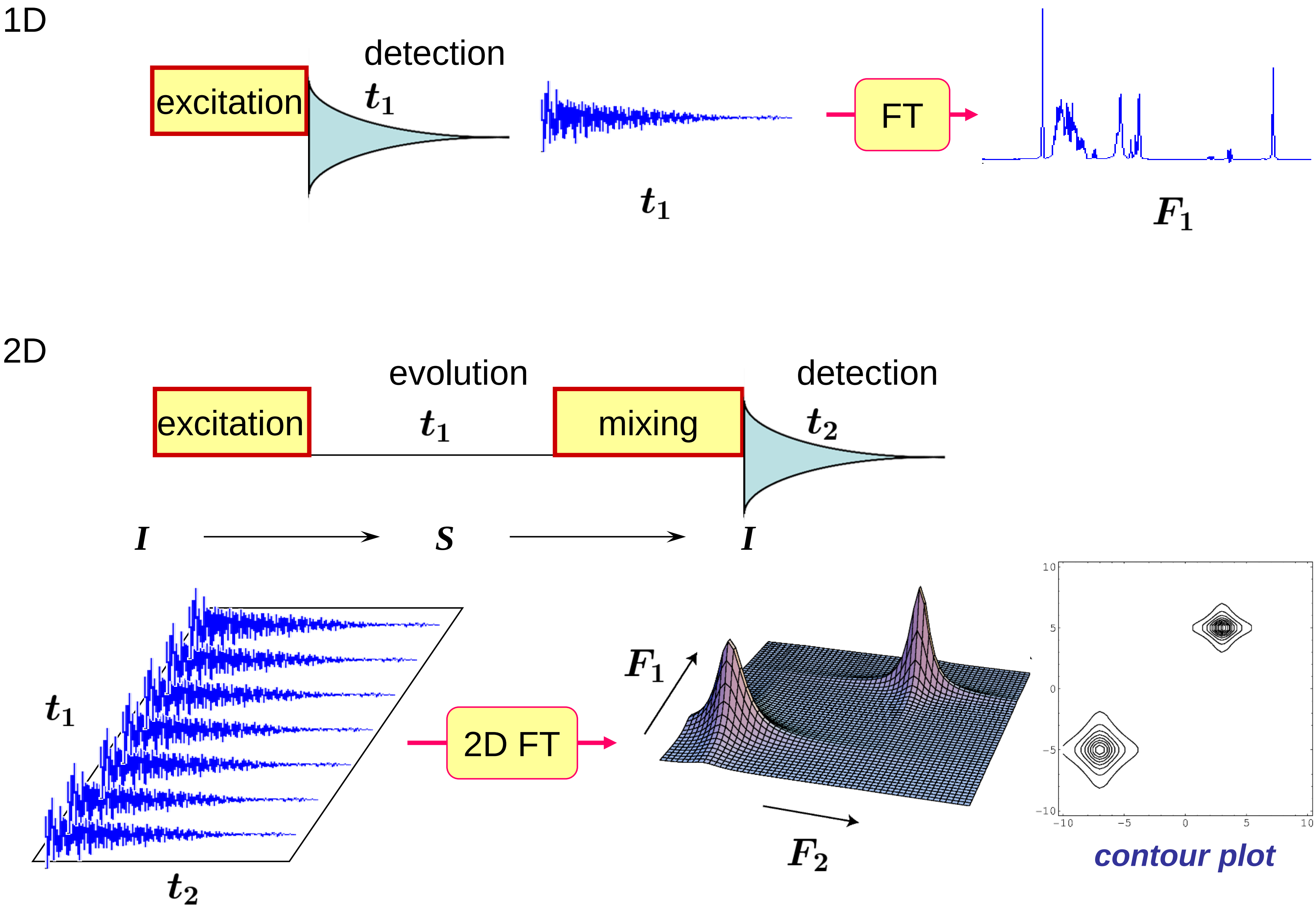
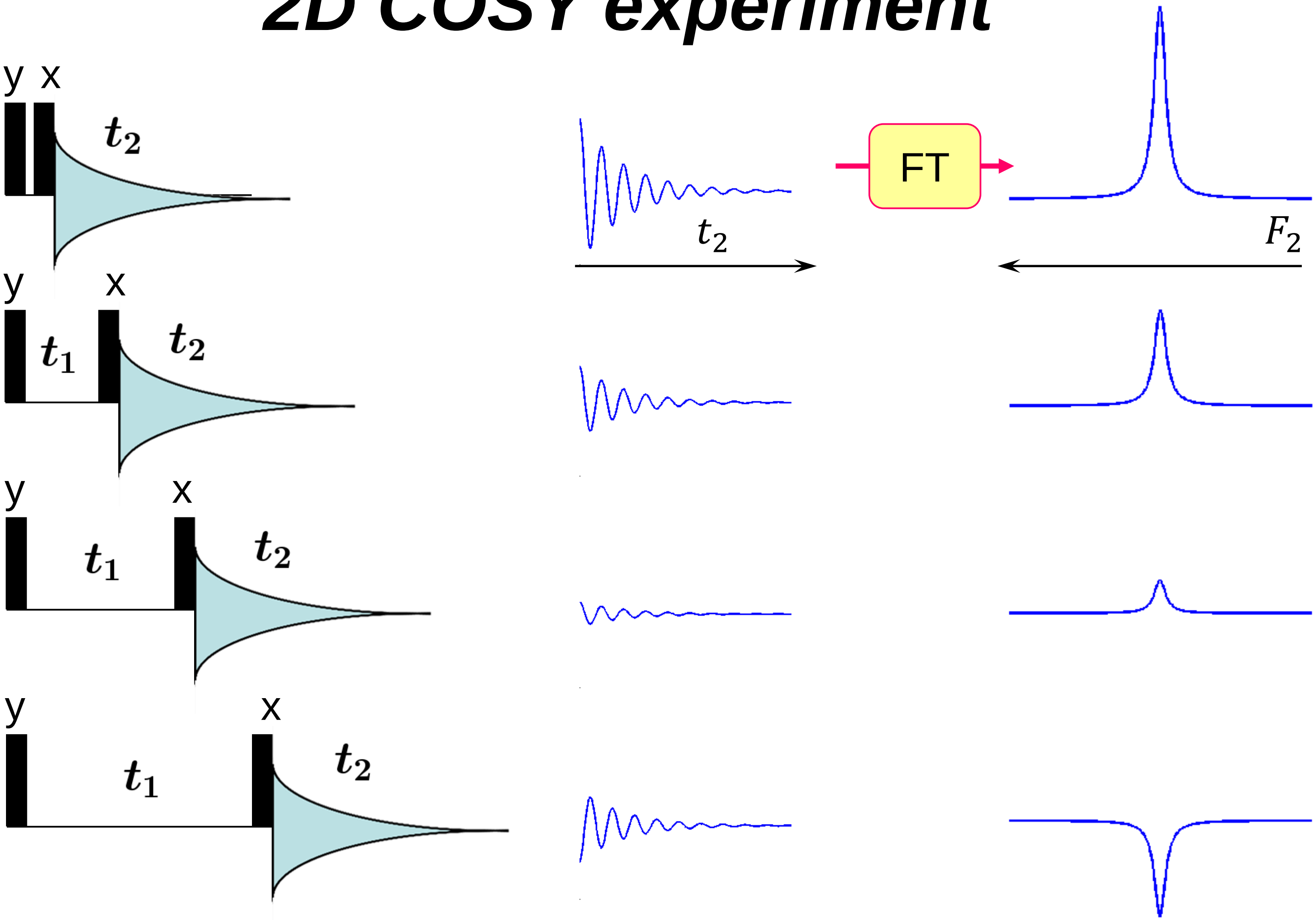
2D Spectroscopy

