Nuclear Magnetic Resonance (NMR)

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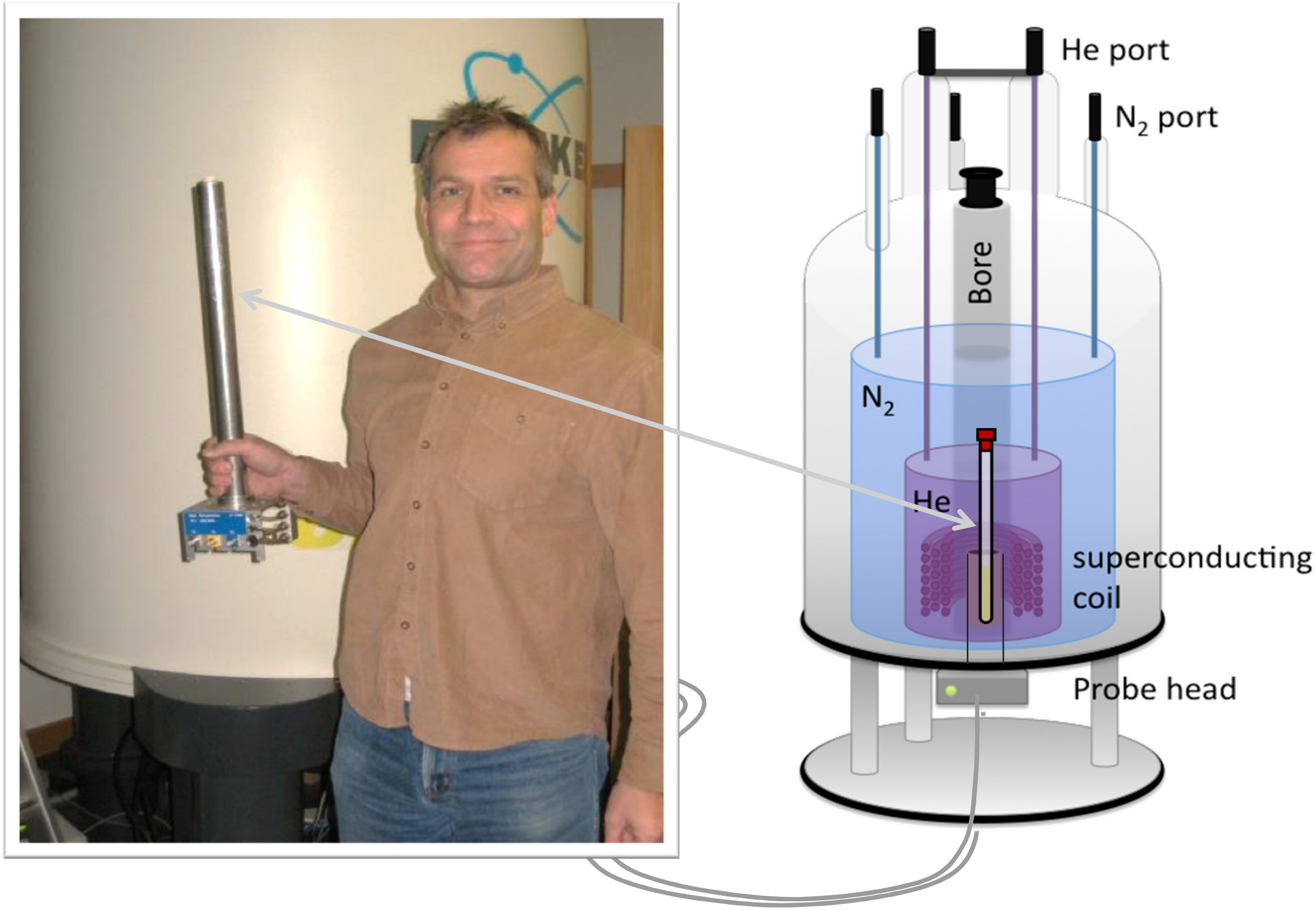
FOR POLYMER RESEARCH

Max Planck Institute for Polymer Research
NMR lecture Manfred Wagner

Nuclear Magnetic Resonance (NMR)



Nuclear Magnetic Resonance (NMR)

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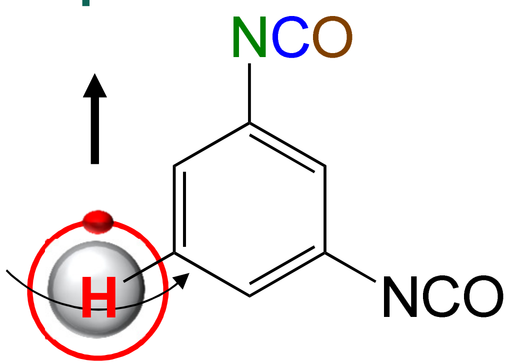
nuclear dipole ^{1}H has a spin number of I = 1/2

magnetic dipole



spinning charge

magnetic dipole

in a magnetic field

$\uparrow B_0$

higher in energy

↓

spin down aligned against B_0

β-state = -½

↑

spin up aligned with B_0

lower in energy

α-state = +½

nuclear dipole with spin number I = ½ are also ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P.....

