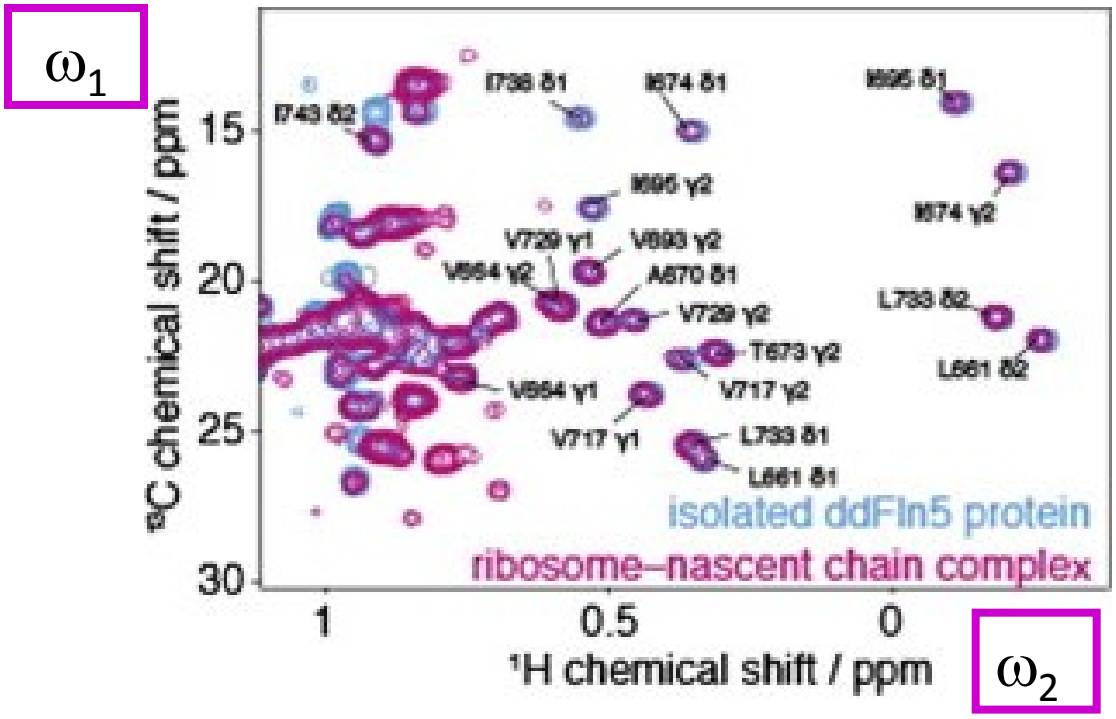
2D FT-NMR Spectra



The 2D spectrum is the graphical representation of a function, $S(\omega_1,\omega_2)$, of two independent frequency variables

2D FT-NMR

There are different kind of experiments:

- to resolve overlapping signals
- to increase sensitivity
- to get information not afforded by 1D methods

2D FT-NMR: experiment stages

A 2D FT-NMR experiment consist of 4 main stages

preparation | evolution | mixing | detection

preparation

During preparation, the spin system is prepared in a non equilibrium coherent state, which is going to evolve during the following stages.
In the simplest experiments (e.g. COSY) the preparation is a single pulse

evolution

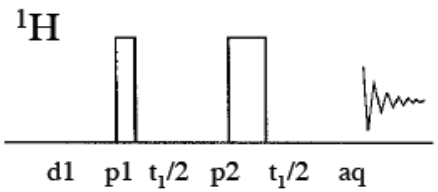
During evolution (t1), the spin system evolves freely under the relevant spin Hamiltonian (e.g., chemical shift and scalar). During the evolution time the frequency labelling for the dimension 1 takes place.

mixing

The various kinds of experiments differentiate according to the mixing process

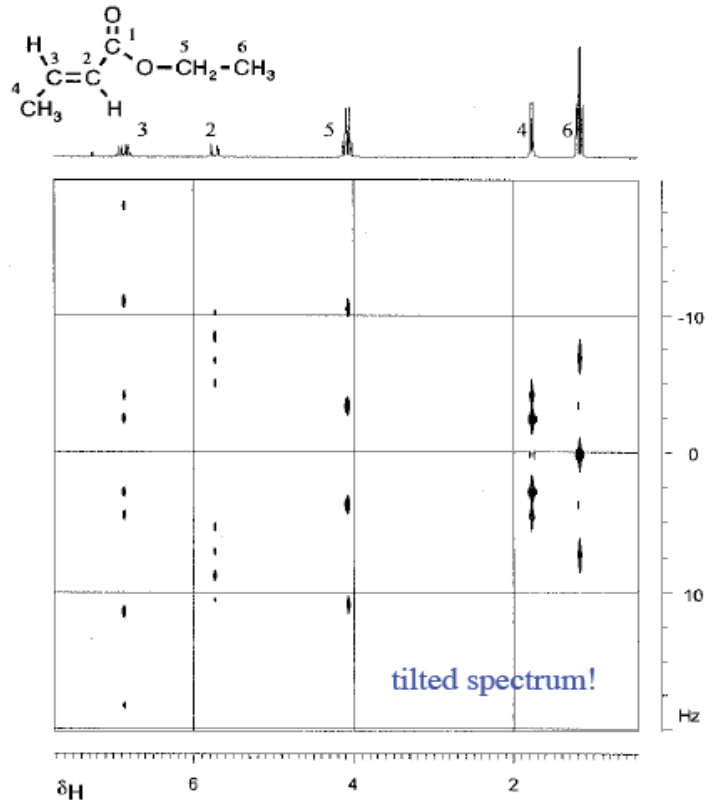
1.experiments for separarting different intractions (e.g. shift and coupling constants: J resolved experiments) along two orthogonal dimensions, thus resolving too crowded 1D spectra. (In this case the mixing process is bypassed)