- COSY
- TOCSY
- HSQC a HMQC
- HMBC
- HETCOR

Principle of 2D spectroscopy



2D COSY experiment

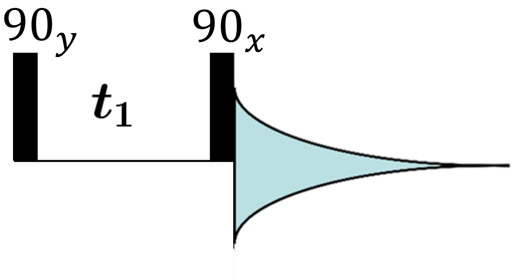


Evolution in t_1 is encoded in signal intensity detected during t_2

t_1, F_1 → ***indirect domain***

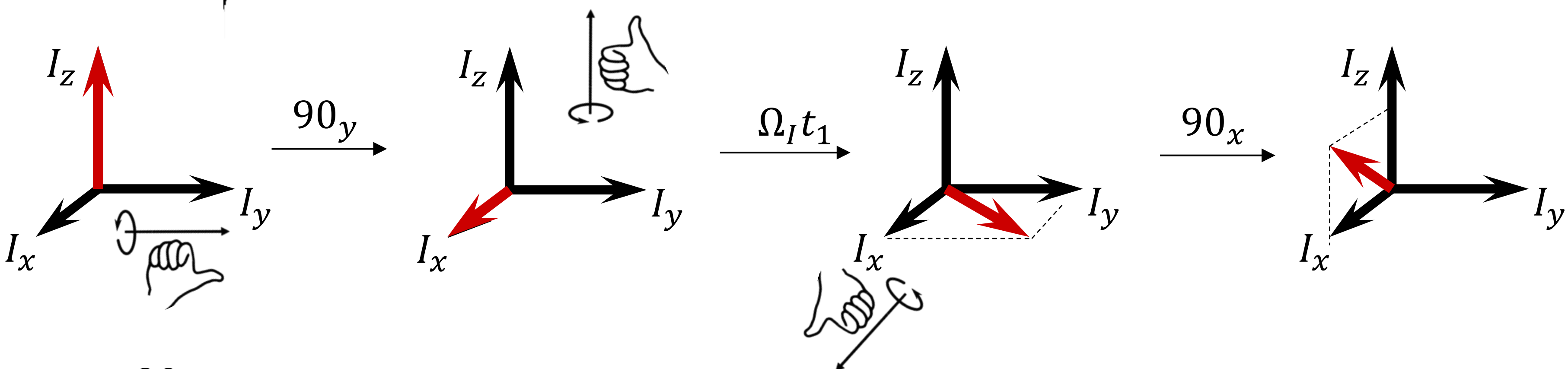
t_2, F_2 → ***direct domain***

2D COSY experiment

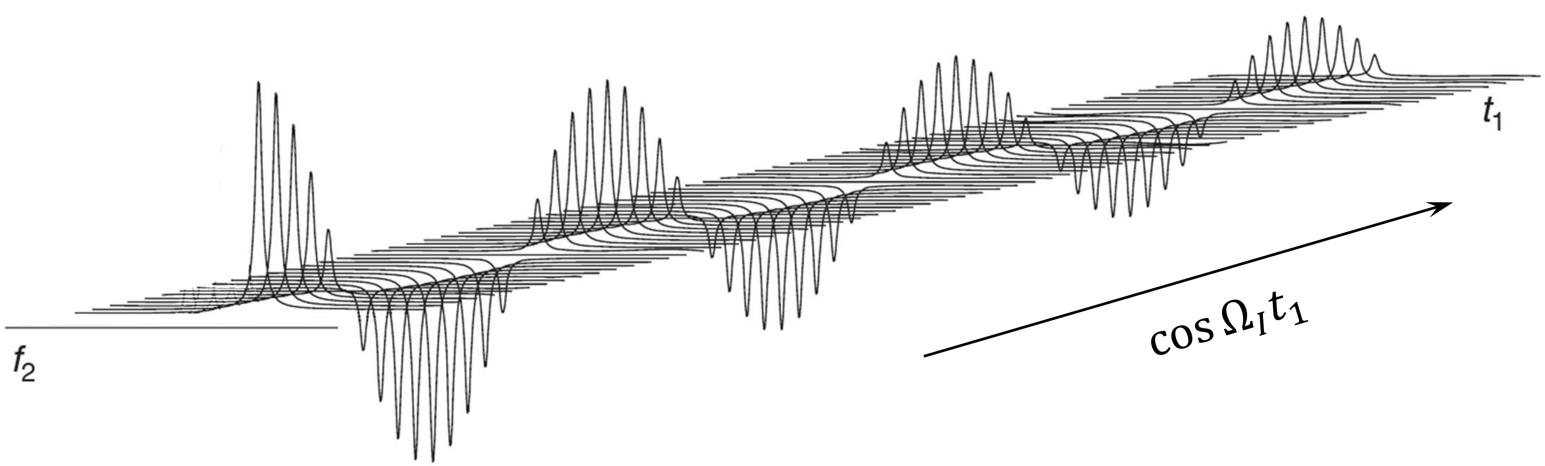


Non-interacting spins

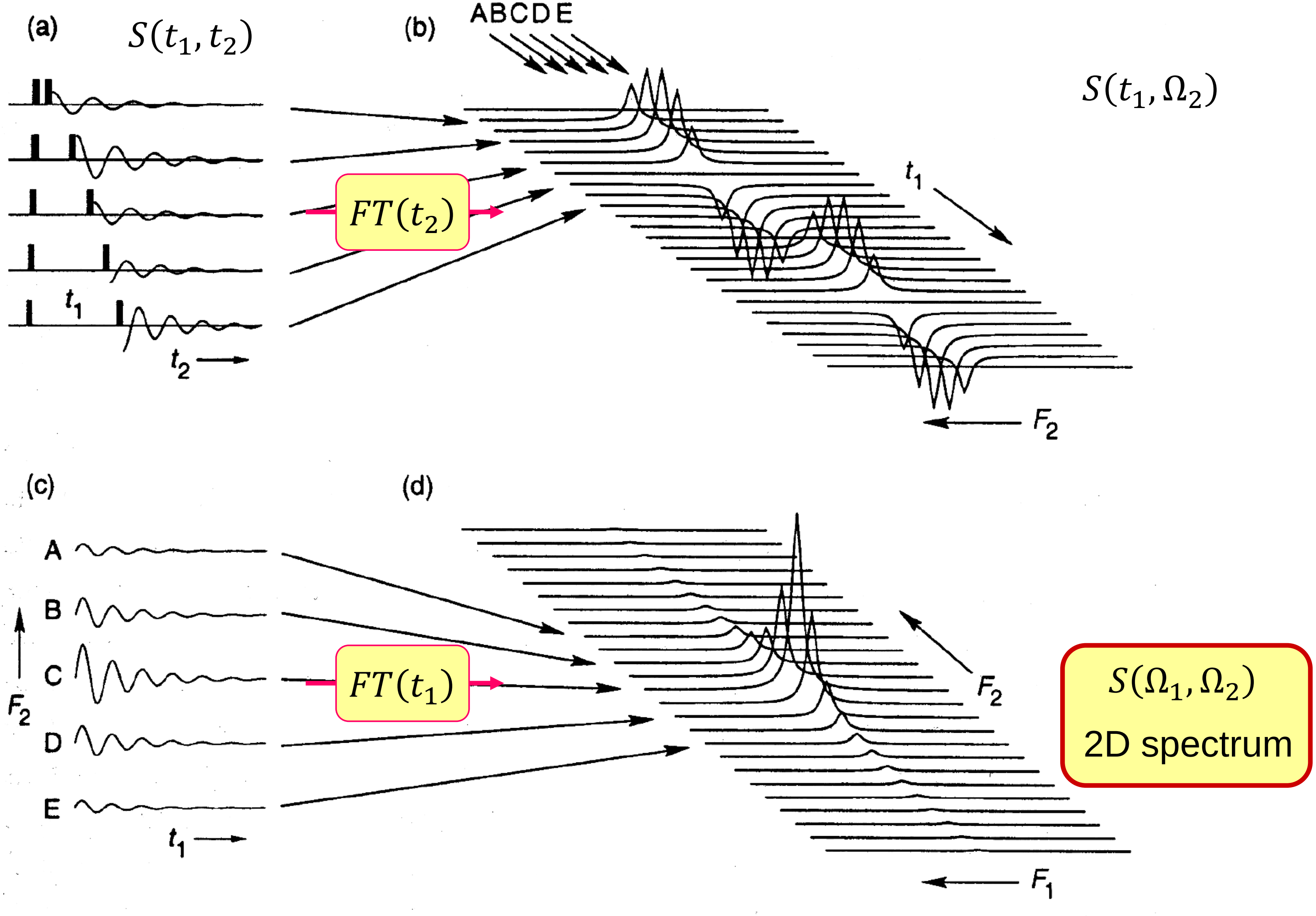
magnetization



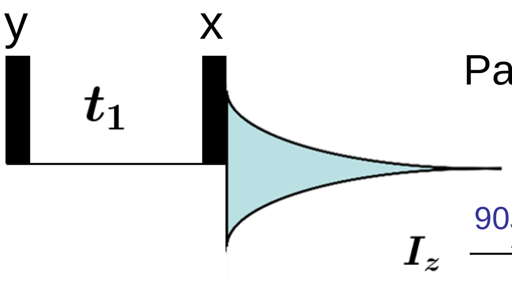
$$I_z \xrightarrow{90_y} I_x \xrightarrow{\Omega_I t_1} I_x \cos \Omega_I t_1 + I_y \sin \Omega_I t_1 \xrightarrow{90_x} I_x \cos \Omega_I t_1 + I_z \sin \Omega_I t_1$$



2D COSY experiment



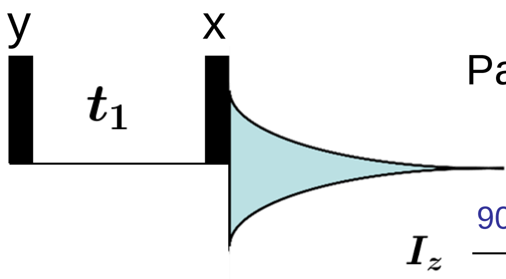
COSY – product operators



Pair of spins with J-coupling (homonuclear)

$$I_z \xrightarrow{90_y}$$

COSY – product operators



Pair of spins with J-coupling (homonuclear)

$$I_z \xrightarrow{90_y} I_x \xrightarrow{\text{chem. shift}} I_x \cos\Omega_I t_1 + I_y \sin\Omega_I t_1 \xrightarrow{\text{J coupling}}$$

$$I_x \cos\Omega_I t_1 \cos\pi J t_1 + 2I_y S_z \cos\Omega_I t_1 \sin\pi J t_1 + I_y \sin\Omega_I t_1 \cos\pi J t_1 - 2I_x S_z \sin\Omega_I t_1 \sin\pi J t_1$$

$$\xrightarrow{90_x}$$

$$I_x \cos\Omega_I t_1 \cos\pi J t_1 - 2I_z S_y \cos\Omega_I t_1 \sin\pi J t_1 + I_z \sin\Omega_I t_1 \cos\pi J t_1 + 2I_x S_y \sin\Omega_I t_1 \sin\pi J t_1$$

$$I_x \frac{1}{2}[\cos(\Omega_I - \pi J)t_1 + \cos(\Omega_I + \pi J)t_1]$$

F_2: Ω_I *inphase doublet, dispersive*

F_1: Ω_I *inphase doublet, dispersive*

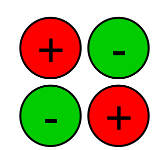
diagonal peak

$$2I_z S_y \frac{1}{2}[\sin(\Omega_I - \pi J)t_1 - \sin(\Omega_I + \pi J)t_1]$$

Ω_S *antiphase doublet, absorptive*

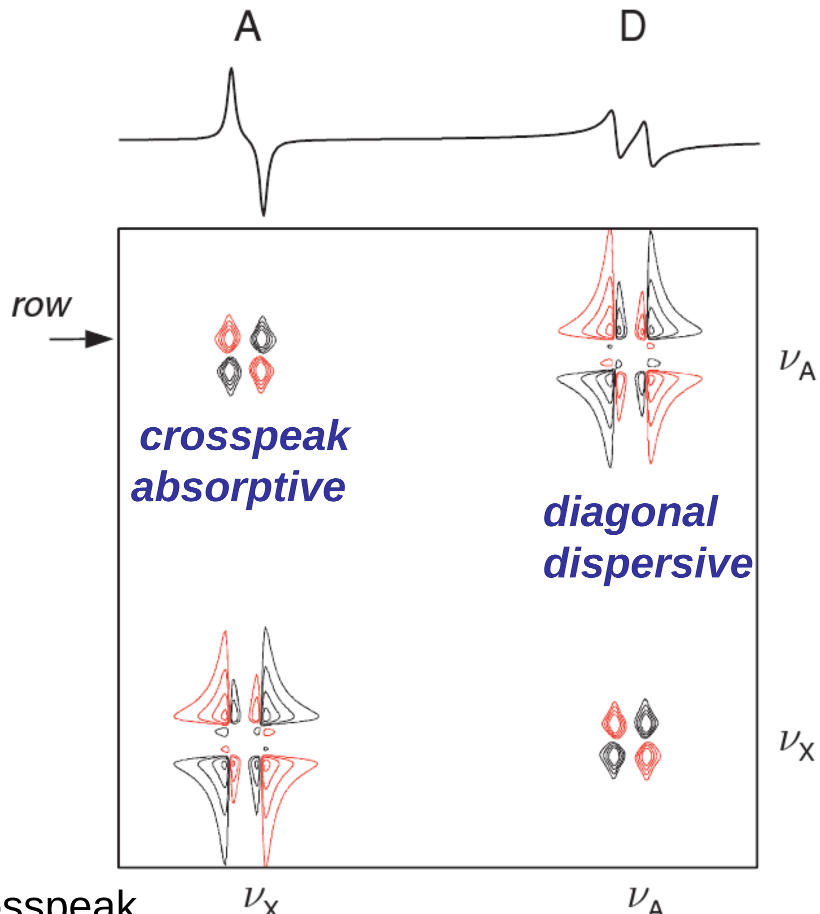
Ω_I *antiphase doublet, absorptive*

crosspeak



Measurement and data processing conditions $\Rightarrow$ loss of spectral resolution

COSY phase sensitive



Crosspeak

$$2I_zS_y\frac{1}{2}[\sin(\Omega_I-\pi J)t_1-\sin(\Omega_I+\pi J)t_1]$$

Diagonal peak $$I_x\frac{1}{2}[\cos(\Omega_I-\pi J)t_1+\cos(\Omega_I+\pi J)t_1]$$

