https://www.epfl.ch/schools/sb/research/isic/platforms/nuclear_magnetic_resonance/

Thank you for your attention

Questions?

hyoshihara@ethz.ch