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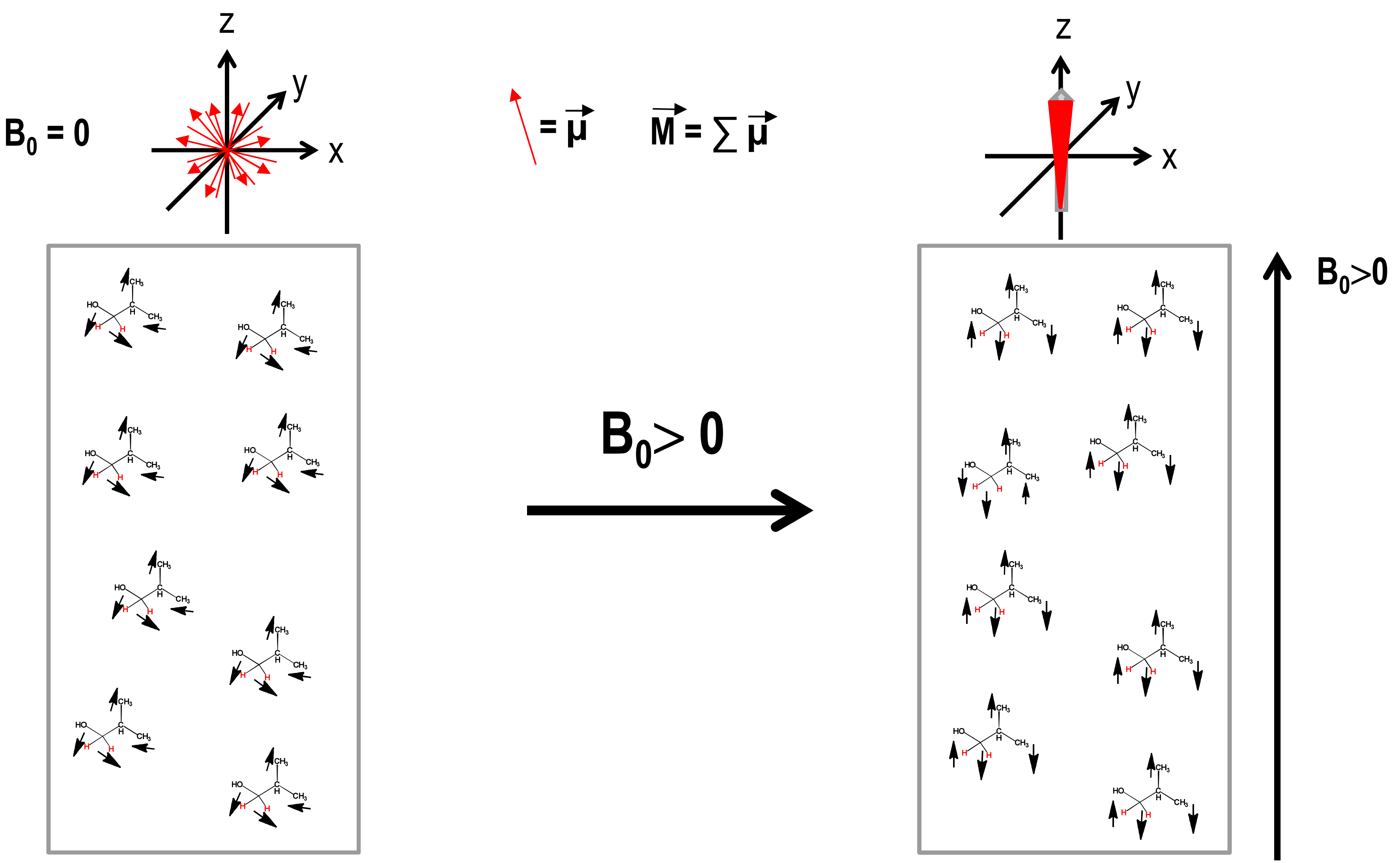