Quadrature detection in the indirect domain

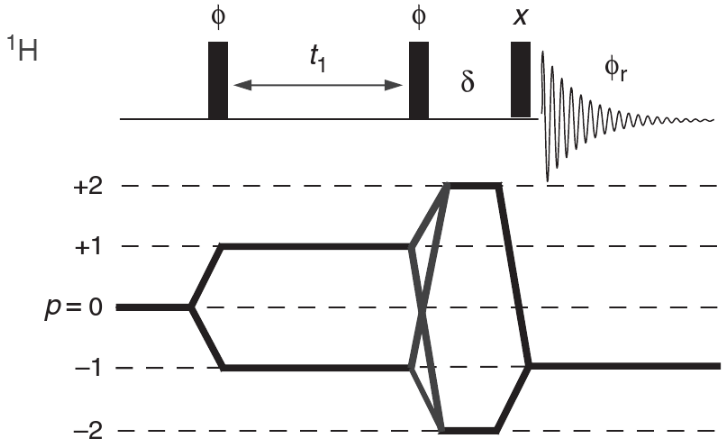
- ***measure of COS and SIN modulated data***
- ***two measurements for one t_1 increment, with different pulse phases***

long measurement time

FID in the indirect domain is truncated

- ***heavy apodization to suppress truncation artefacts***
- ***Crosspeak intensity builds according to SIN function***
- ***As a result, final lineshapes are not Lorentz curves and are broad = lower resolution***
- ***In addition, diagonal peaks are suppressed***

DQF-COSY



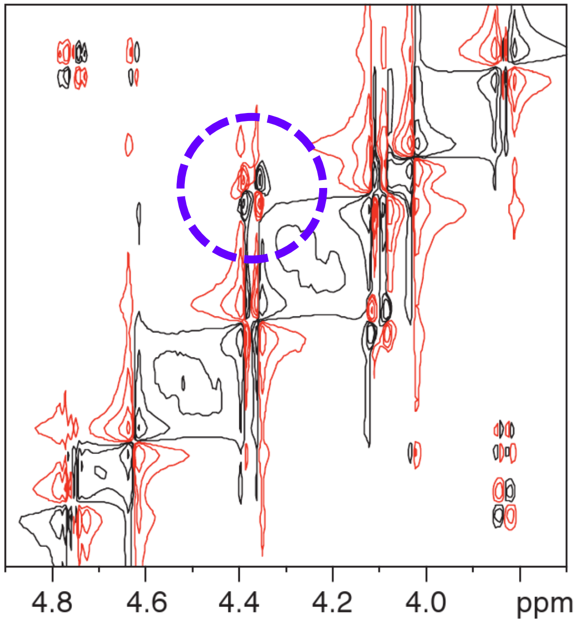
double-quantum filtered

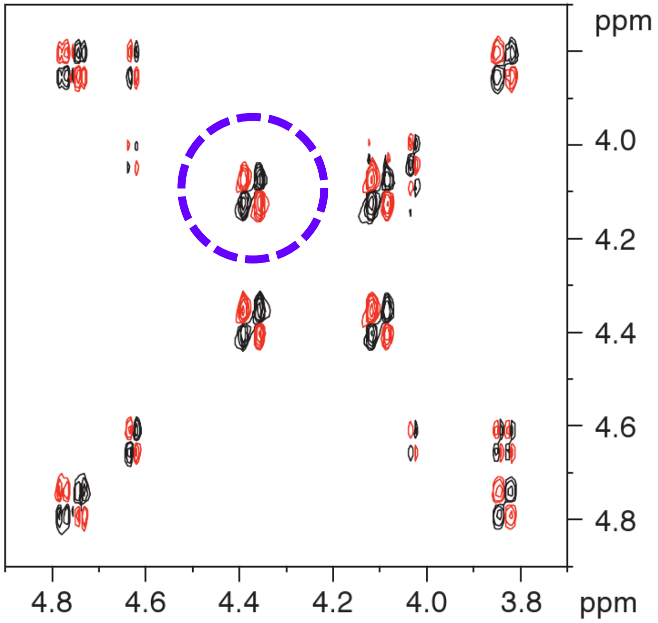
diagonal peaks have the same phase as crosspeaks

Improved resolution near the diagonal

Suppression of signals without couplings

COSY

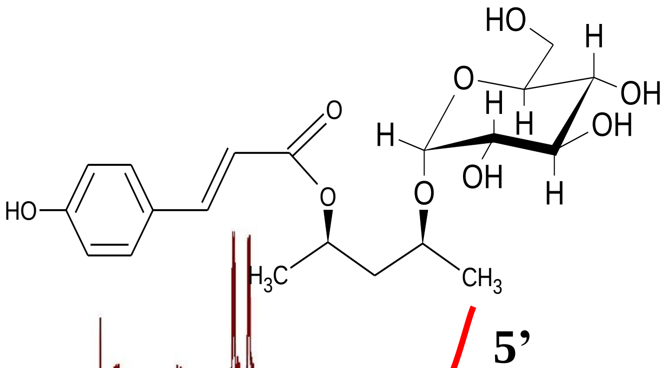


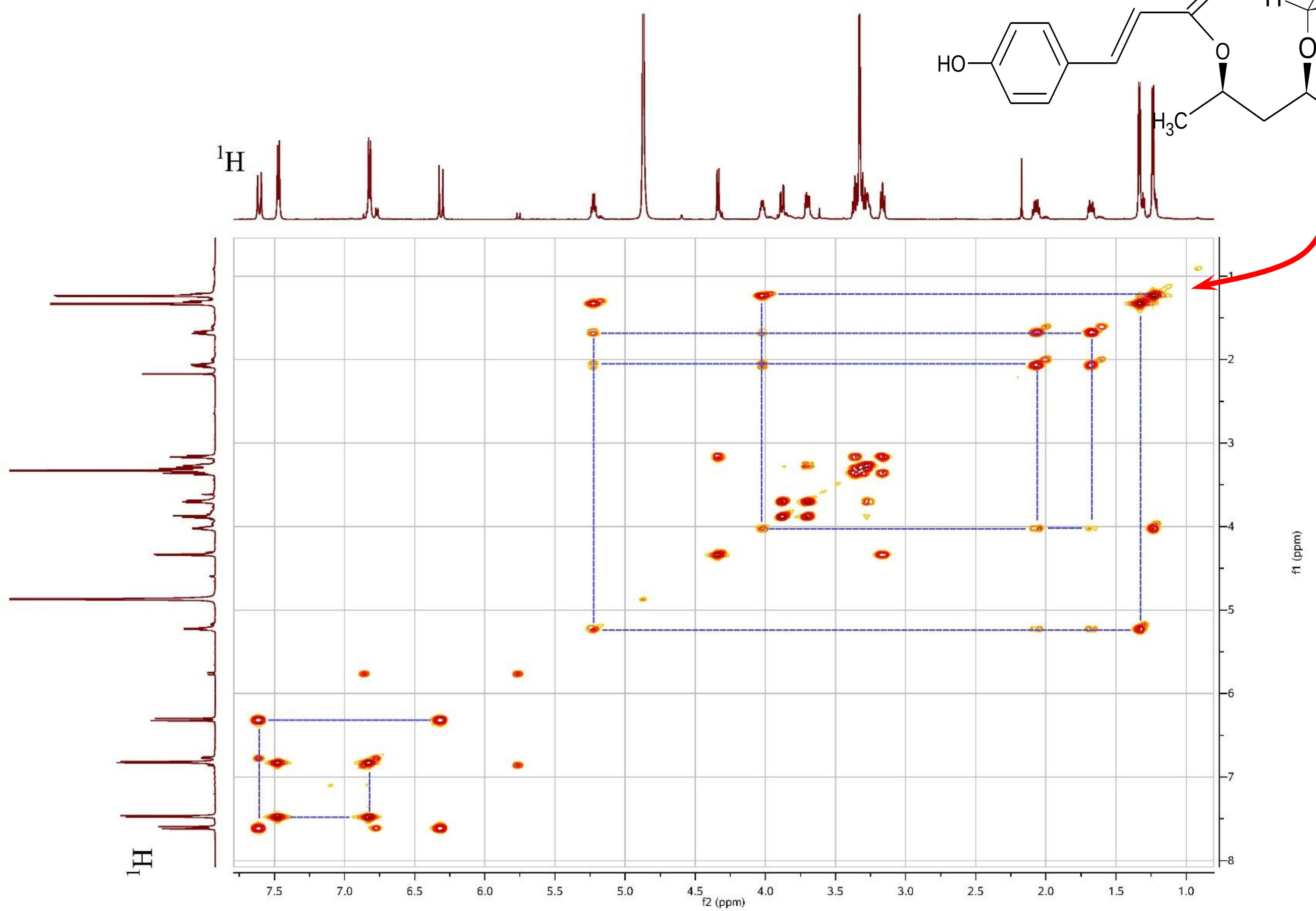


DQF-COSY

COSY

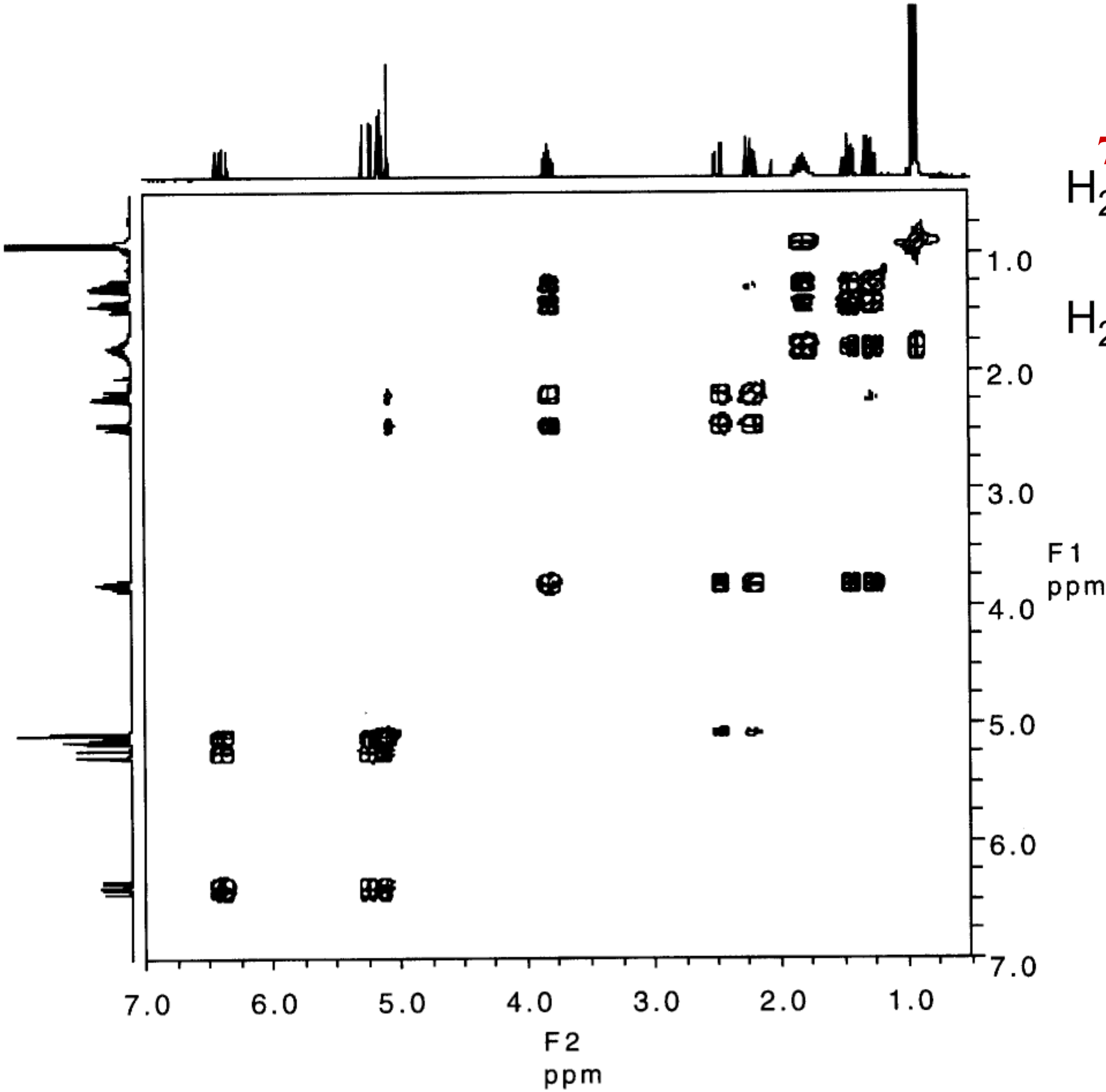
Correlations between signals of protons with J-coupling