J-Resolved



p1: $(x)_4, (y)_4, (-x)_4, (-y)_4$
p2: $x, -x, y, -y, (y, -y, -x, x)_2, -x, x, -y, y$
aq: $(x)_2, (-x)_2, (y)_2, (-y)_2$

ns = 4 x 128; 20 min.
16 x 256; 2.5 h



mixing

2. experiments for correlating the transitions of coupled spins (e.g., COSY). During the mixing there is the coherence transfer (transverse magnetization or multi quantum coherences) from one transition to another. The mixing is very short, one pulse (COSY) or a short sequence.

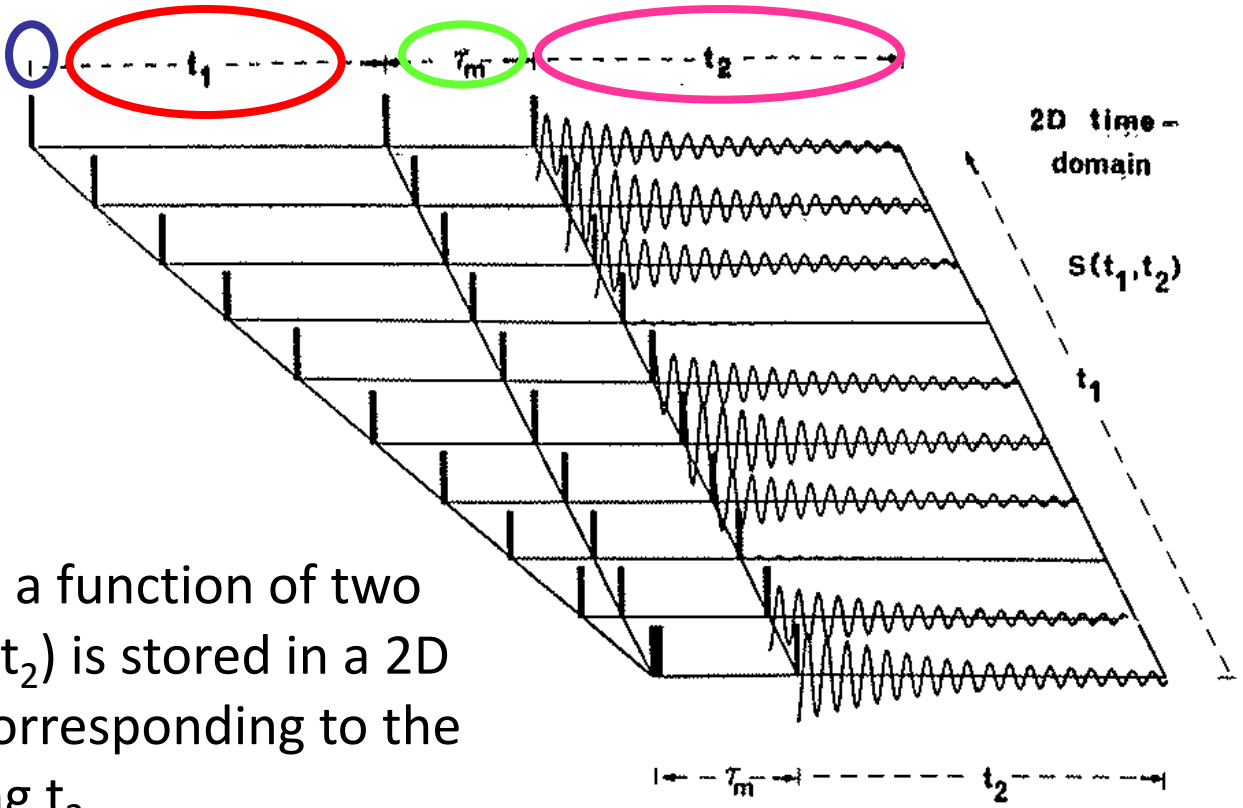
3. experiments for the study of dynamic processes, such as chemical exchange or NOE (extended mixing time during which the incoherent magnetization exchange takes place)

detection

During the detection period (t_2) the conventional acquisition of the time signal, originated by the transverse magnetization, is carried out.

