Young Belgian Magnetic Resonance Scientist 2011

YBMRS 2011

21st - 23rd NOVEMBER

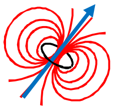
BLANKENBERGE

10TH

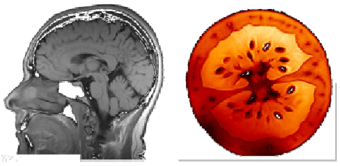
ANNIVERSARY

Educational Session 1	10.30 – 11.30
Short Break	11.30 – 11.40
Educational Session 2	11.40 – 13.00
Lunch	13.00 – 14.00
Opening & Session 1	14.00 – 15.40

MAGNETIC RESONANCE

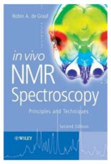


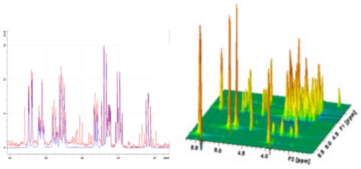
« Imaging » MRI



« High field » NMR Spectroscopy

Liquid (high resolution) state

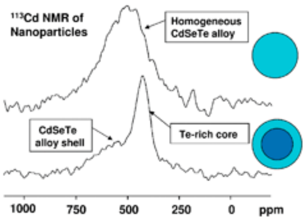




« Low-field » TD NMR

EPR Spectroscopy/Imaging

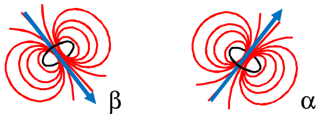
Solid state



Back to basics

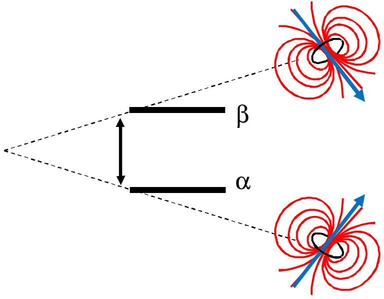
Spin ½

no external magnetic field



both orientations are
energy equivalent
and
equally possible

magnetic field B_0

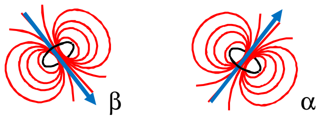


depending on the
orientation of its
magnetic field a
nucleus obtains a
different energy

Back to basics

Spin ½

no external magnetic field



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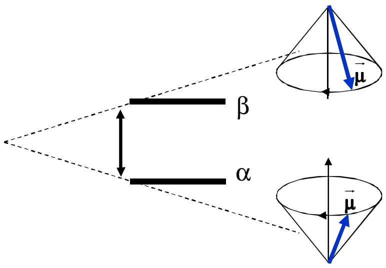


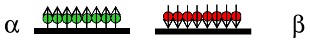
precession around
the external field
with $\omega_0 = \gamma B_0$

Back to basics

Many Spins

no external magnetic field

$\Delta N = 0$

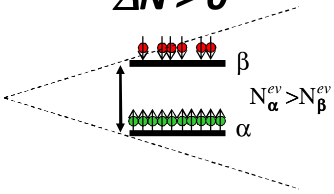


$N_{\alpha}^{ev} = N_{\beta}^{ev}$

both orientations are
energy equivalent
and
equally possible

magnetic field B_0

$\Delta N > 0$



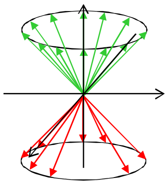
$N_{\alpha}^{ev} > N_{\beta}^{ev}$

depending on the
orientation of its
magnetic field a nucleus
obtains a different
energy