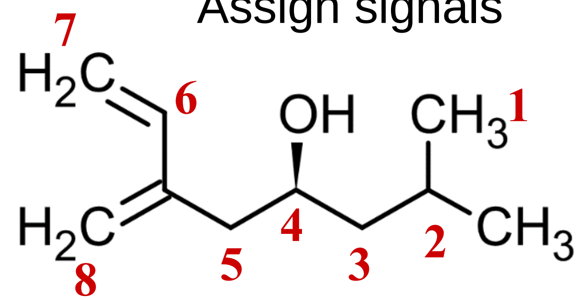


crosspeak = there is J-coupling between the corresponding protons
Only „one step“ transfers

COSY

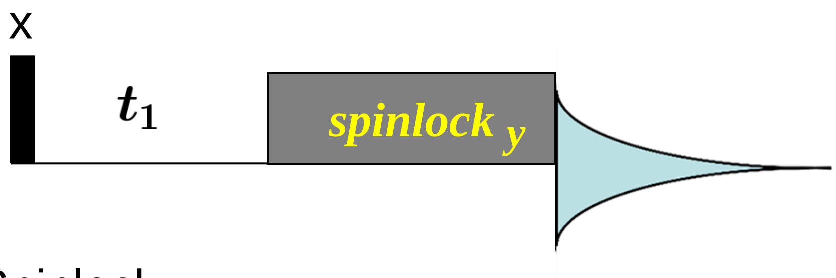


Assign signals

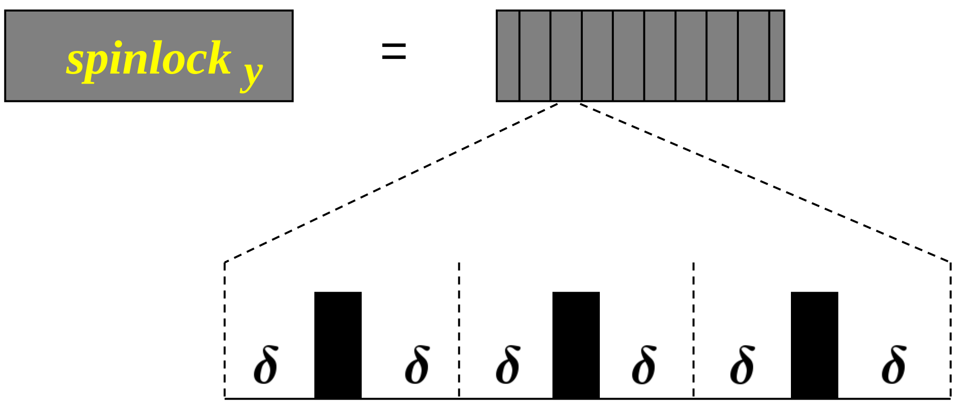


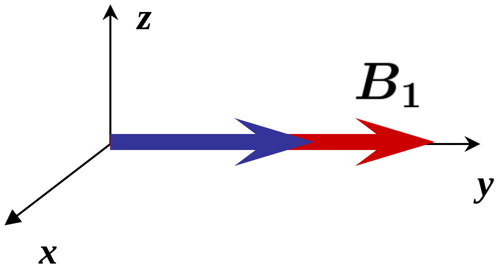
ipsenol

TOCSY



Spinlock





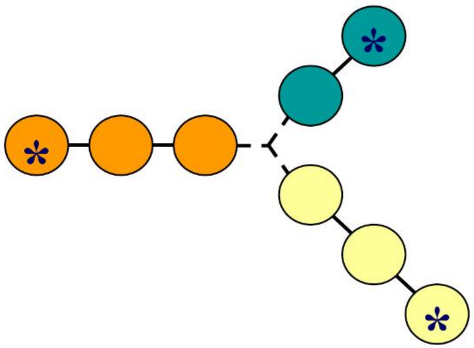
Magnetization "locked" in the **y**-axis

- ***Chemical shift does not evolve***
- ***J coupling remains active***

All spins effectively feel the same magnetic field B_1 and have the same energy levels quantized w.r.t. this field (in the rotating frame)

polarization freely flows between **all spins** connected by the J-couplings

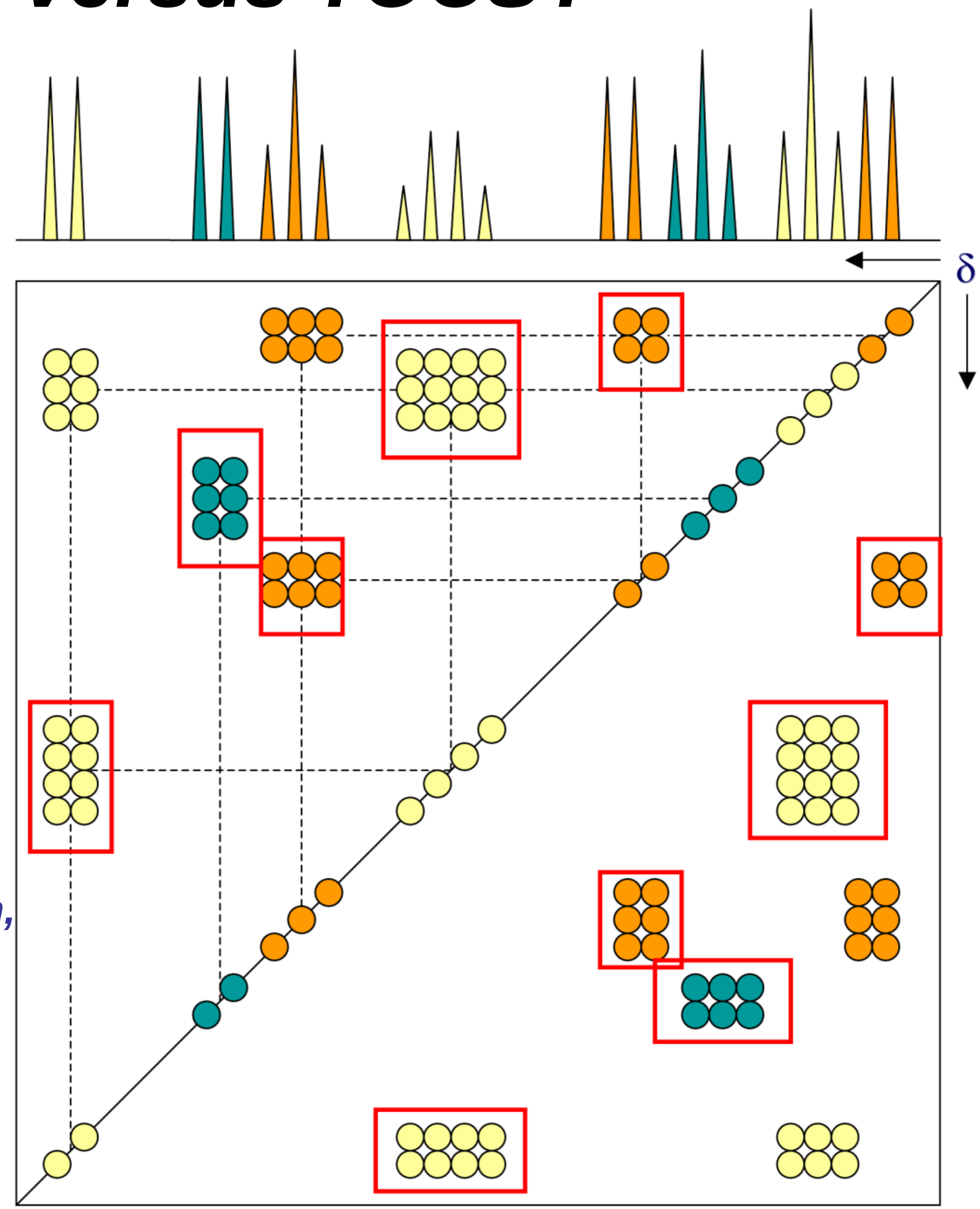
COSY versus TOCSY



Identification of spin systems

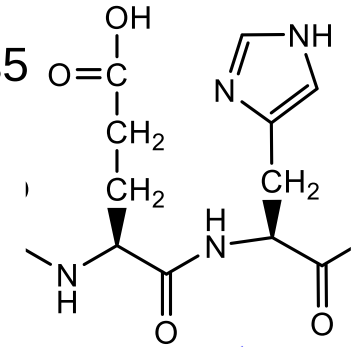
Side chains

crosspeak = proton belongs to
that particular spin system,
a network of J-couplings