In a 2D experiment many FIDs are acquired. They differ only for the length of the t_1 interval

The signal, which is a function of two time variables, $s(t_1,t_2)$ is stored in a 2D matrix , with row corresponding to the FIDs acquired during t_2



NOESY

COSY $(90°)_y - t_1 - (90°)_y - t_2$

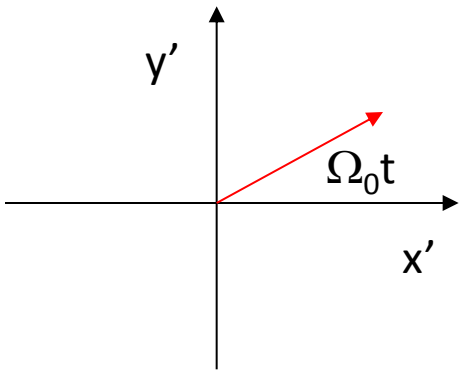
Frequency labelling during the evolution time (t_1)

Let's consider a sample with only one kind of spins, e.g., il ^{1}H di HOD, and therefore only one signal.

One starts from equilibrium, with the magnetization aligned along z

M is brough along x' by a $(90°)_y$ pulse

Due to the chemical shift, the magnetization precesses in the transverse plane with angular frequency Ω_A.



during the interval t_1 the magnetization evolves because of the chemical shift and has components in the tranverse plane:

$M_{x'} = M_0 \cos(\Omega_A t_1)$ and $M_{y'} = M_0 \sin(\Omega_A t_1)$

A second $\pi/2$ pulse along y' brings the Mx' component along –z, whereas leaves unaltered the component along y' $M_{y'} = M_0 \sin(\Omega_A t_1)$, which will be acquired during t_2.

The first FID, for a very short t_1, has very low intensity.

t_1 is incremented at the next experiment and the corresponding FID has starting intensity $M_0 \sin(\Omega_A t_1)$ and equation

$M_0 \sin(\Omega_A t_1) \exp(-i\Omega_A t_2)$

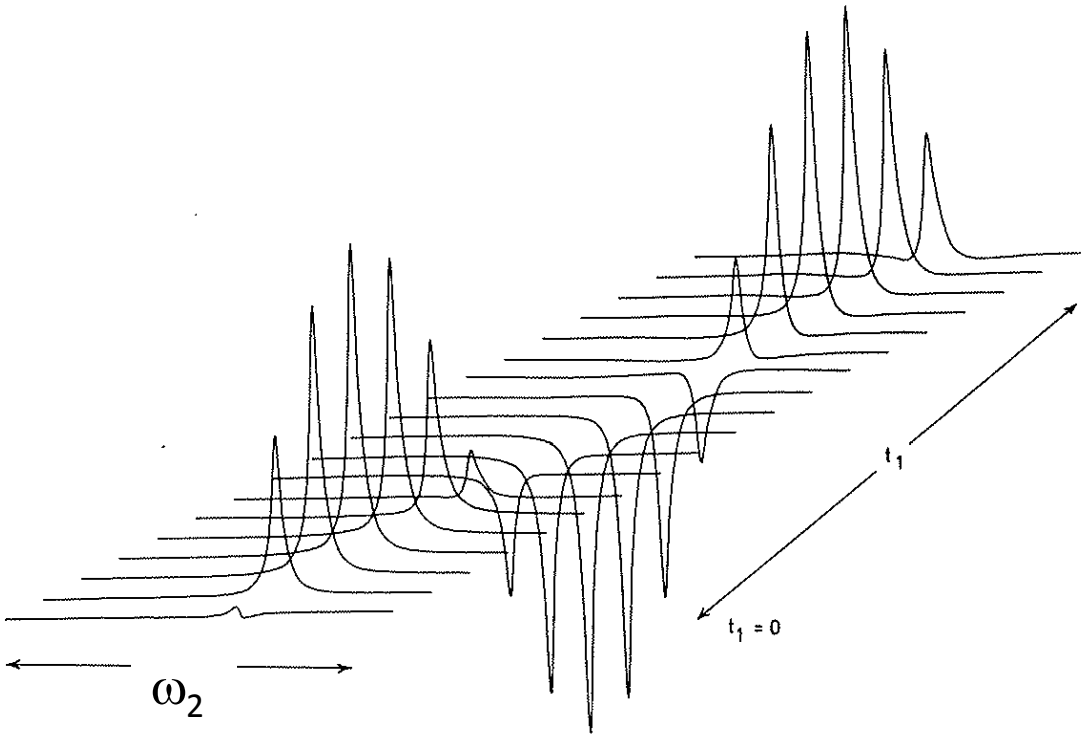
This procedure is repeated for a certain numer of t_1.