Back to basics

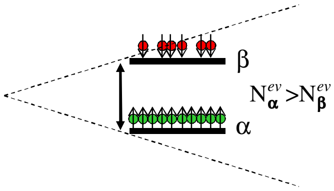
Many Spins

precession



Snapshot
orientation defined only
in the direction // B0

magnetic field B_0



$N_{\alpha}^{ev} > N_{\beta}^{ev}$

depending on the
orientation of its
magnetic field a nucleus
obtains a different
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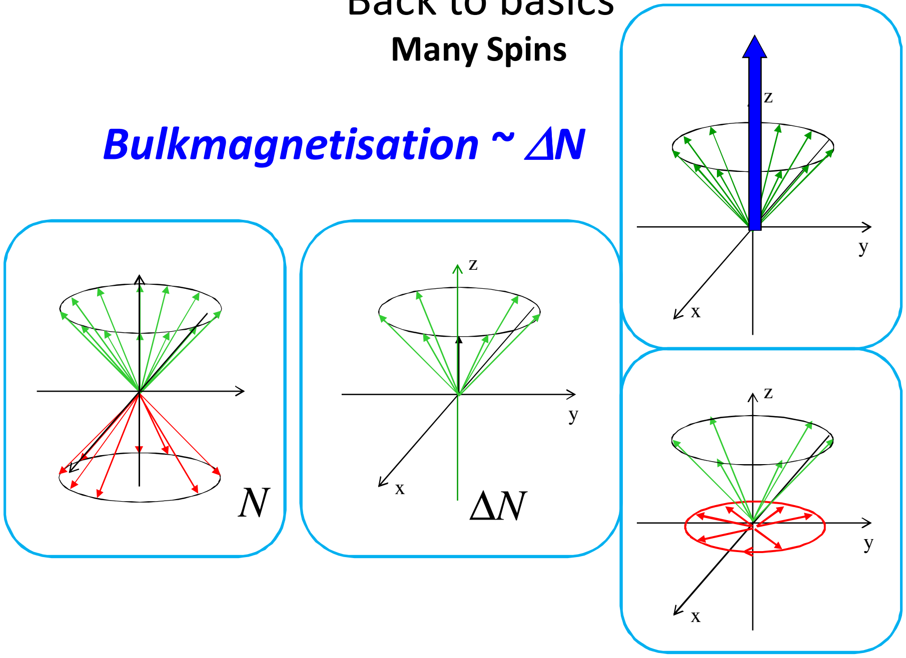
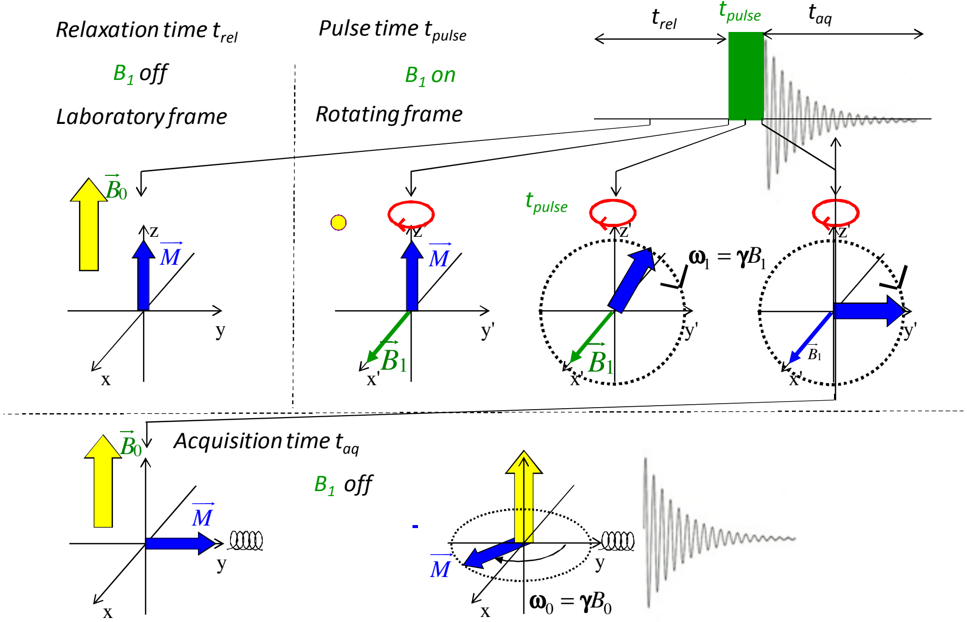
Back to basics

Many Spins

Bulkmagnetisation ~ ΔN



The NMR measurement



Relaxation time t_{rel}

B_1 off

Laboratory frame

Pulse time t_{pulse}

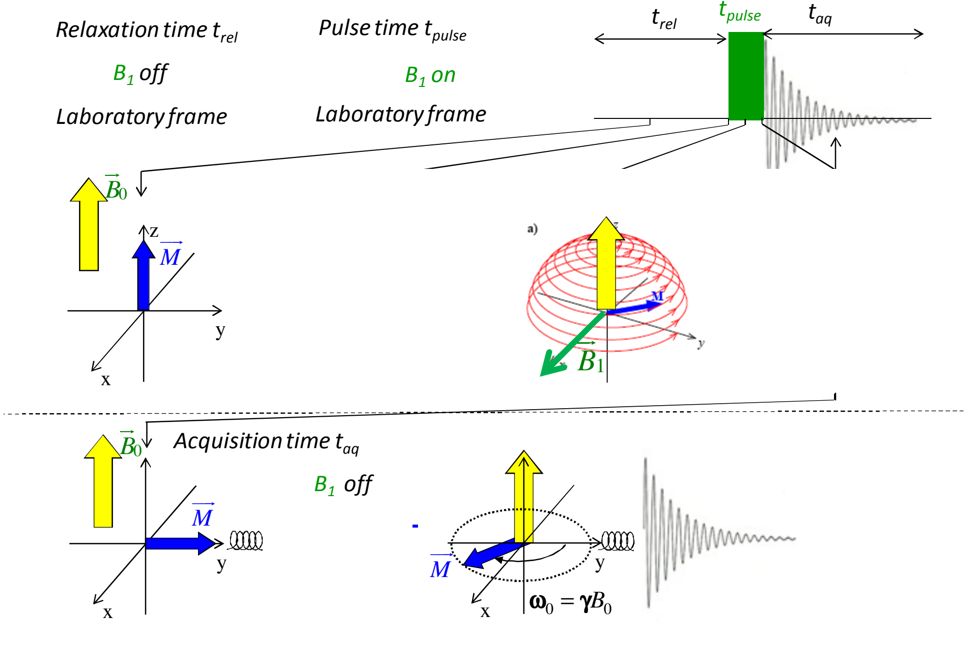
B_1 on

Rotating frame

Acquisition time t_{aq}

B_1 off

The NMR measurement



Relaxation time t_{rel}

B_1 off

Laboratory frame

Pulse time t_{pulse}

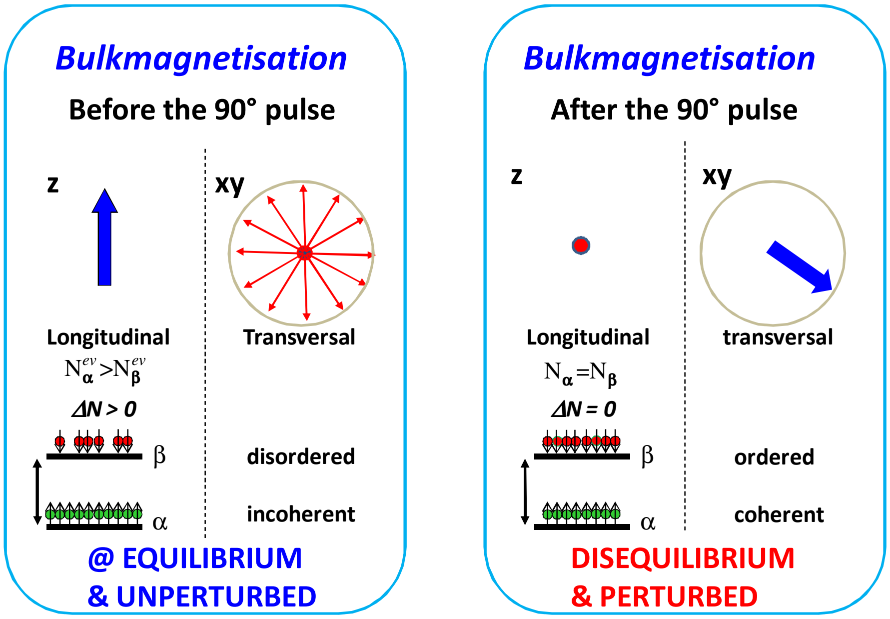
B_1 on

Laboratory frame

Acquisition time t_{aq}

B_1 off

NMR relaxation :