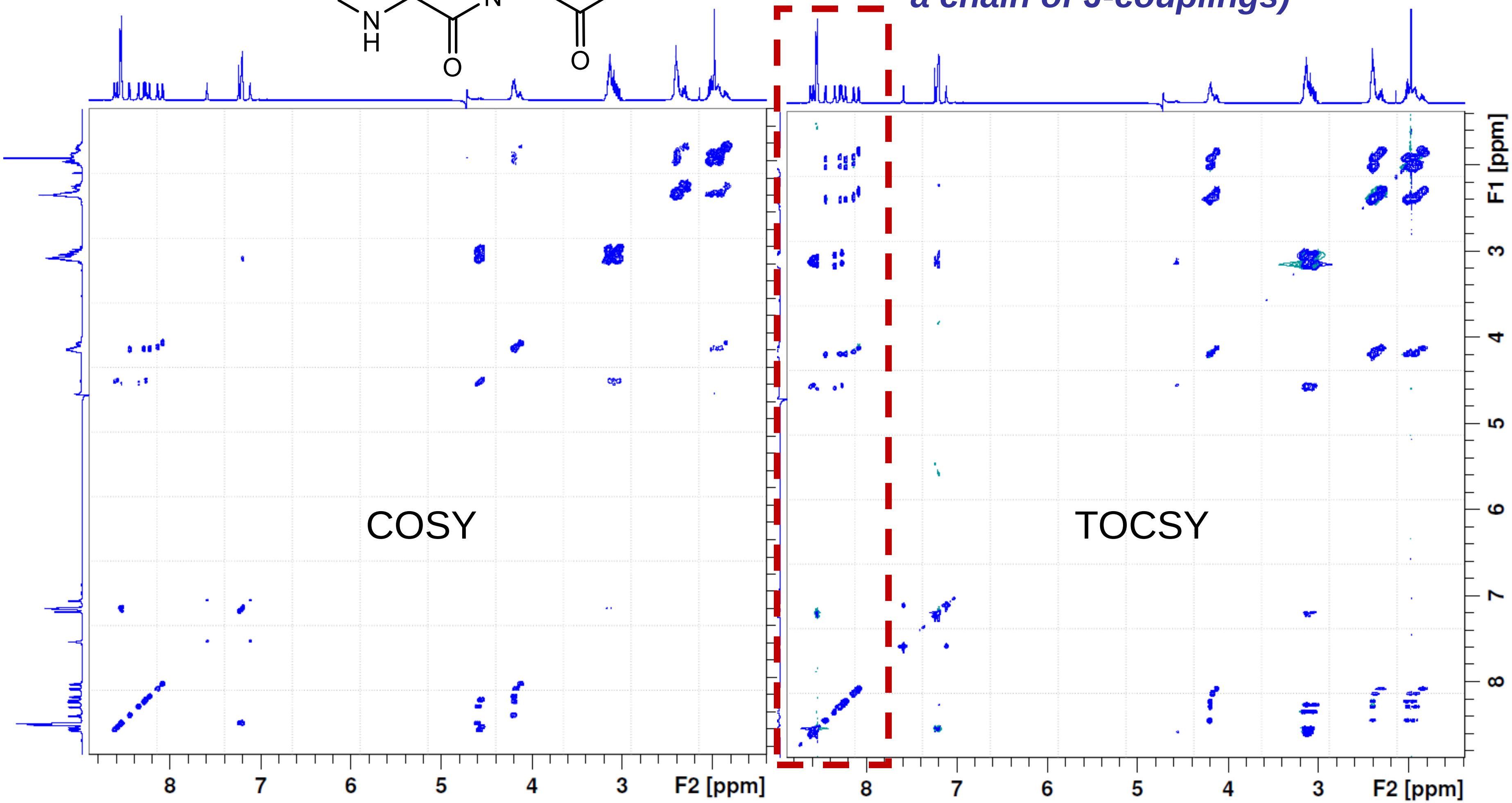


COSY versus TOCSY

Peptide Glu5-His5

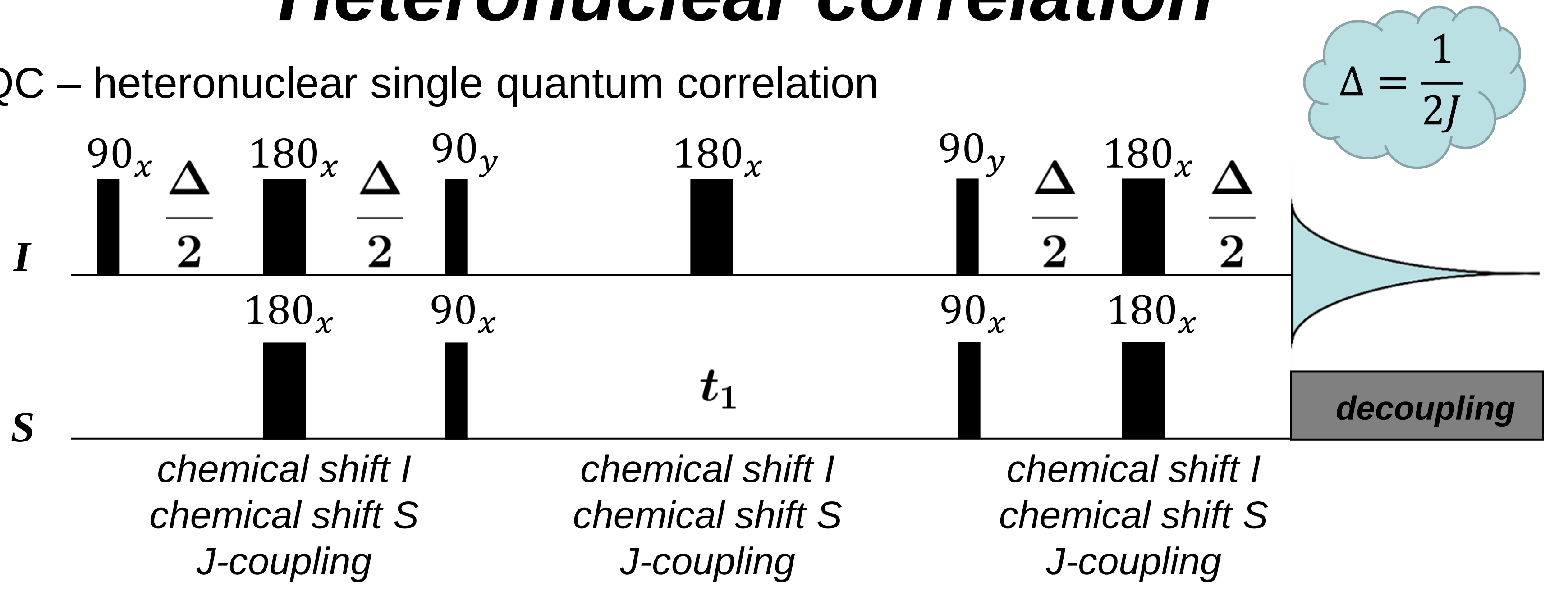


Amidic protons reveal chemical shifts of all other protons within the side chain (provided there is a chain of J-couplings)



Heteronuclear correlation

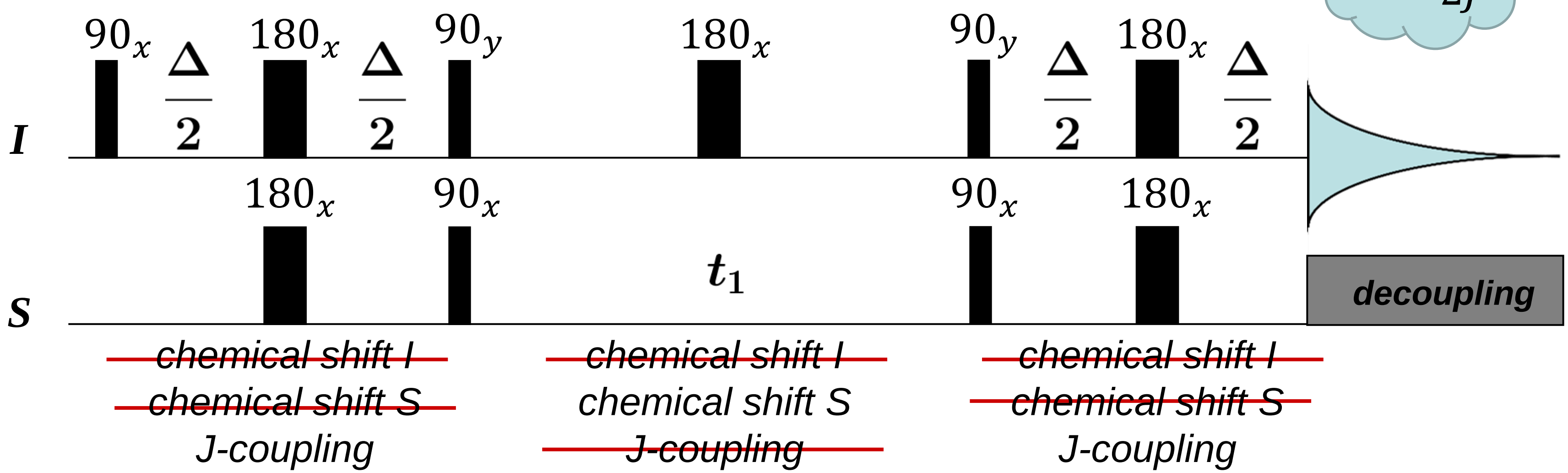
HSQC – heteronuclear single quantum correlation



Heteronuclear correlation

HSQC – heteronuclear single quantum correlation

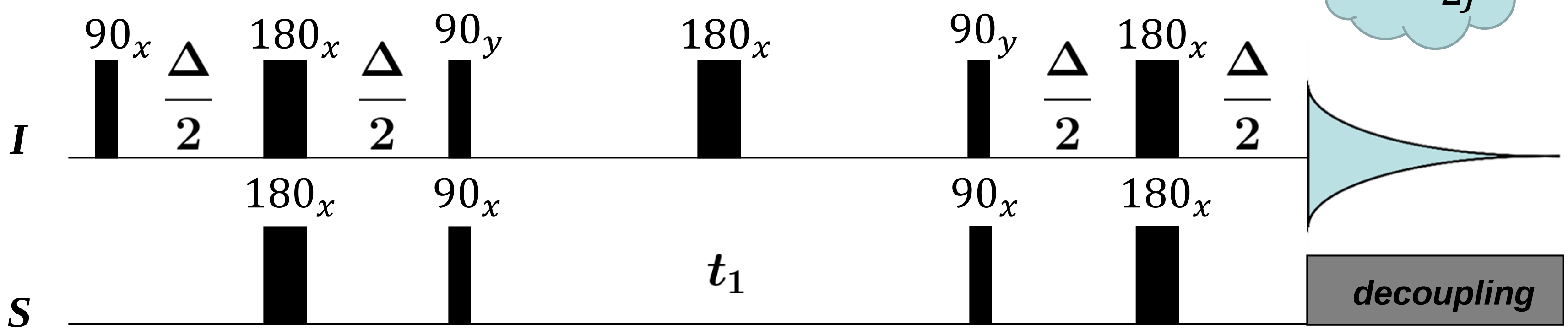
$$\Delta = \frac{1}{2J}$$



Heteronuclear correlation

HSQC – heteronuclear single quantum correlation

$$\Delta = \frac{1}{2J}$$



~~chemical shift I~~	~~chemical shift I~~	~~chemical shift I~~
~~chemical shift S~~	chemical shift S	~~chemical shift S~~
J-coupling	~~J-coupling~~	J-coupling

$$I_z \xrightarrow{I:90_x} -I_y \xrightarrow[S:180_x]{I:180_x} I_y \xrightarrow{\pi J \Delta} -2I_xS_z \xrightarrow[S:90_x]{I:90_y} -2I_zS_y \xrightarrow{I:180_x} 2I_zS_y \xrightarrow{\Omega_S t_1}$$

$$2I_zS_y \cos\Omega_S t_1 - 2I_zS_x \sin\Omega_S t_1 \xrightarrow[S:90_x]{I:90_y} 2I_xS_z \cos\Omega_S t_1 - 2I_xS_x \sin\Omega_S t_1$$