The trasnverse relaxation cannot be neglected, thus the signal function of the two time variables is:

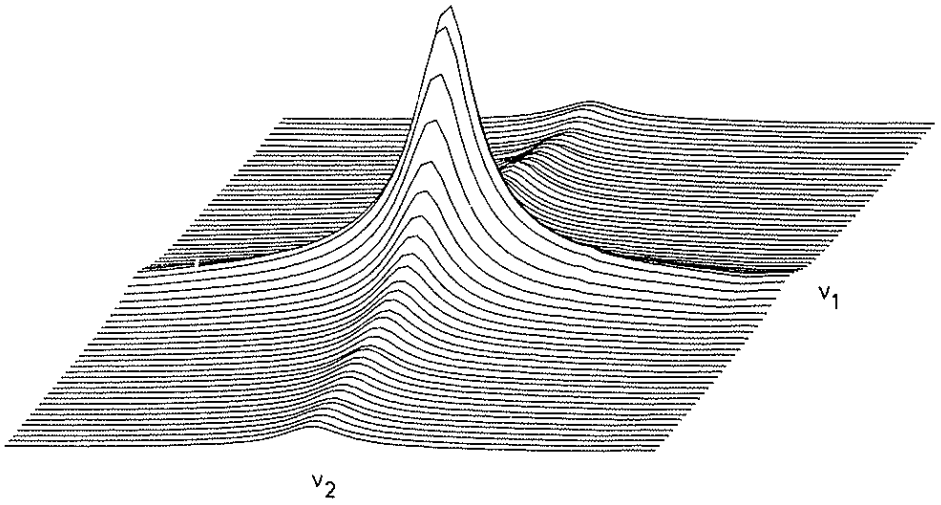
$$s(t_1,t_2) = M_0 \sin(\Omega_A \mathbf{t_1}) \exp(-\mathbf{t_1}/T_{2A}) \exp(-i\Omega_A \mathbf{t_2}) \exp(-\mathbf{t_2}/T_{2A})$$

After obtaining the FIDs, the **FT** is carried out **in dimension 2**, obtaining spectra, the intensity of which is a function of t_1

$$s(t_1,\omega_2)= M_0\sin(\Omega_A t_1)\exp(-t_1/T_{2A})\cdot T_{2A}/[1+(\omega_2-\Omega_A)^2T_{2A}^2]$$