Bulkmagnetisation

Before the 90° pulse

z

xy

Longitudinal

$N_\alpha^{ev} > N_\beta^{ev}$

ΔN > 0

Transversal

β

α

disordered

incoherent

@ EQUILIBRIUM
& UNPERTURBED

Bulkmagnetisation

After the 90° pulse

z

xy

Longitudinal

$N_\alpha = N_\beta$

ΔN = 0

transversal

β

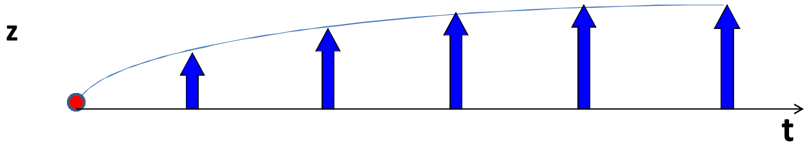
α

ordered

coherent

DISEQUILIBRIUM
& PERTURBED

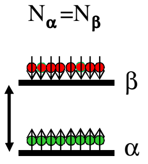
NMR relaxation : return to equilibrium



Longitudinal relaxation

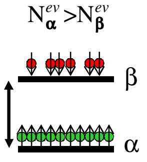
M$_z$ is the bulkmagnetisation at any time t → M_z^t
M$_0$ is the bulkmagnetisation at equilibrium (t = ∞) → M_z^{eq}

$$\frac{dM_z^t}{dt} = R_1\left(M_z^{eq} - M_z^t\right) \qquad M_z^t = M_z^{eq}\left(1 - pe^{-R_1 t}\right) \qquad R_1 = (T_1)^{-1}$$

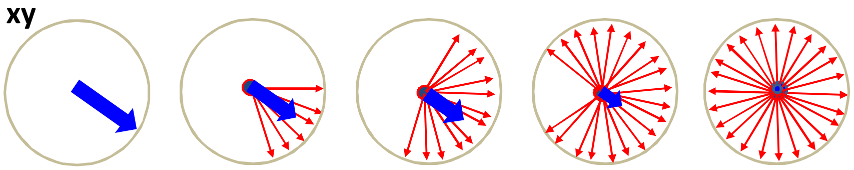


T_1 relaxation
Enthalpic process

Exchange of energy with the surroundings (lattice)



NMR relaxation : return to equilibrium



Transversal relaxation

$$\frac{dM_{x,y}^t}{dt} = -R_2 M_{x,y}^t \qquad M_{x,y}^t = M_{x,y}^0 e^{-R_2 t} \qquad R_2 = (T_2)^{-1}$$

ordered

coherent

loss of order
loss of coherence

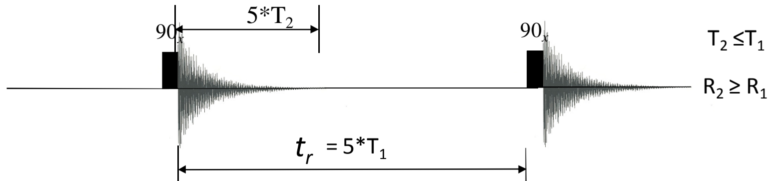
disordered

incoherent

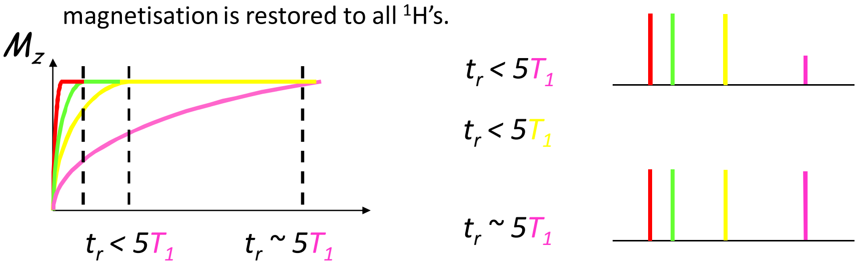
T_2 relaxation
Entropic process
Mutual exchange of energy (spin-spin)
Can be caused by chemical exchange as well!

Importance of T_1 and T_2 for spectroscopy

Full T_1 relaxation is essential for a quantitative response (integrations)



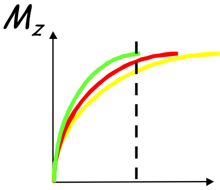
Since each ^1H in a molecule is characterized by a different T_1, one should wait a time t_r equal to 5 times the longest T_1 before equilibrium magnetisation is restored to all ^1H's.