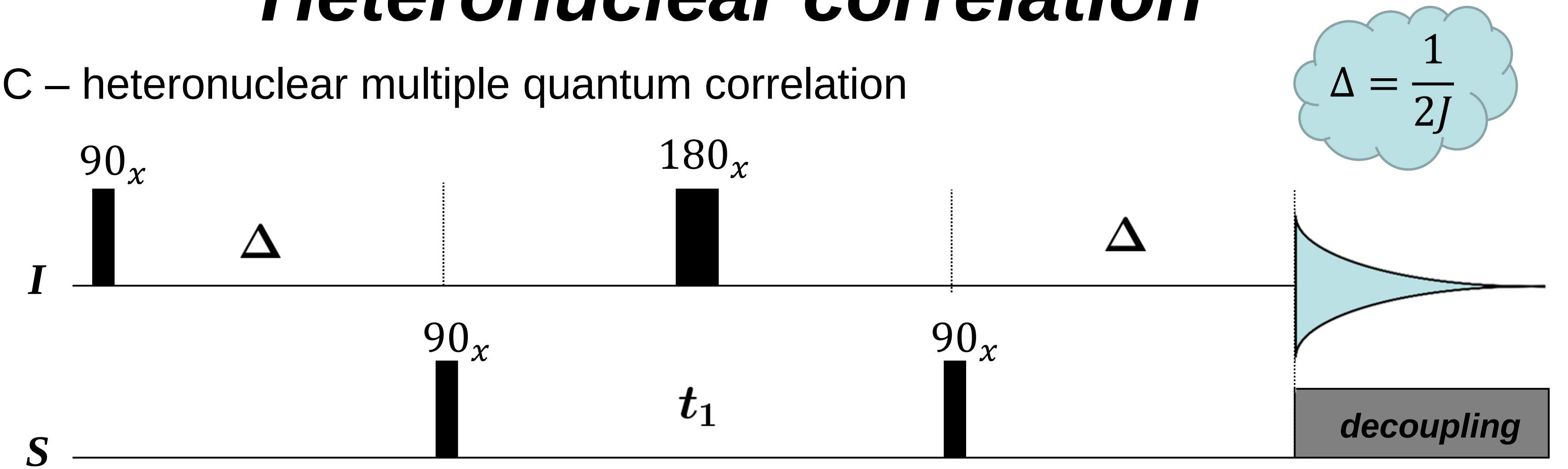
$$\xrightarrow[S:180_x]{I:180_x} -2I_xS_z \cos\Omega_S t_1 - 2I_xS_x \sin\Omega_S t_1 \xrightarrow{\pi J \Delta} -I_y \cos\Omega_S t_1 - \cancel{2I_xS_x \sin\Omega_S t_1}$$

Direct domain Ω_I ***Indirect domain*** Ω_S

Signal of nucleus I modulated by chemical shift of nucleus S

Heteronuclear correlation

HMQC – heteronuclear multiple quantum correlation

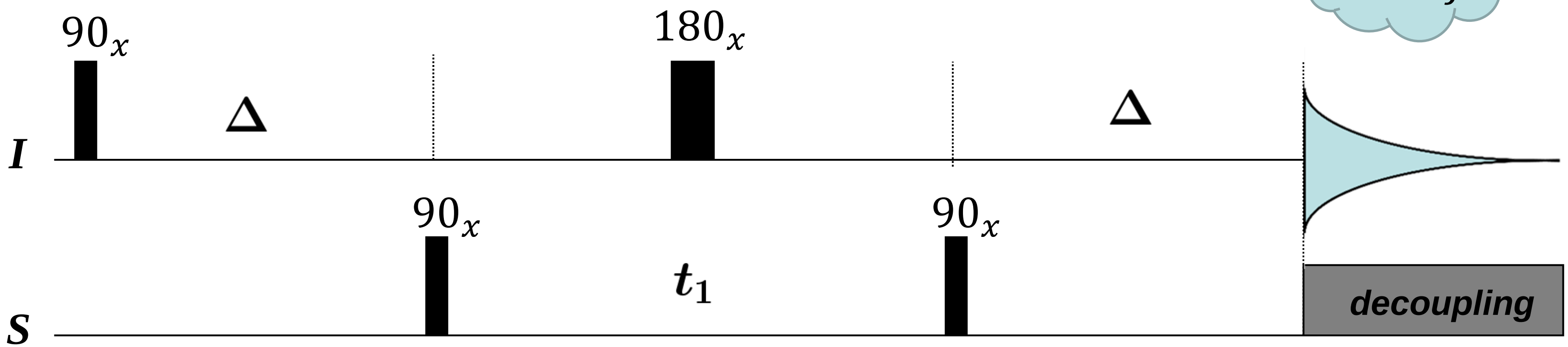


chemical shift I
chemical shift S
J-coupling

Heteronuclear correlation

HMQC – heteronuclear multiple quantum correlation

$$\Delta = \frac{1}{2J}$$



~~*chemical shift I*~~

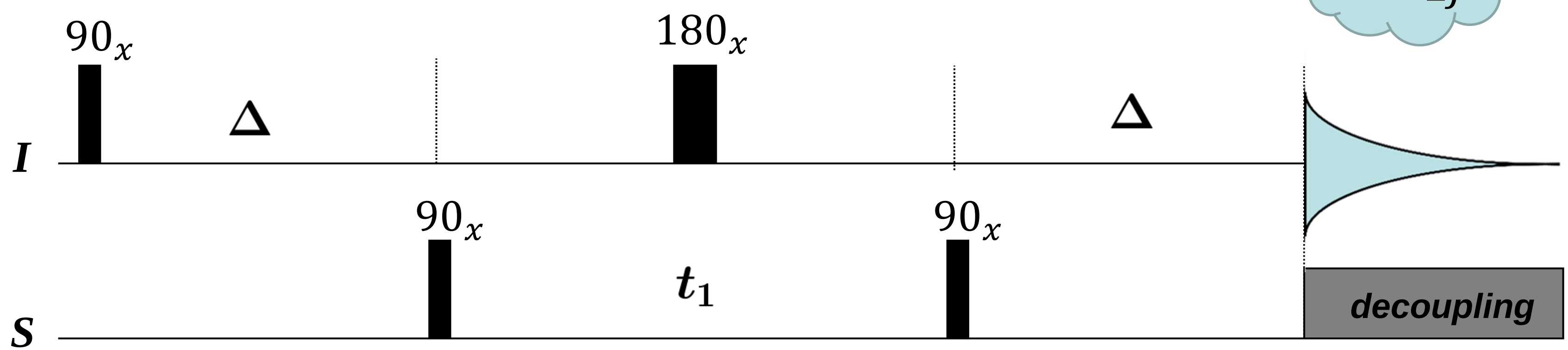
chemical shift S

~~*J-coupling*~~

Heteronuclear correlation

HMQC – heteronuclear multiple quantum correlation

$$\Delta = \frac{1}{2J}$$



~~*chemical shift I*~~
chemical shift S
~~*J-coupling*~~

$$I_z \xrightarrow{I:90_x} -I_y \xrightarrow{\pi J\Delta} 2I_xS_z \xrightarrow[S:90_x]{} -2I_xS_y \xrightarrow{I:180_x} -2I_xS_y \xrightarrow{\Omega_S t_1}$$

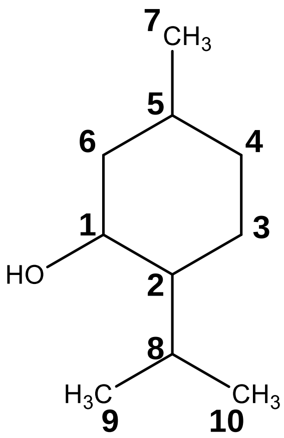
$$-2I_xS_y\cos\Omega_S t_1 + 2I_xS_x\sin\Omega_S t_1 \xrightarrow[S:90_x]{} -2I_xS_z\cos\Omega_S t_1 + 2I_xS_x\sin\Omega_S t_1$$

$$\xrightarrow{\pi J\Delta} -I_y\cos\Omega_S t_1 + \cancel{2I_xS_x\sin\Omega_S t_1}$$

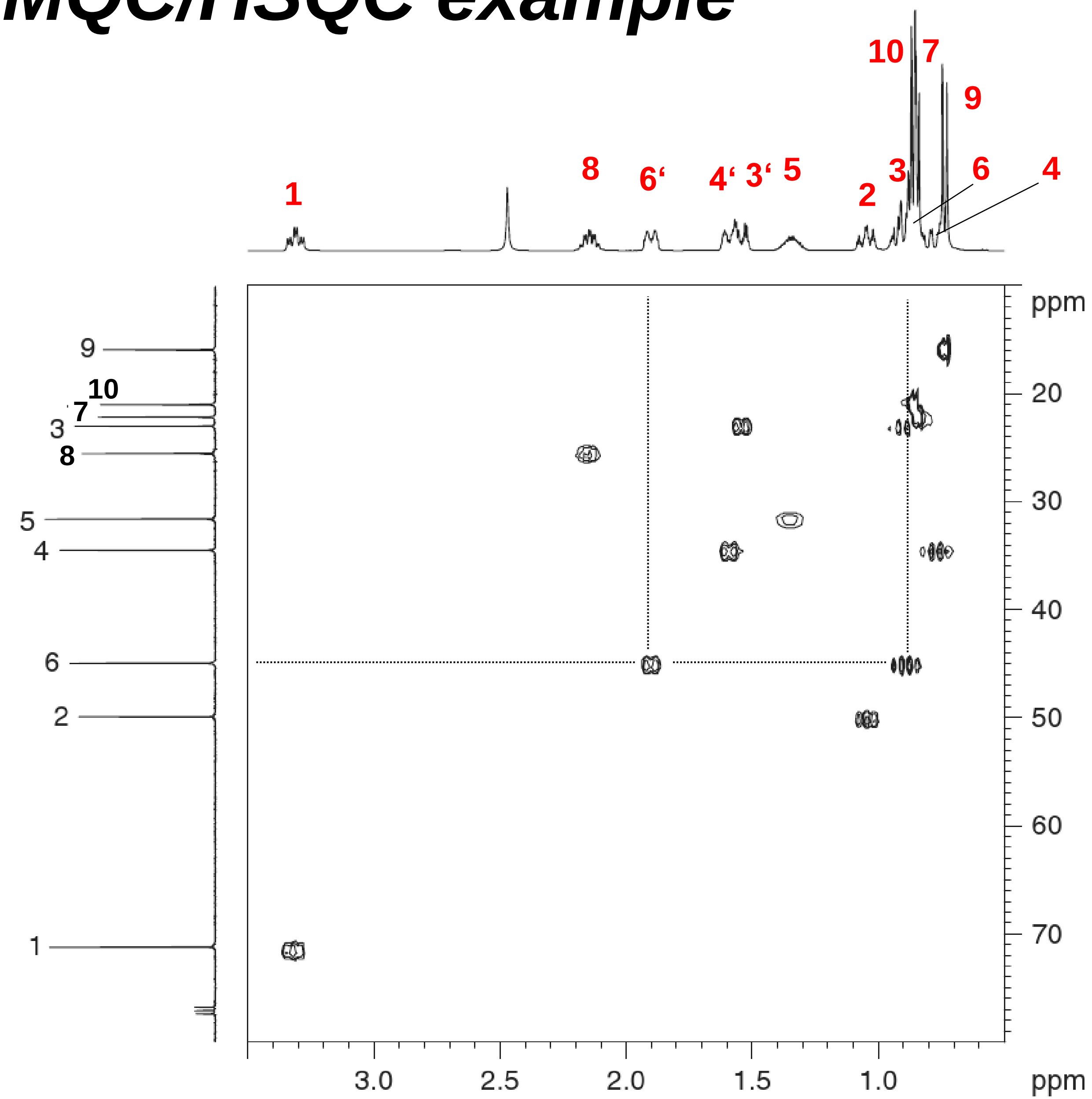
Direct domain Ω_I ***Indirect domain*** Ω_S