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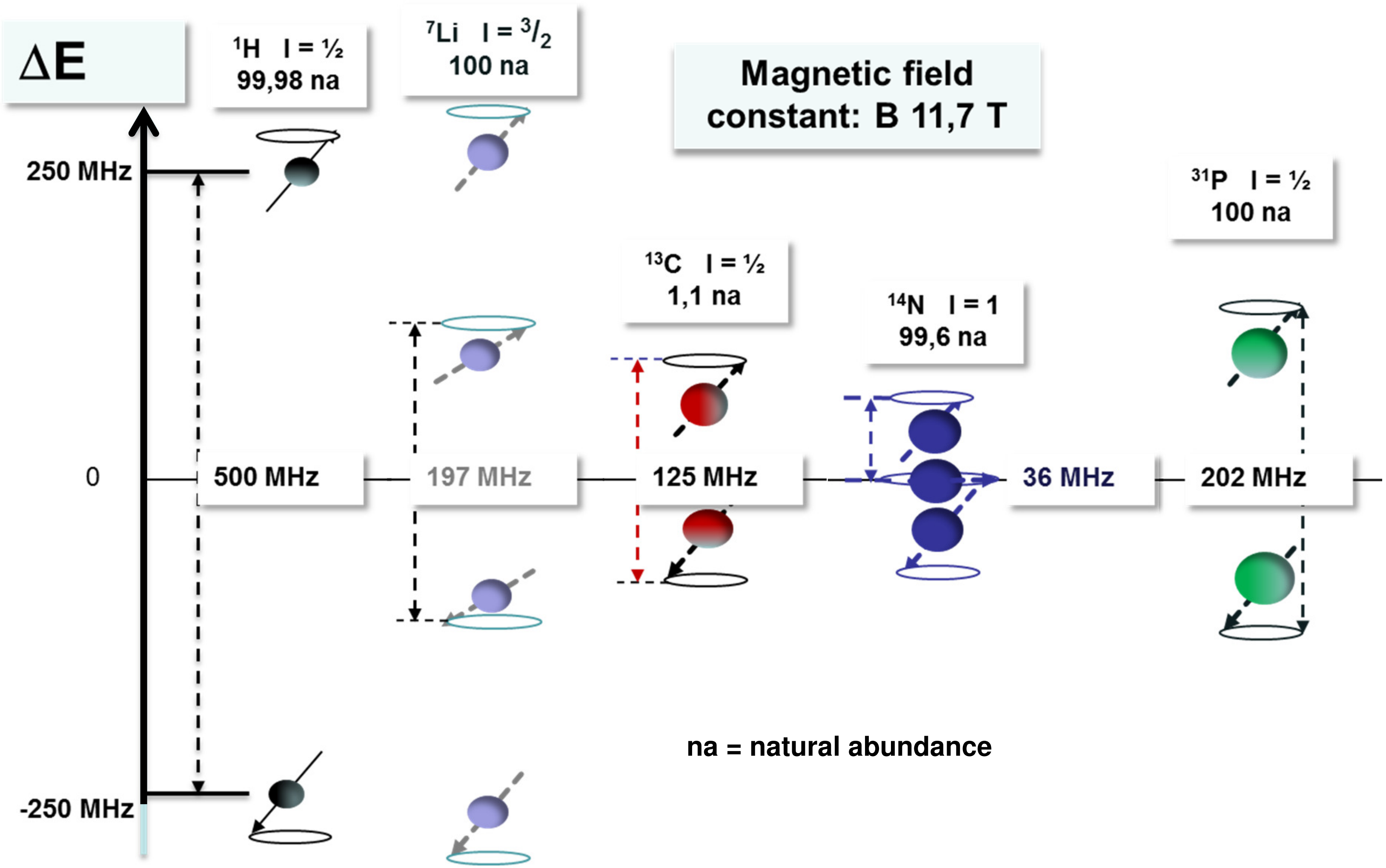

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B_0-Field strength	^{1}H	^{19}F	^{31}P	^{13}C	^{29}Si	^{15}N
2.34 T	100 MHz	94 MHz	40.5 MHz	25.2 MHz	19.9 MHz	10.2 MHz
11.74 T	500 MHz	470 MHz	202 MHz	125.7 MHz	99.4 MHz	50.7 MHz
23.4 T	1000 MHz	940 MHz	405 MHz	252 MHz	199 MHz	102 MHz

MHz

	^{1}H	^{19}F	^{31}P	^{13}C	^{29}Si	^{15}N
natural abundance (na)	99,988	100	100	1,07	4,685	0,364