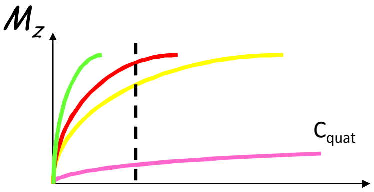
Importance of T_1 and T_2 for spectroscopy

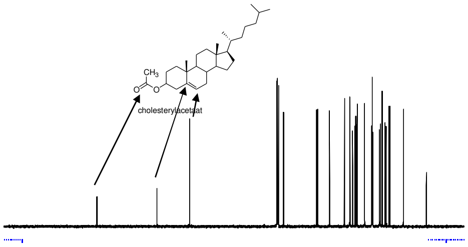
Full T_1 relaxation is essential for a quantitative response (integrations)

^1H NMR, T_1's are generally similar and fairly short



^{13}C NMR, T_1's are generally dissimilar and can be very long



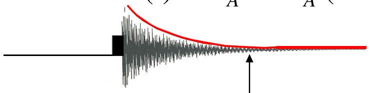


Importance of T_1 and T_2 for spectroscopy

The NMR signal attenuates exponentially with time

$$s(t) \sim M_A \cos\omega_A t(e^{-t/T_2})$$

Small molecules : T_2 ~ 0.2 to 2s



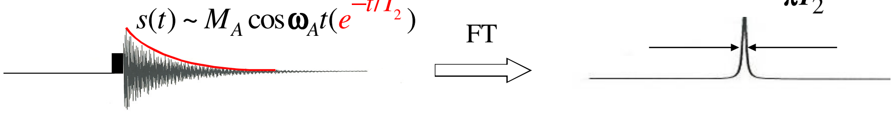
Beyond a time equal to 3 times T_2 88% of the signal has dissipated and mostly noise is measured

The value of T_2 thus determines the time period during which the signal should be monitored

T_2 relaxation determines the resonance line width

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2}$$

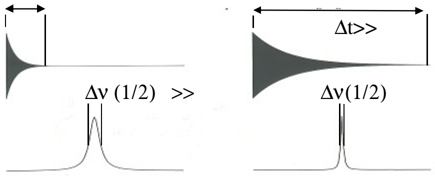
$$s(t) \sim M_A \cos\omega_A t(e^{-t/T_2})$$

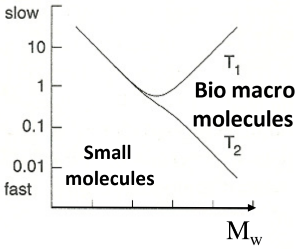


As the value of T_2 shortens, the resonance line will broaden

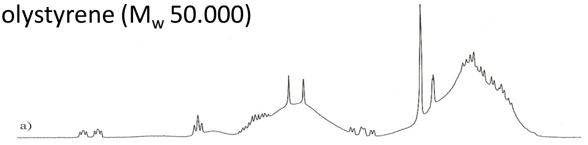
Importance of T_1 and T_2 for spectroscopy

$R_2 = (T_2)^{-1}$ scales linearly with the molecular weight Mw





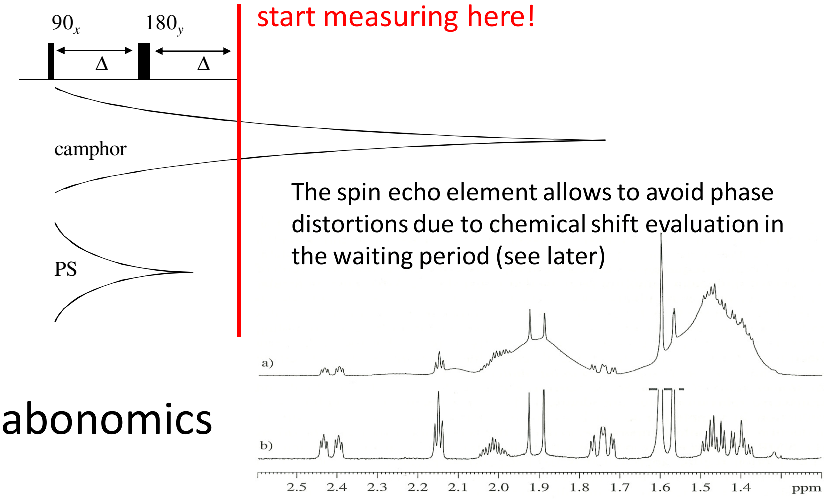
Camphor + polystyrene (M_w 50.000)



As the molecular weight increases, the lines become much broader, affecting resolution and information contents

Importance of T_1 and T_2 for spectroscopy

Spin echo sequence



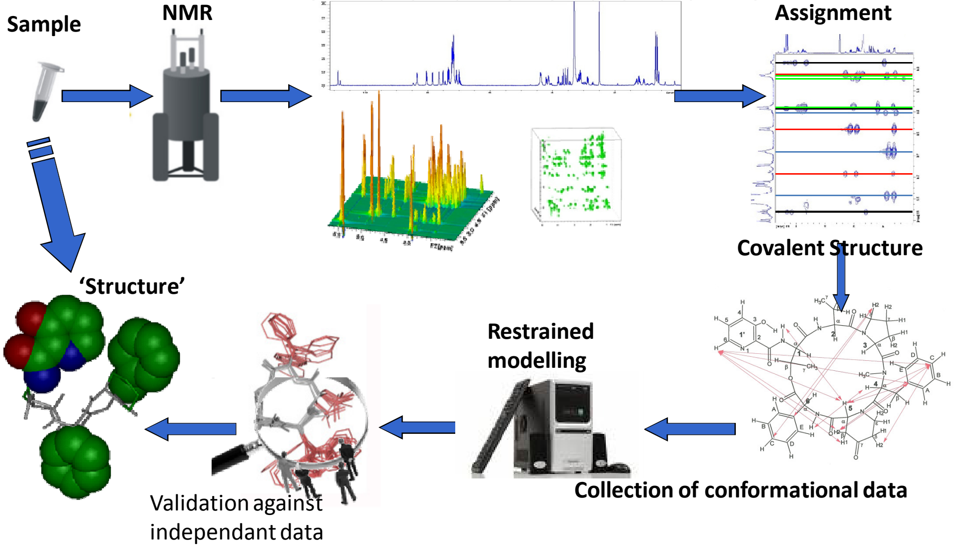
start measuring here!