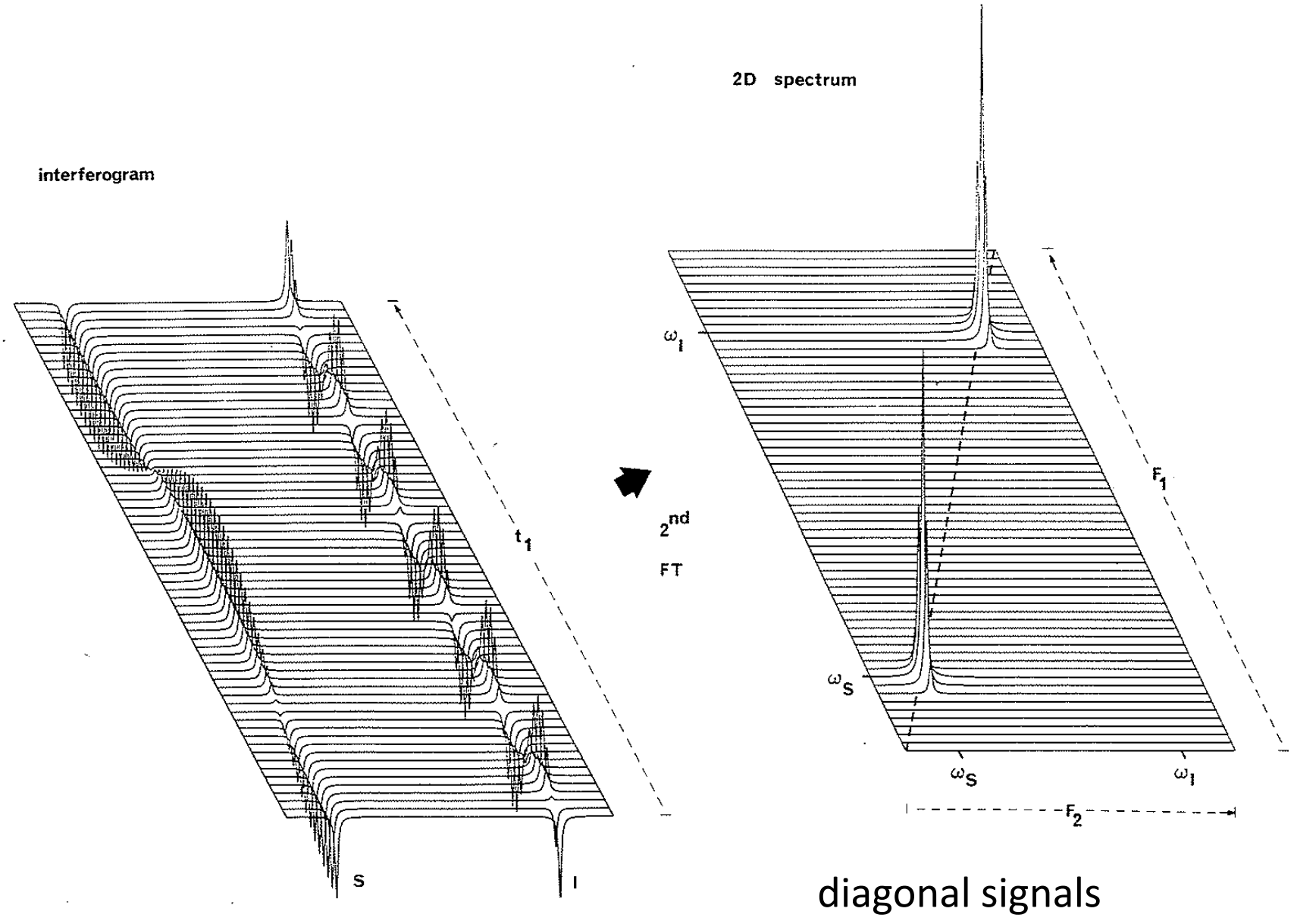


Now the FT is carried out along the columns (dimension 1) and the 2D peak is obtained



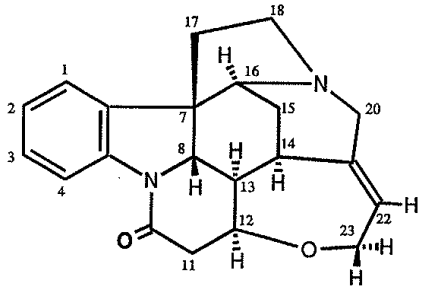
$$S(\omega_1,\omega_2)= M_0T_{2A}/[1+(\omega_2-\Omega_A)^2T_{2A}^2]\cdot T_{2A}/[1+(\omega_1-\Omega_A)^2T_{2A}^2]$$