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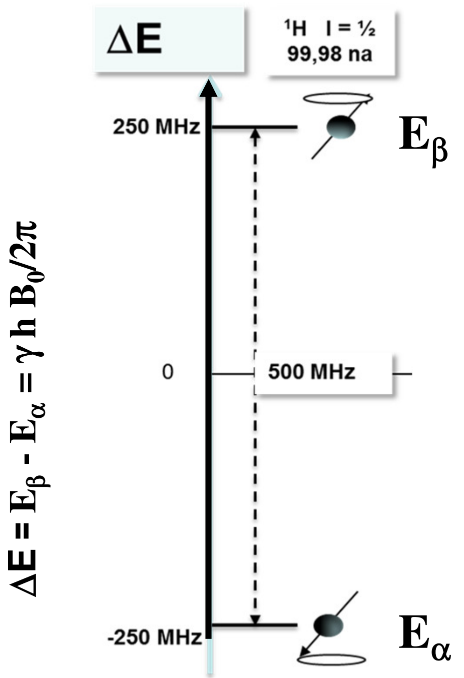
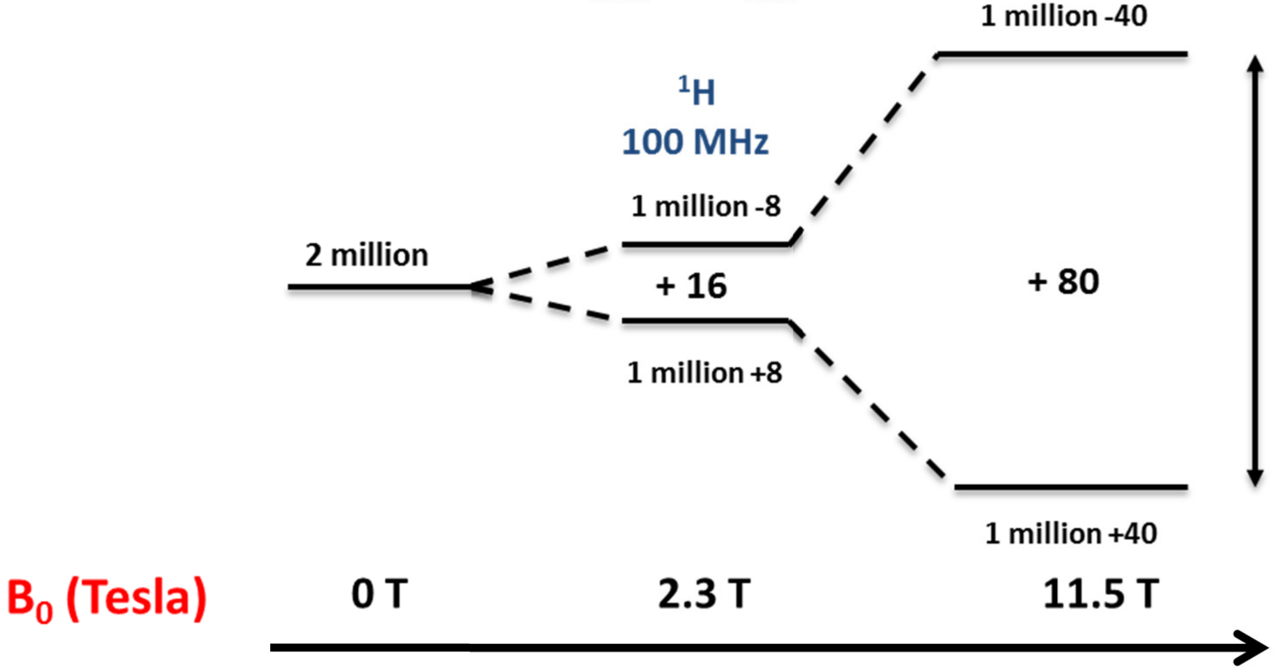
$$\frac{N_\beta}{N_\alpha} = e^{-\Delta E/k_B T} \approx 1 - \frac{\Delta E}{k_B T} = 1 - \frac{\gamma \hbar B_0}{k_B T}$$





k_B = Boltzmann constant = 1,3805 10^{-23}JK^{-1}
T = absolute temperature in K
h = h/2π = 6,6256 10^{-34}Js
B_0 = magnetic flux density in Tesla
γ = magnetogyric relation (for ^{1}H) = 26,7522 10^7 rad T^{-1}s^{-1}

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One example to calculate the sensitivity

$$\frac{^{1}H}{^{13}C} = \frac{(\gamma_{^{1}H})^3}{(\gamma_{^{13}C})^3} = \frac{(26{,}7513)^3}{(6{,}7262)^3} = (3{,}977)^3 = \sim 64$$

$$\frac{^{1}H}{^{13}C} = \frac{99{,}98}{1{,}07} \times \sim 64 = \sim 5900$$

factor related to na

Nuclear Magnetic Resonance (NMR)