The spin echo element allows to avoid phase distortions due to chemical shift evaluation in the waiting period (see later)

Metabonomics

Polystyrene with Mr 50.000 and camphor, T_2 PS ~ 5ms << T_2 camphor ~0.5s

NMR: Structure elucidation



Sample

NMR

Assignment

Covalent Structure

Collection of conformational data

Restrained modelling

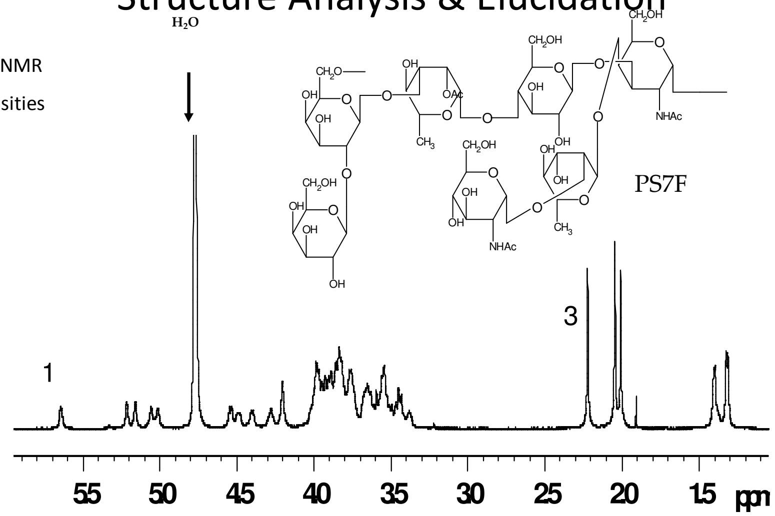
Validation against independant data

'Structure'