The signal with equal ω_2 and ω_1 coordinates is a **diagonal peak**

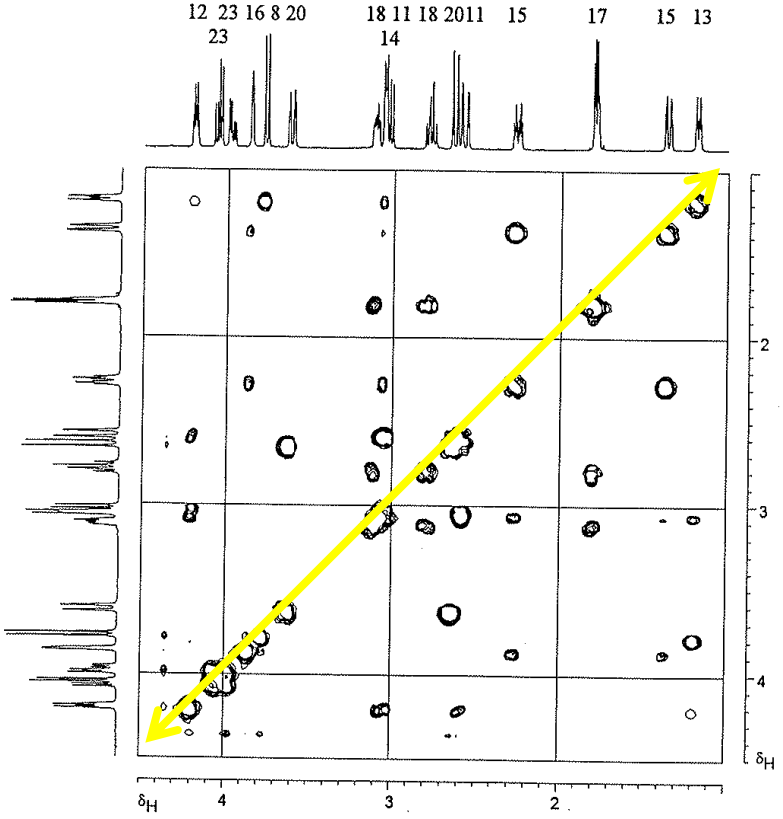


diagonal signals

strychnine 10 %



COSY

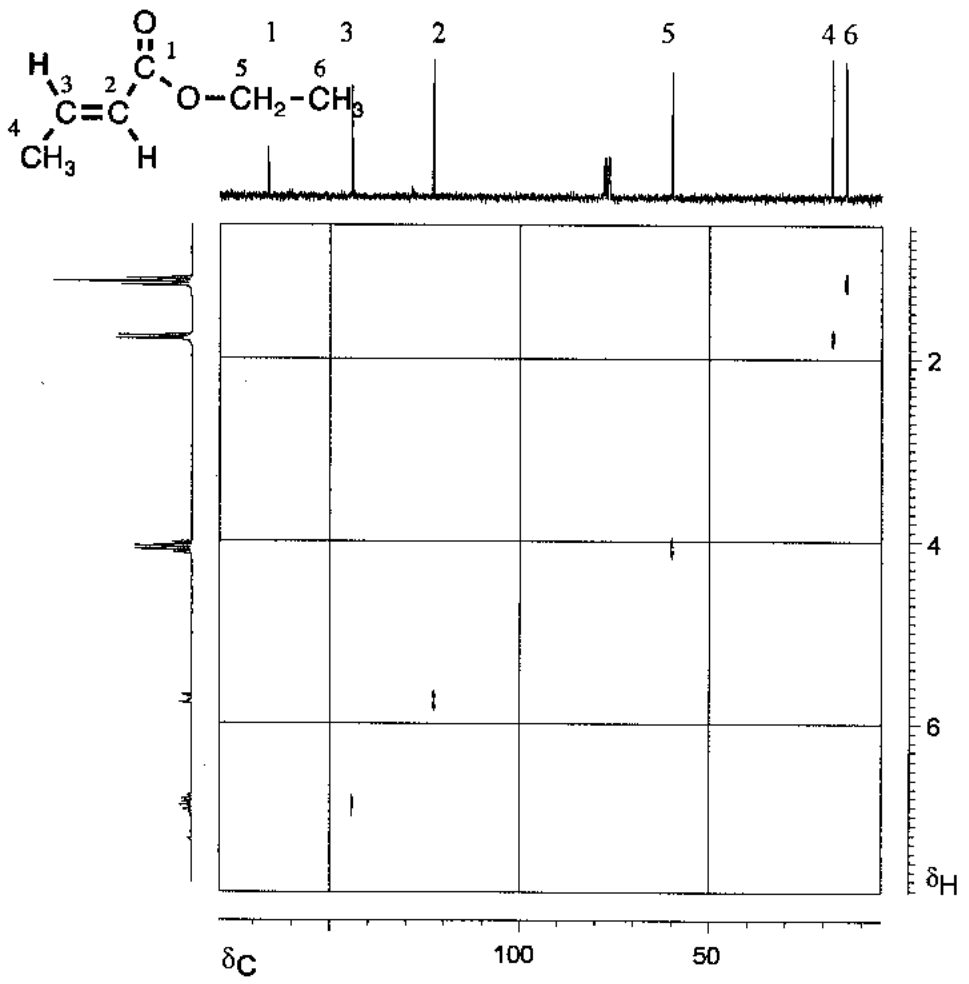


In the case of COSY experiment for a system of two nuclei, with different chemical shifts, Ω_A and Ω_B, there are the **diagonal peaks**, at $\omega_1 = \omega_2 = \Omega_A$ and $\omega_1 = \omega_2 = \Omega_B$. If they are scalarly couppled, **cross-peaks**, i.e. extradiagonal peaks, appear symmetrically with respect to the diagonal, at $\omega_1 = \Omega_A$ e $\omega_2 = \Omega_B$ and at $\omega_1 = \Omega_B$ e $\omega_2 = \Omega_A$, due to the coherence transfer caused by the second 90° che costituisce il processo di mixing.

ethyl crotonate

HETCOR

heteronuclear
correlation
spectroscopy



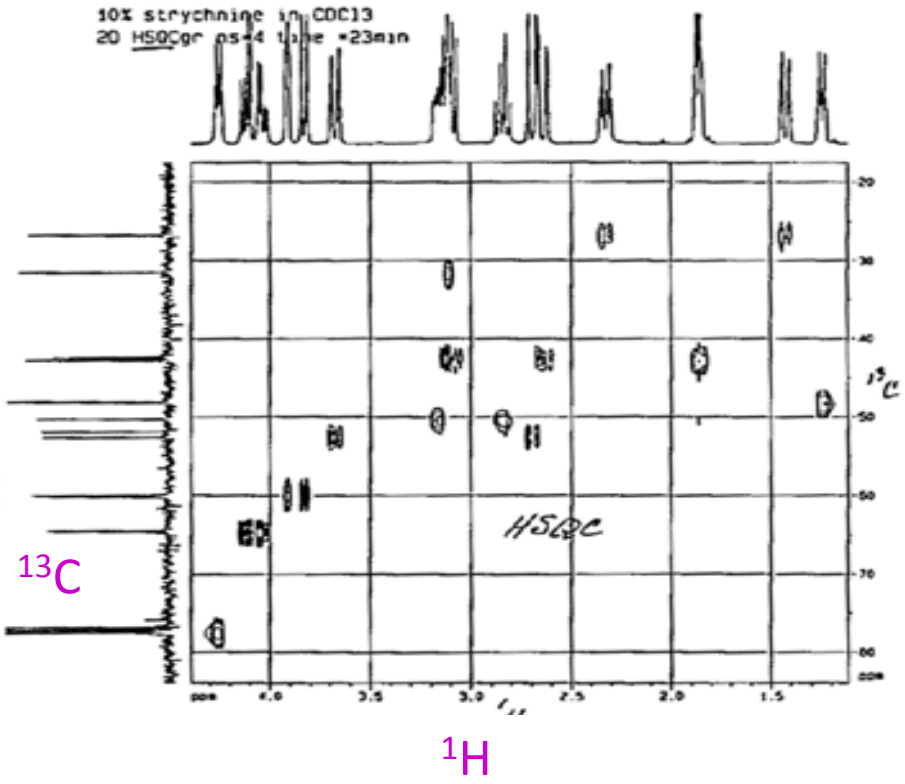
heteronuclear correlation:
cross-peaks only