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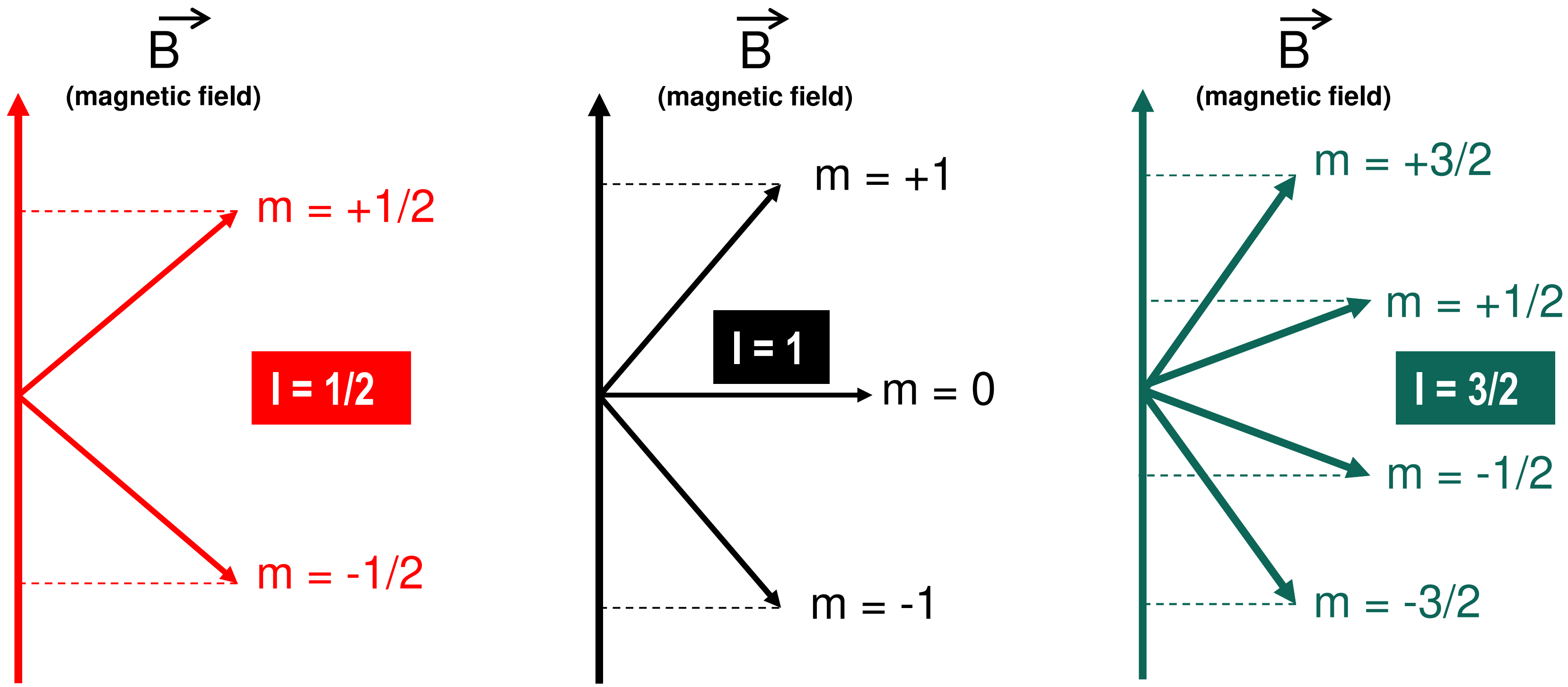

I = 1/2

^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{29}Si, ^{31}P…

I = 1,2,3..

^{2}H, ^{6}Li, ^{10}B, ^{14}N…

I = 3/2, 5/2..

^{7}Li, ^{9}Be ^{11}B, ^{17}O, ^{23}Na, ^{25}Mg, ^{27}Al...

m = I, I-1, I-2, …, -I   => 2I+1 possibilities

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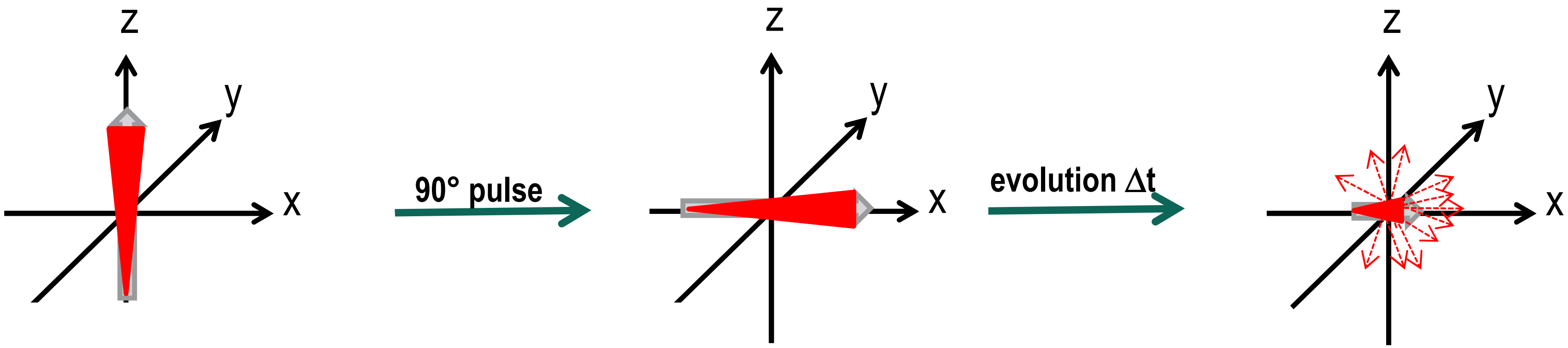