Structure Analysis & Elucidation

1D ^{1}H NMR

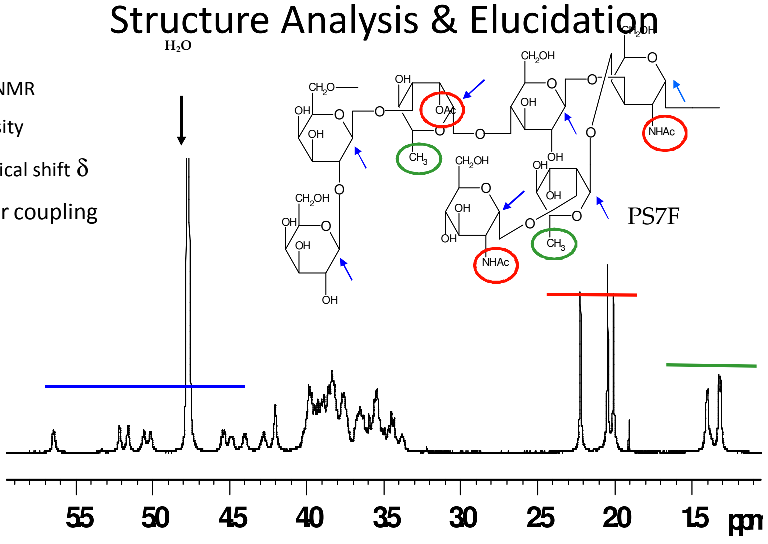
- Intensities



Structure Analysis & Elucidation

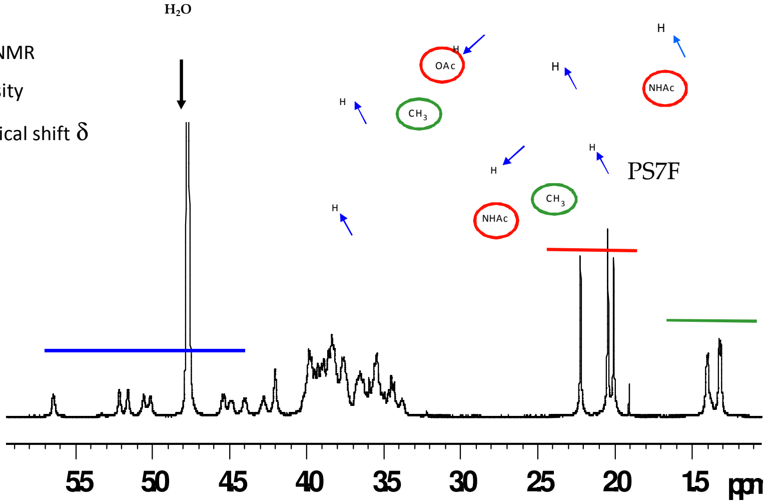
1D ^{1}H NMR

- Intensity
- Chemical shift δ
- Scalar coupling



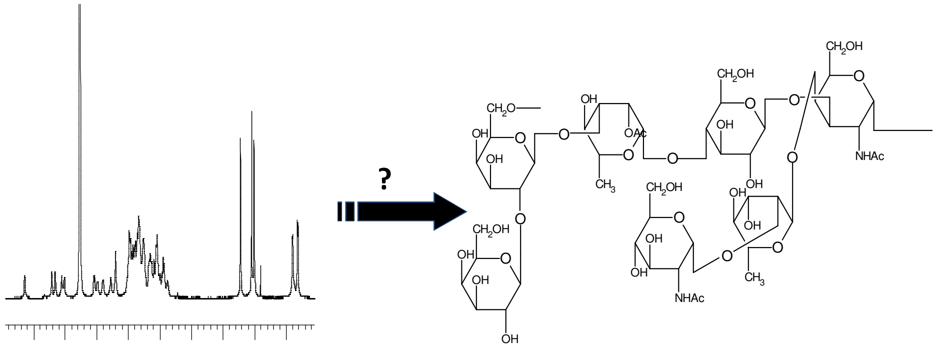
Structure Analysis & Elucidation

1D ^1H NMR
- Intensity
- Chemical shift δ



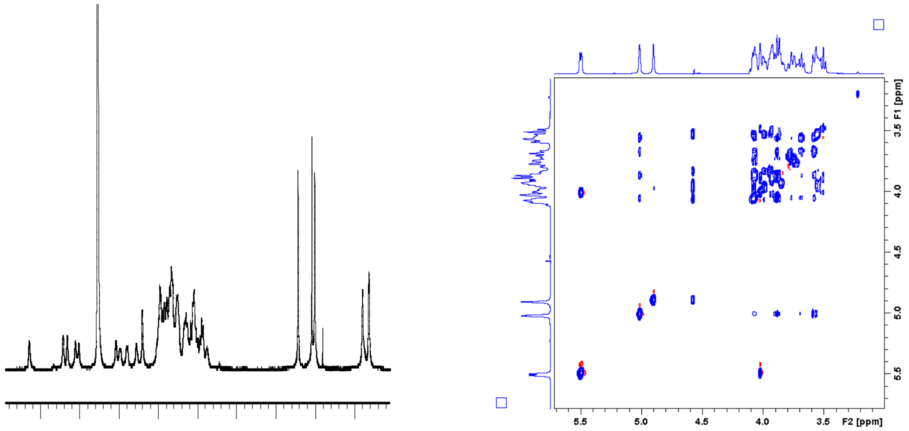
Structure Analysis & Elucidation

Collection of ^1H and ^{13}C signals



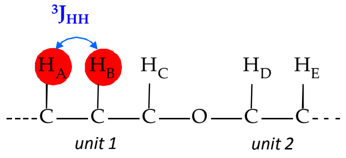
Covalent Structure/Conformation

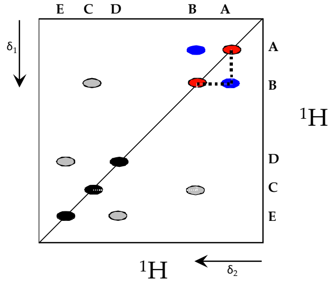
Structure Analysis & Elucidation



Structure Analysis & Elucidation

Scalar coupling interaction between chemically nearby ^{1}Hs



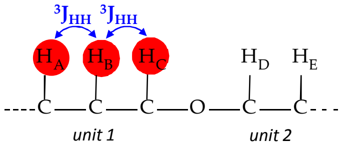


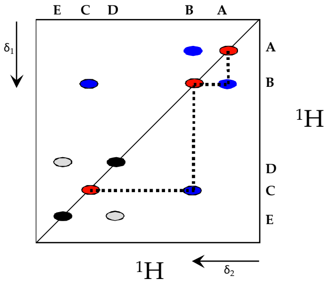
^{1}H-^{1}H COSY : Correlating neighbouring hydrogens

- Individual ^{1}H's must be within three chemical bonds
- Only ^{1}H's from the same unit are correlated due to the glycosidic links

Structure Analysis & Elucidation

Scalar coupling interaction between chemically nearby ^{1}Hs





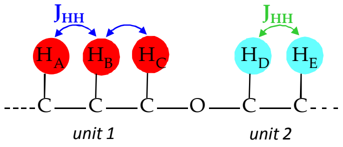
^{1}H-^{1}H COSY : Correlating neighbouring hydrogens

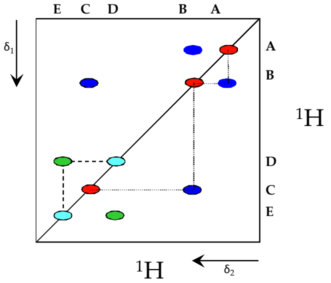
— **Individual ^{1}H's must be within three chemical bonds**
— **The glycosidic links interrupt the correlation sequence**

→ **Only ^{1}H's from the same unit are correlated due to the glycosidic links**

Structure Analysis & Elucidation

Scalar coupling interaction between chemically nearby ^{1}Hs





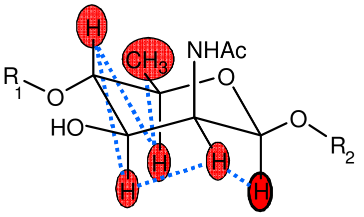
^{1}H-^{1}H COSY : Correlating neighbouring hydrogens

— **Individual ^{1}H's must be within three chemical bonds**
— **The glycosidic links interrupt the correlation sequence**

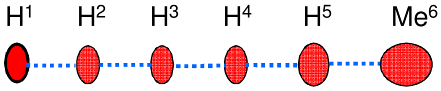
→ **Only ^{1}H's from the same unit are correlated due to the glycosidic links**

Structure Analysis & Elucidation

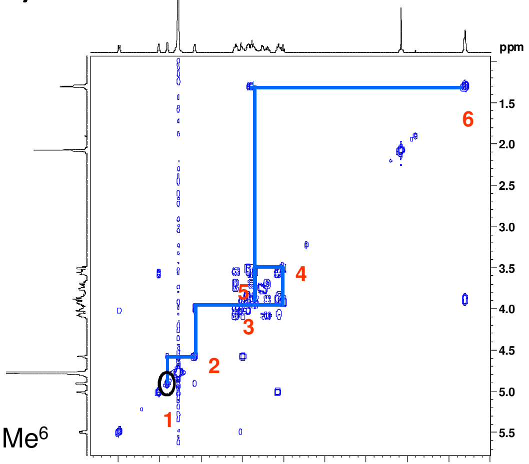
COSY



Which ^{1}Hs belong to the same unit?



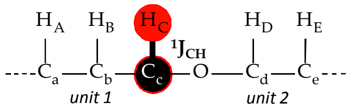
Information on carbon skeleton incomplete