signal of nucleus I modulated by chemical shift of nucleus S

HMQC/HSQC example

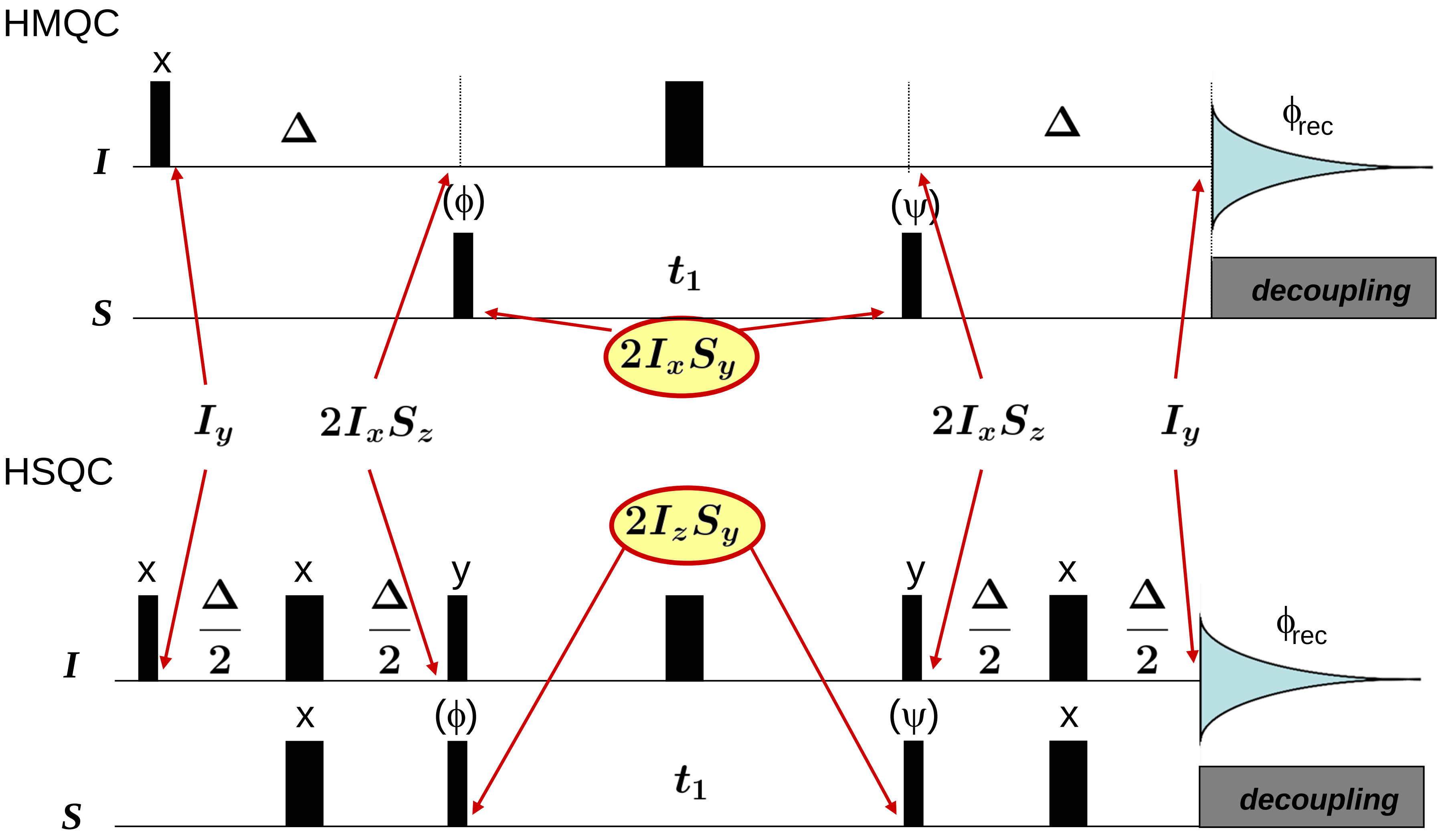


menthol



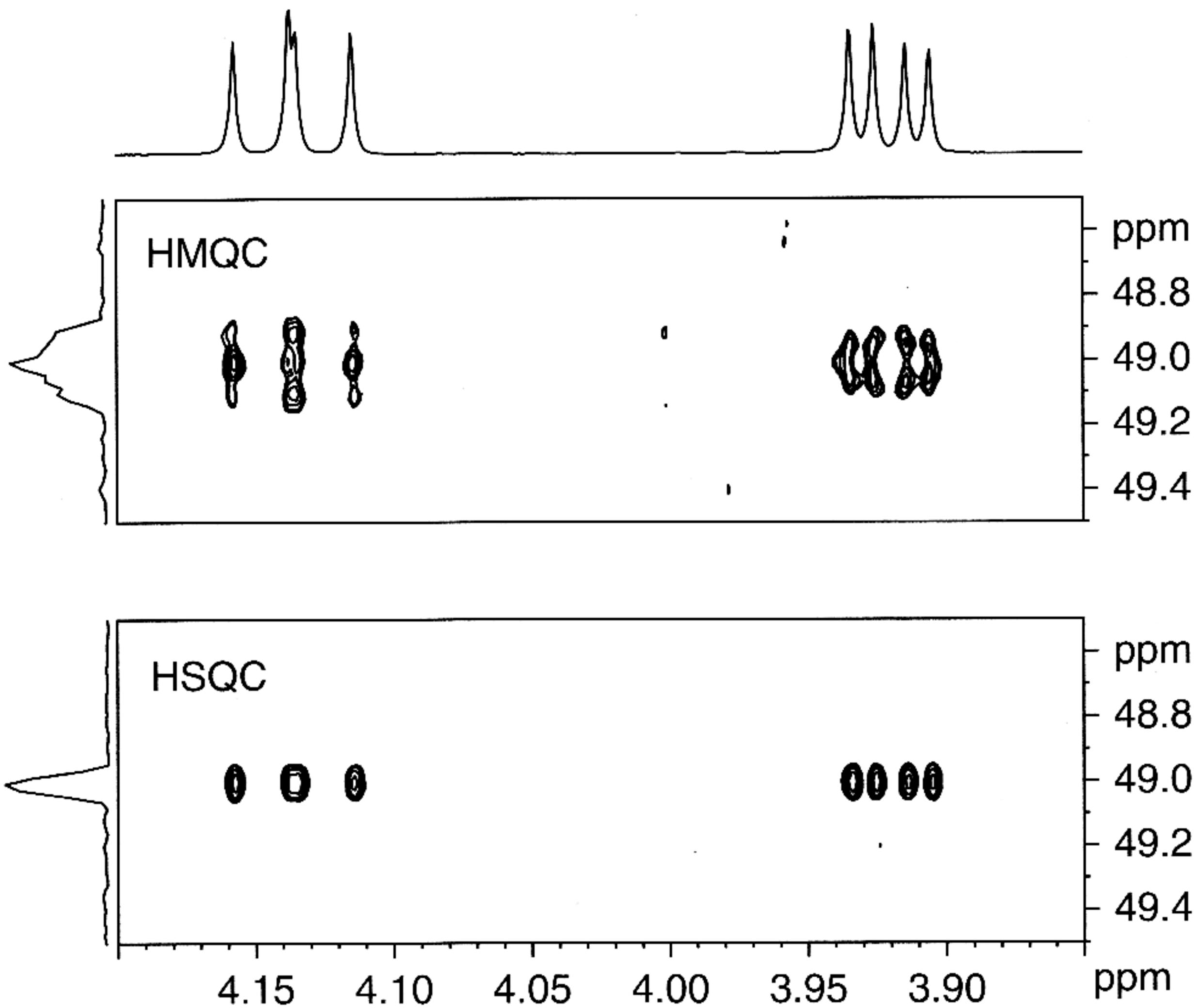
Transfer assignments from ^{1}H onto ^{13}C