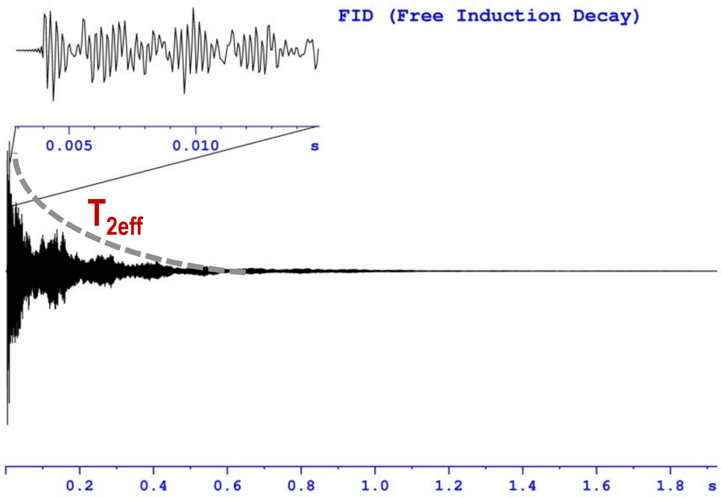

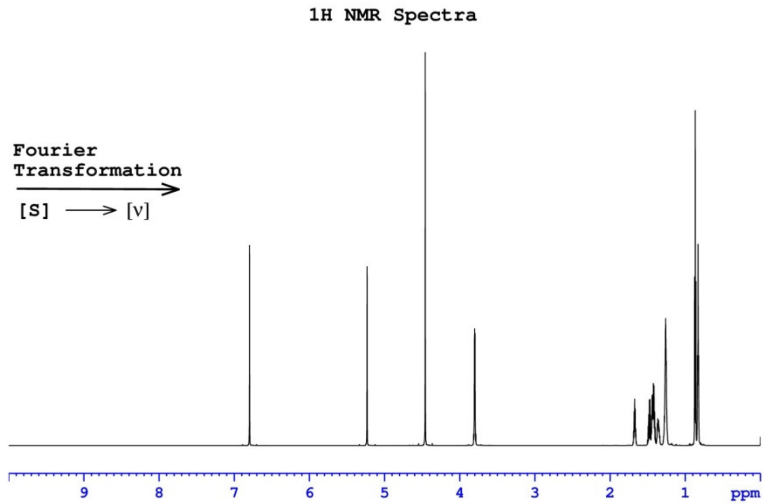

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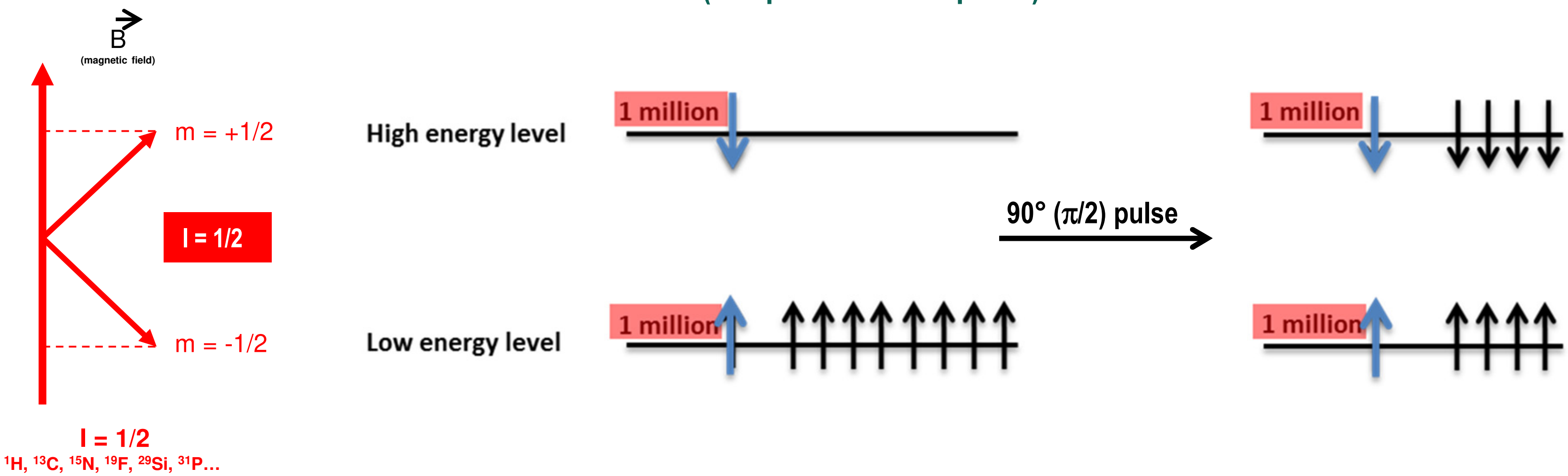
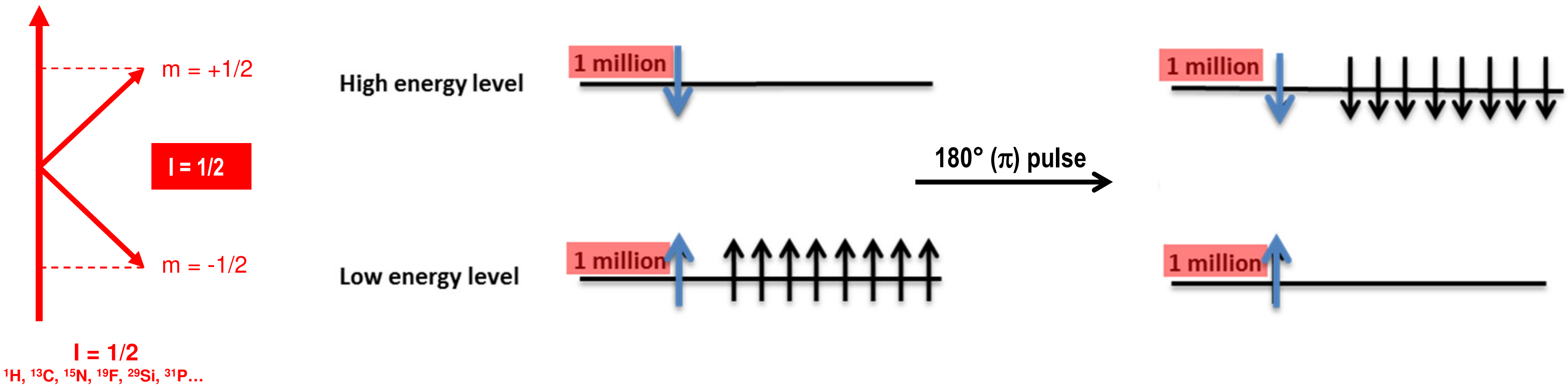
How does it looks like (90° pulse or 180° pulse)? For 50 MHz ^{1}H