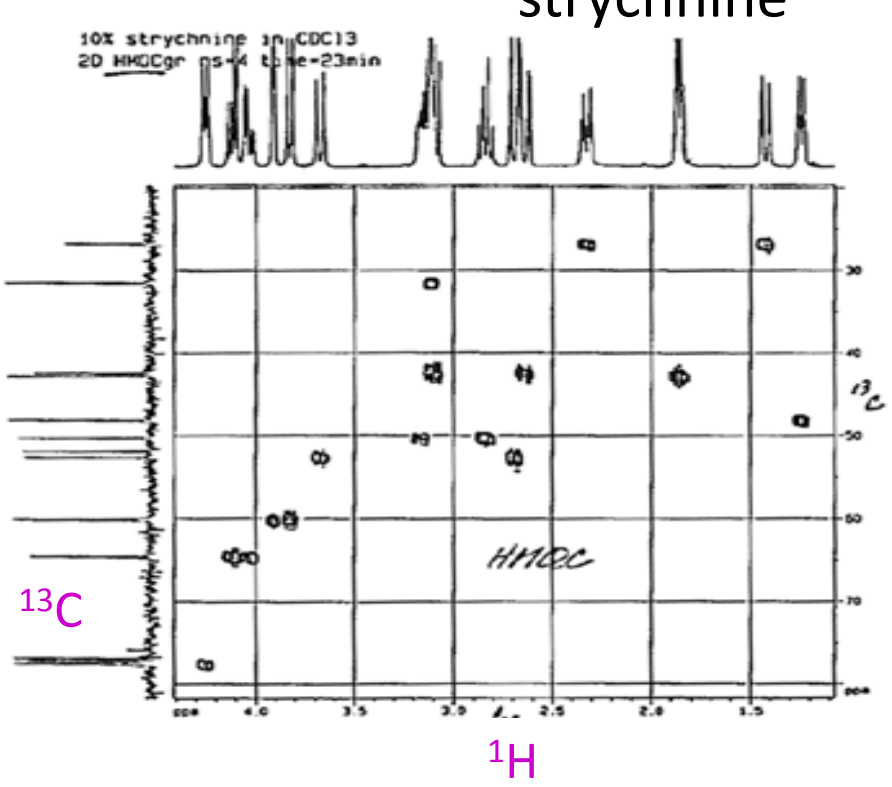
direct detection

Inverse Heteronuclear Correlation

enhanced sensitivity because both excitation and detection are carried on ^{1}H ($\gamma_H^{5/2}$)

strychnine





HSQC

from INEPT

HMQC

from DEPT

Sensitivity of 2D NMR Spectra

The 2D NMR spectra usuallly take longer than 1D and therefore it is important to optimize sensitivity

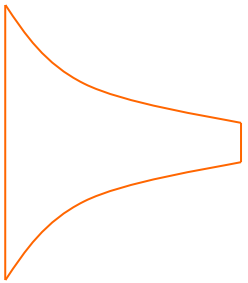
The signal of a 1D spectrun is proportional to the number of samples points times the average height of the signal “envelope”, “weighted” by a suited function (e.g. matched filter) in the time domain, $0 < t_2 < t_{2Max}$

S: signal intensity

n: number of scans

N: number of sampled points

$$S = n \cdot N \cdot \langle sh \rangle$$



<sh> average value of the ‘’envelope” height, h, mulripplied by the weighting function

The 2D signal is proportinal to the total number of sampled points (in both dimendiond) and to the average height of the weighted signal's ”envelope” in the time domain, i.e. in teh intervals : $0< t_1< t_{1Max}$ and $0< t_2 <t_{2Max}$.

$$S = n \cdot N_1 \cdot N_2 \cdot \langle sh \rangle$$

For best sensitivity matched filters are used in both dimendions.

It is possible to obtain the same sensitivity (intended as S/N per unit time) for 1D and 2D spectra provided:

I. the transverse decay an the decay due to the static magnetic field inhomogeneities are negligible during the evoluion time t_1

II. the instrumental stabiity is good so that the t_1 noise is negligible

III. same number of peaks, thus the intensit of one line of the 1D spectrum is not distributed among several 2D peaks

The I criterium requires low resolution in t_1. In the case high resolution in t_1 is needed, a little decay of sensitivity in the 2D spectrum must be reckoned

The III criterium is satisfied in heterocorrelated maps

Optimization of the 2D Spectra

The height of the ”envelope” depends on the interval for relaxation between two subsequent scans. Ideally it is on the order of $3T_1$ in order to avoid the longitudinal interference between the subsequent experiments, which leads to t_1 noise. However, it is more common to work at the steady state, using 4 or more più dummy scan before the true accumulations.

It is better to use the least possible resolution in the 1 dimension, to shorten the experiment time and reduce the efect of the signal decay in t_1. Few datapoints cause the nasty Gibbs oscillation after the FT due to signal truncation. For these reason apodization function are used. A sensible choise are the Gaussian functions. The further zero filling procedure improves the digital resolutioin of the final spectrum.