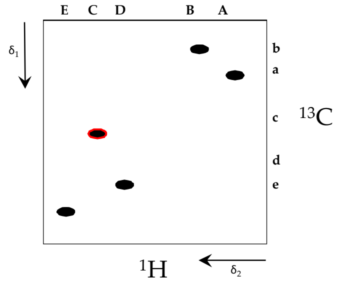


Also TOCSY

Structure Analysis & Elucidation

Scalar coupling interaction between directly bonded ^{1}H and ^{13}C



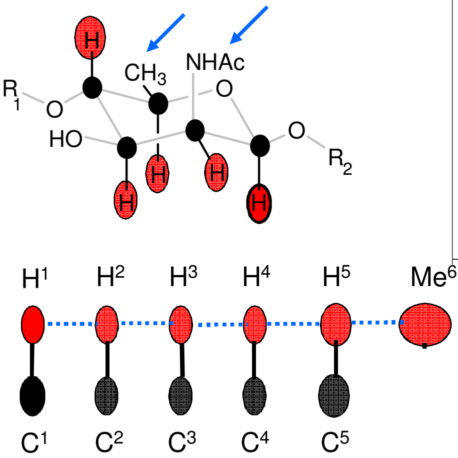


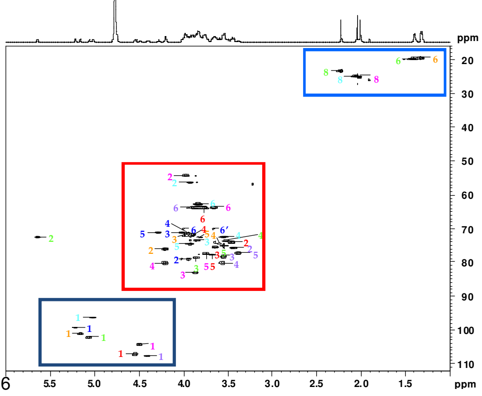
^{1}H-^{13}C HSQC : Single Bond Correlation

- — Establishing C—H bonding pairs in the molecule
- — No quaternary carbons
- — CH_3, CH_2, CH can be independantly established (meHSQC)

Structure Analysis & Elucidation

^{1}H-^{13}C HSQC PS7F

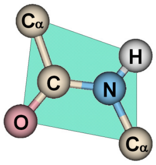


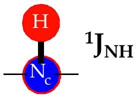


Basic elements of the carbon skeleton of the saccharide units identified but exact linkage still unknown

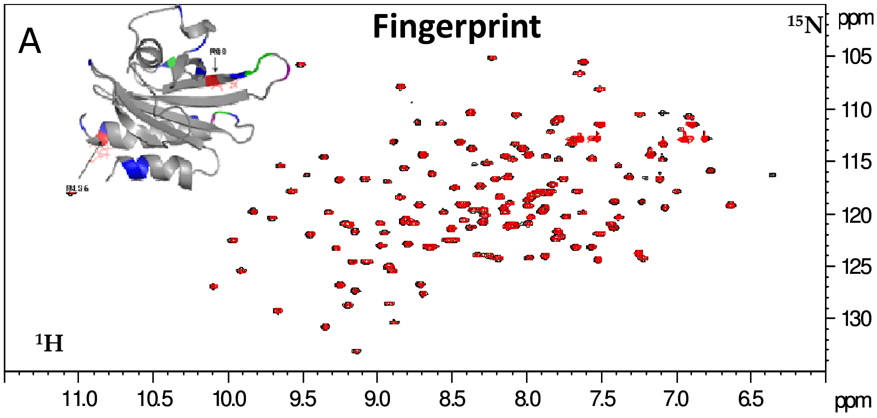
Structure Analysis & Elucidation

^{1}H-^{15}N HSQC: the protein NMR workhorse



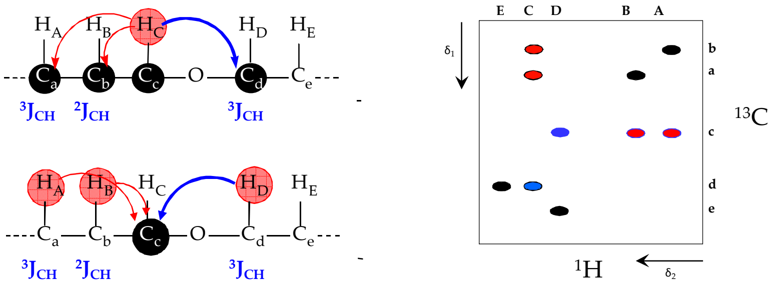


Human profilin (139 amino acids)



Structure Analysis & Elucidation

Scalar coupling interaction between nearby ^{1}Hs and ^{13}Cs

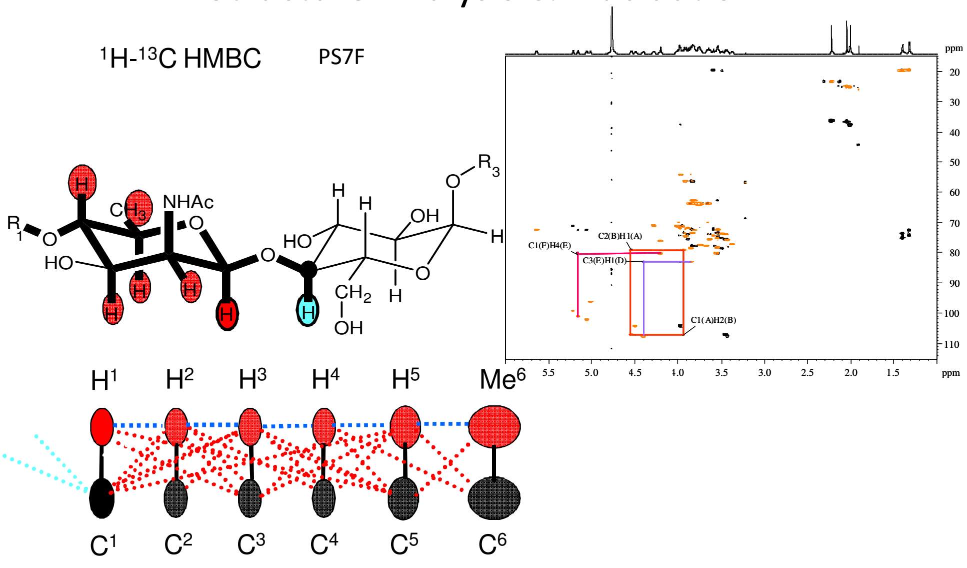


^{1}H-^{13}C HMBC : Multiple Bond Correlation

- Quaternary carbons identified
- Allows the immediate vicinity of the carbon skeleton to be inferred
- Connections across glycosidic linkages established