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Chemical shift and references
^{1}**H** with 1% TMS (tetramethylsilane) in $CDCl_3$ = 0 ppm -in water, TMSP instead of TMS
^{7}**Li** with 1 mol LiCl in D_2O = 0 ppm
^{11}**B** with 15% $BF_3.Et_2O$ in $CDCl_3$ at = 0 ppm
^{13}**C** with 1% TMS (tetramethylsilane) in $CDCl_3$ = 0 ppm
^{15}**N** with $MeNO_2$ + 10% $CDCl_3$ = 0 ppm
^{17}**O** with D_2O = 0 ppm
^{19}**F** with CCl_3F or fluorobenzene
^{27}**Al** with 1.1 m $Al(NO_3)_3$ in D_2O = 0 ppm
^{29}Si with 1% TMS (tetramethylsilane) in $CDCl_3$ = 0 ppm
^{31}**P** with H_3PO_4 external = 0 ppm
^{117}**Sn** , $^{1\ 19}$**Sn** with Me_4Sn + 5% C_6D_6 = 0 ppm
^{41}**K**, ^{71}**Ga**, ^{85}**Rb**, ^{101}**Ru**, ^{133}**Cs**, ^{207}**Pb** and many others

nuclei	γ_n in 10^7 rad s^{-1}*T^{-1}	Resoncance frequency at 11,7T	Natural abundance (na)
^{1}H	+26.7513	500 MHz	99,98 %
^{2}H = D	+4.1065	76 MHz	0,02 %
^{7}Li	+10.3962	194 MHz	92 %
^{11}B	+8.5847	160 MHz	80 %
^{13}C	+6.7262	125 MHz	1,07
^{15}N	-2.7116	51 MHz	0.37 %
^{17}O	-3.6264	68 MHz	0.038 %
^{19}F	+25.1662	470 MHz	100 %
^{27}Al	+6.9763	130 MHz	100 %
^{29}Si	-5.3190	99 MHz	4.7 %
^{31}P	+10.8291	202 MHz	100 %
^{117}Sn	-9.5888	178 MHz	7.7 %
^{119}Sn	-10.0317	187 MHz	8.6 %

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NMR nuclei available in our house

^{1}H, ^{2}H, ^{6}Li, ^{7}Li, ^{10}B, ^{11}B, ^{13}C, ^{14}N, ^{15}N, ^{17}O, ^{19}F, ^{23}Na, ^{25}Mg, ^{27}Al, ^{29}Si, ^{31}P, ……^{39}K, ^{43}Ca,

^{49}Ti,……..^{119}Sn,……^{133}Cs……^{195}Pt…..^{205}Tl..

- Chemical shift (specific information about structure)
- Indirect Coupling constant (coupled nuclei over electron bonds in the neighborhood, inside a certain distance, between one and five bonds) ⟶ through bond
- Integration (complete amount inside a tube),
- Dipole coupling (NOE) ⟶ through space,
- Relaxation time (broadening of signals (T_2), spin-lattice relaxation (T_1)), related to dynamic behavior,
- Diffusion in solution (filtered NMR),

Nuclear Magnetic Resonance (NMR)