Heteronuclear correlation



ϕ = x, -x; ψ = x, x, -x, -x; ϕ_{rec} = x, -x, -x, x

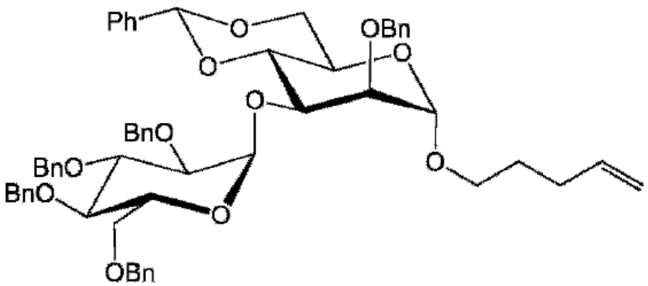
HMQC versus HSQC

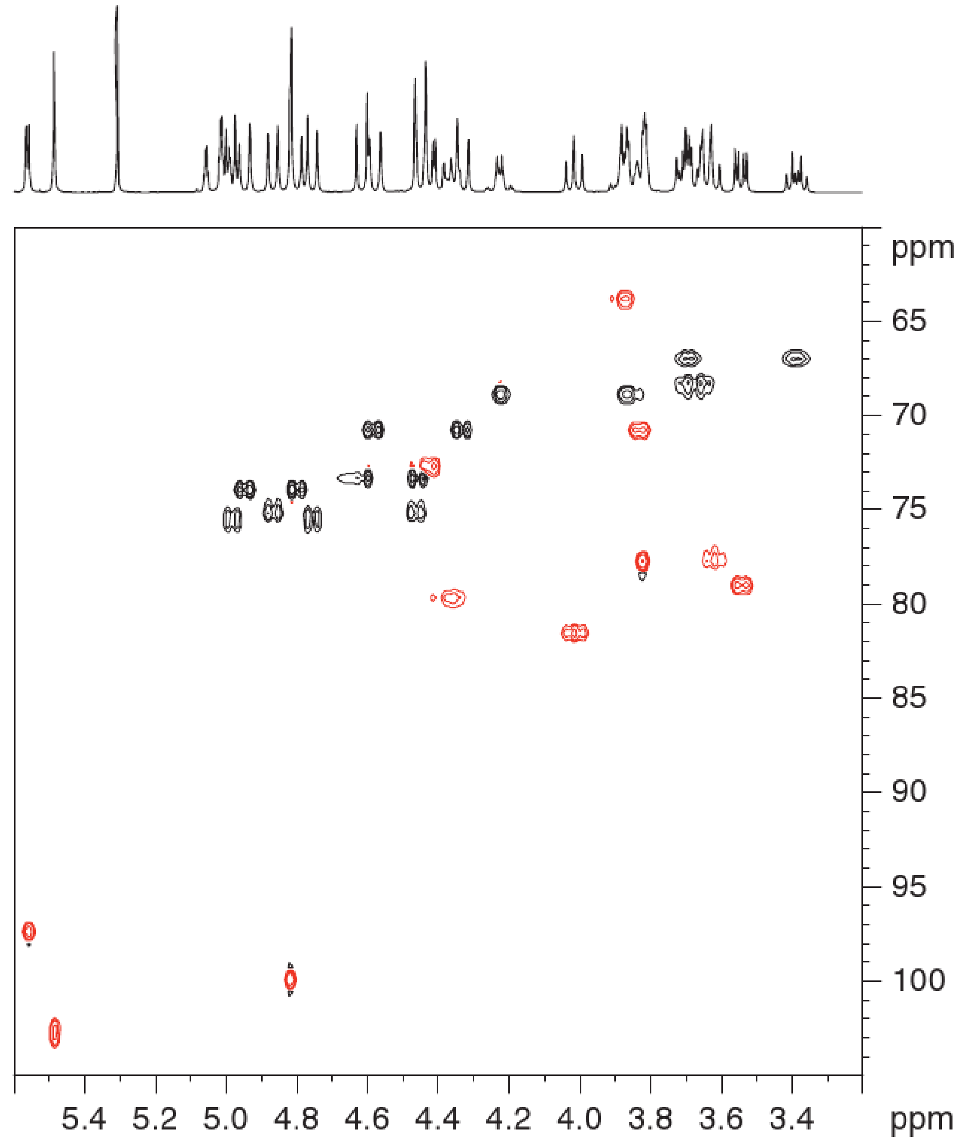


evolution in the indirect domain is influenced by J couplings to other protons

Clean spectrum, improved resolution

Multiplicity edited HSQC





combination of DEPT a HSQC

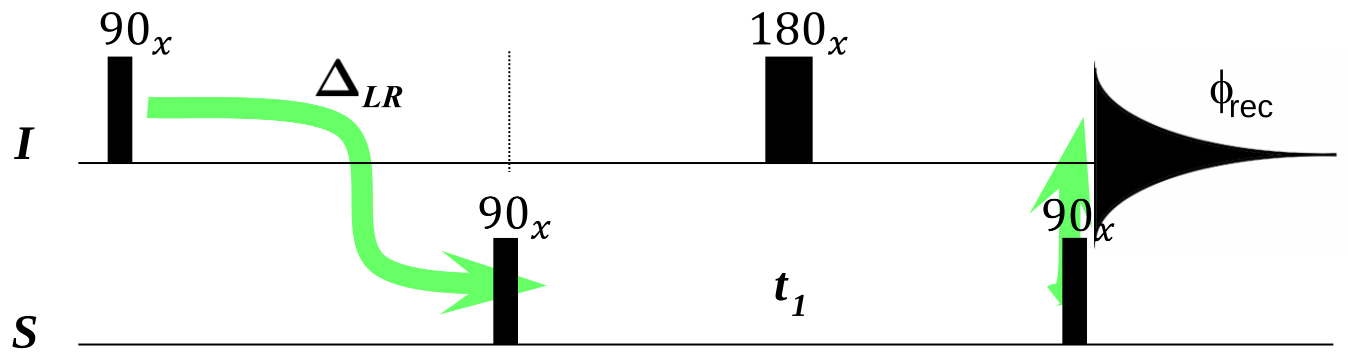
CH a CH_3 **positive**

CH_2 **negative**

HMBC

Heteronuclear Multiple-Bond Correlation

$$\Delta_{LR} = \frac{1}{2J_{LR}}$$



< 8 Hz

> 63 ms

create multiple-quantum Coherences on S

Let them evolve according to chemical shifts

Convert them to single-quantum coherences on I

$$I_z \xrightarrow{I:90_x} -I_y \xrightarrow{\pi J \Delta} 2I_xS_z \xrightarrow[S:90_x]{} -2I_xS_y \xrightarrow{I:180_x} -2I_xS_y \xrightarrow{\Omega_S t_1}$$

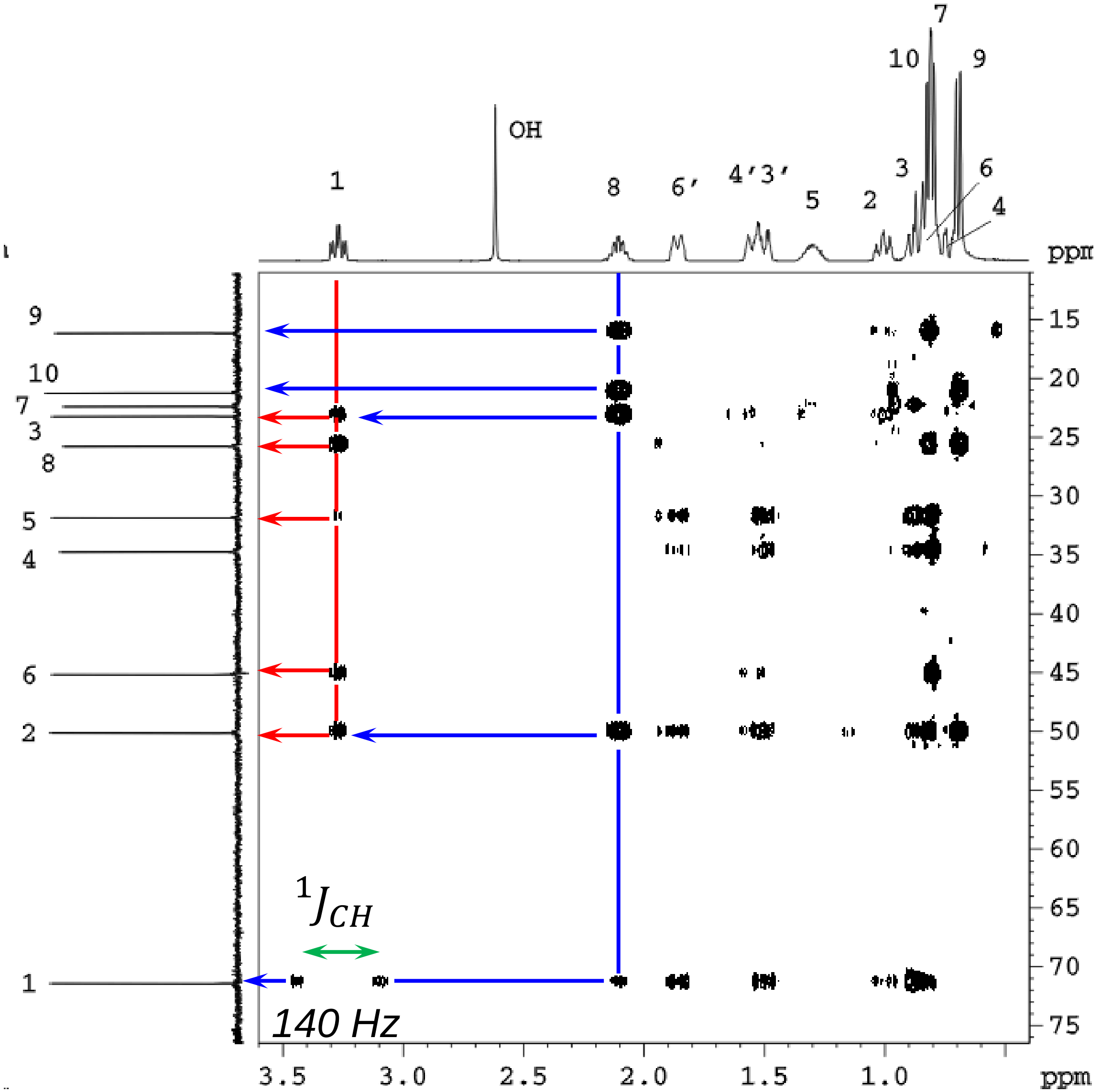
$$-2I_xS_y \cos\Omega_S t_1 + 2I_xS_x \sin\Omega_S t_1 \xrightarrow[S:90_x]{} -2I_xS_z \cos\Omega_S t_1 + 2I_xS_x \sin\Omega_S t_1$$

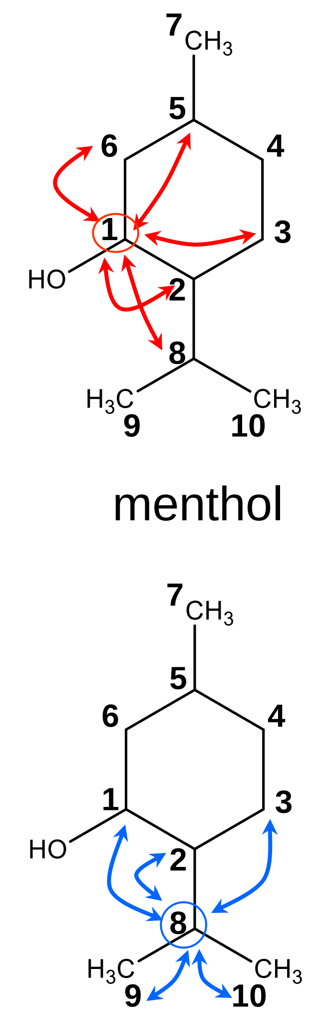
- Relaxation losses
- Refocusation is skipped

- Anti-phase coherence is detected
- Decoupling cannot be used
- magnitude mode

Experiment HMBC

heteronuclear ^{1}H-^{13}C correlation through „2 and more“ chemical bonds

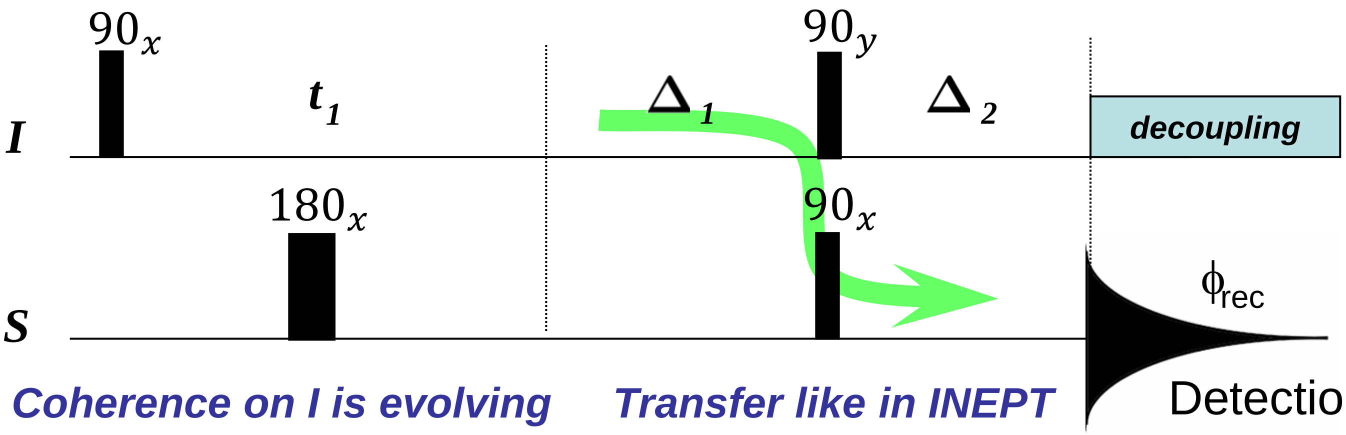




menthol

HETCOR

Heteronuclear correlation



Detection on hetero-nucleus

Coherence on I is evolving according to chemical shift

Transfer like in INEPT + refocusation

Only suitable for sensitive nuclei