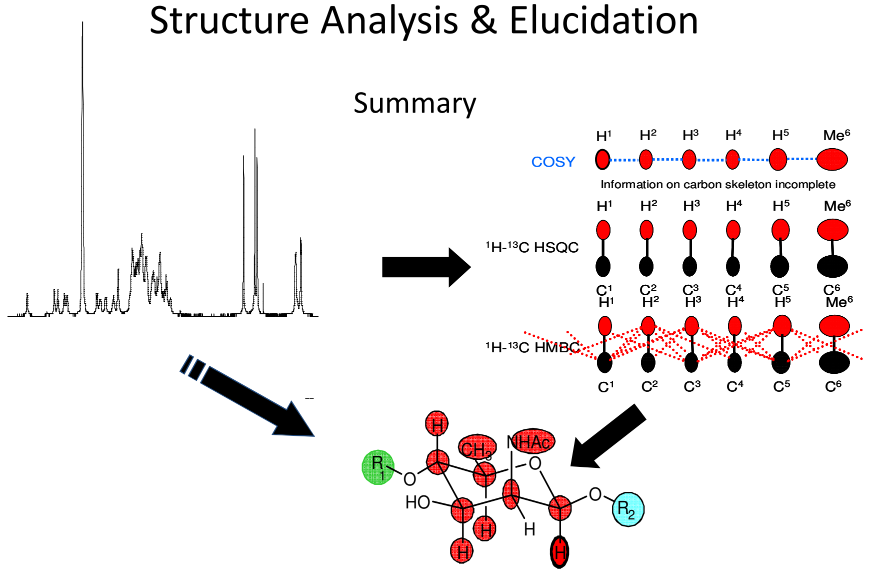
Structure Analysis & Elucidation

^{1}H-^{13}C HMBC PS7F



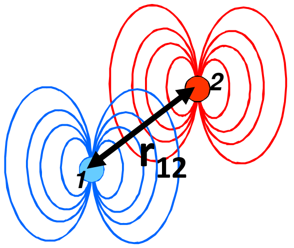
Structure Analysis & Elucidation

Summary



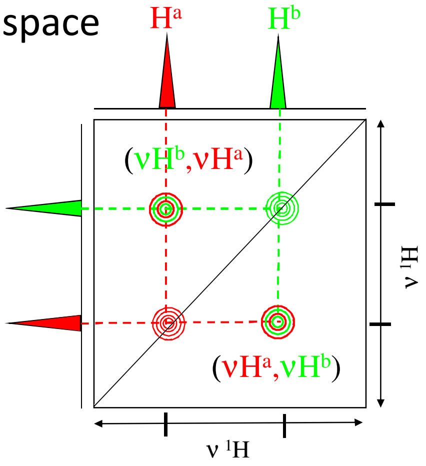
Structure Analysis & Elucidation
The NOE effect: an alternative coupling through space

NOESY/ROESY



$$I_{NOE1,2} = k(r_{1,2})^{-6}$$

Since I_{NOE} ~r^{-6}, only inter proton distances < 5Å canbe measured!



NOE between
A and B

Each cross-peak results from an inter ^{1}H distance shorter than 5A

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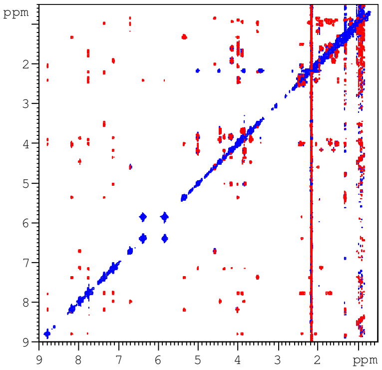
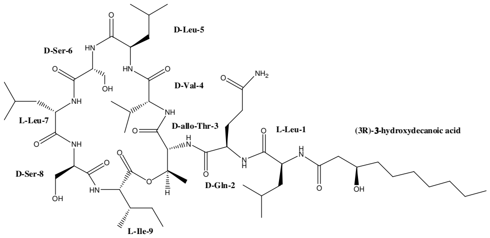
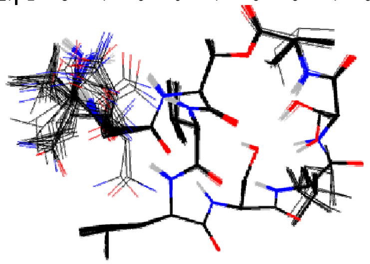
NOESY/ROESY





Distribution of 127 distance restraints

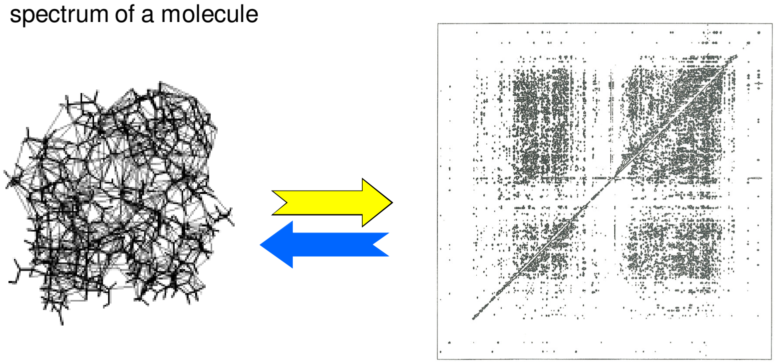
	Alk	Leu1	Gln2	Thr3	Val4	Leu5	Ser6	Leu7	Ser8	Ile9	Total
intra	4	5	6	3	7	11	8	6	8	12	**70**
inter i-->i±1	4	7	6	13	8	7	4	4	8	9	**35**
inter I-->>i±1	2	3	1	6	9	1	6	6	4	6	**22**



Structure Analysis & Elucidation

The NOESY spectrum as the basis for solution structure determination

The molecular structure, defined at the level of a dense network of inter hydrogen distances, is thus encoded in the 2D NOESY spectrum of a molecule



NMR based structure determination consists in extracting the dense network of distances from the NOESY spectrum and computing the corresponding 3D structure