Quadrature in ω_1

There are two strategies:

to run a parallel experiment to collect the cosine component

The t_1 at which the cosine component is obtained may be either the same of the sine component

or interspersed with those of the sine component

The two schemes are close to those employed for the conventional quadrature detection: simultaneous sampling (SHR: States, Haberkorn, Ruben) or not simultaneous sampling (TPPI: time proportional phase incrementation).

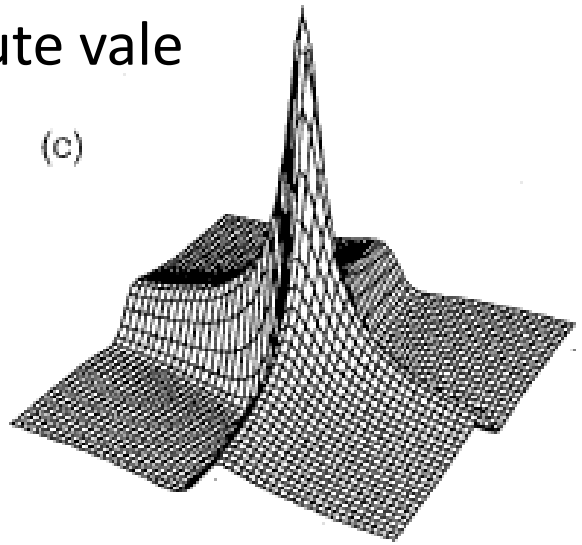
After the first FT the signal is phased and the imaginary part is deleted, thus the signals after the second transform can be phased as pure absorption obtaining the socalled **phase-sensitive spectra**

The advantage is that there is further information concerning the relative phase of the signals and close signals are more easily distinguished

The disadvatage is that a double amount of computer and disk memory is used

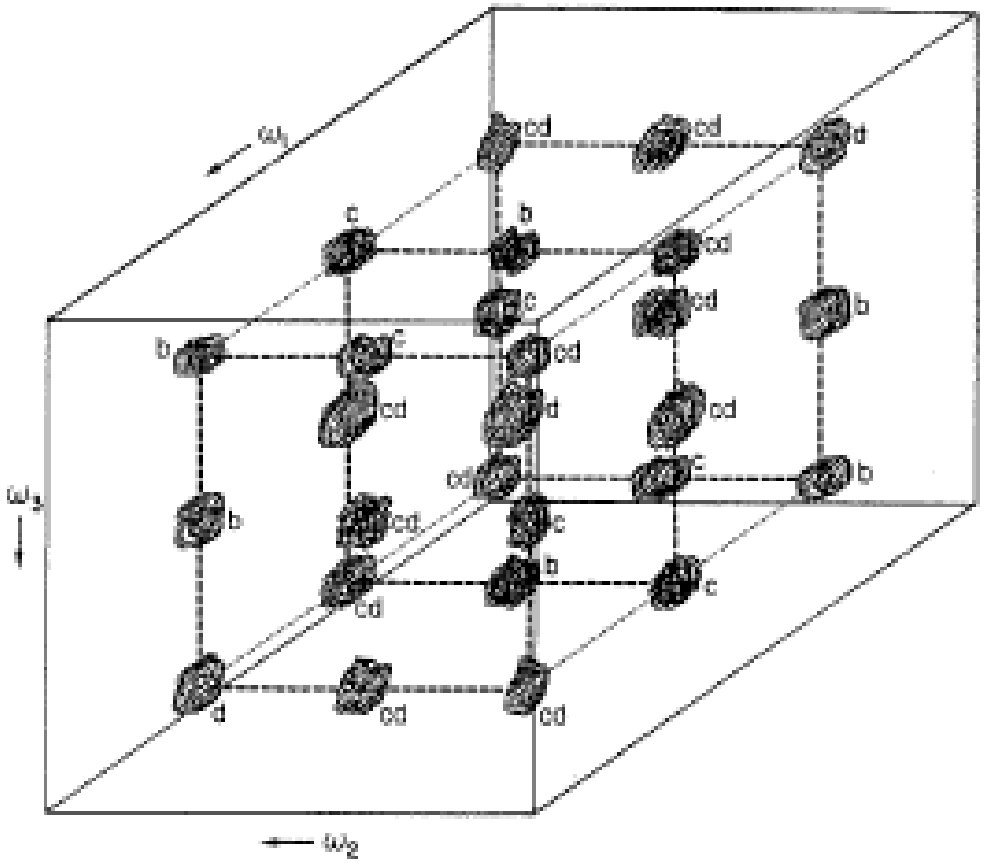
It is more common to acquire the sine and cosine component in the same FID during a series of scans acquired according the EXORCYCLE phase cycle.

The peaks obtained after both FT display a mixture of absorption and dispersion, so taht cannot be phased as pure absorption,
and therefore are presented as absolute vale



mixture of absorption and dispersion

3D: COSY-COSY



after t_1 a further evolution time is inserted