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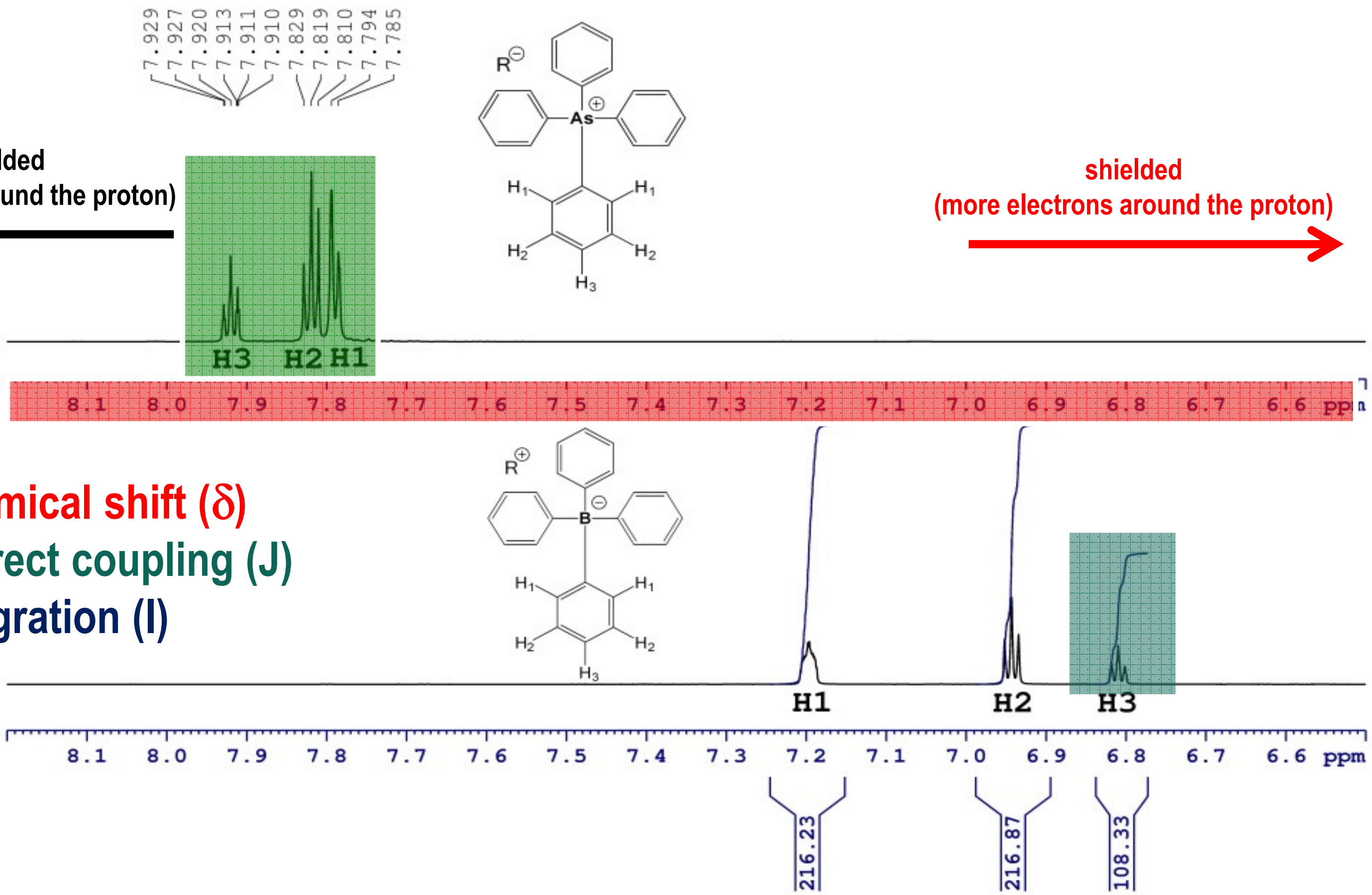
one example ^{1}H NMR of organic molecules with charges

deshielded
(less electrons around the proton)

shielded
(more electrons around the proton)



a. Chemical shift (δ)
b. Indirect coupling (J)
c. Integration (I)

Nuclear Magnetic Resonance (NMR)

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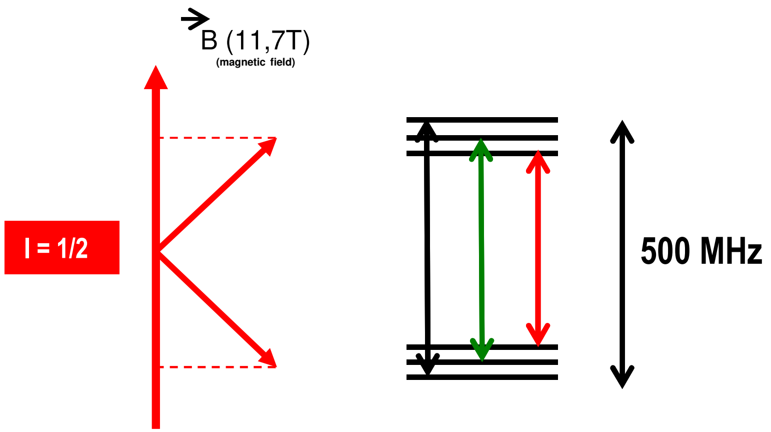
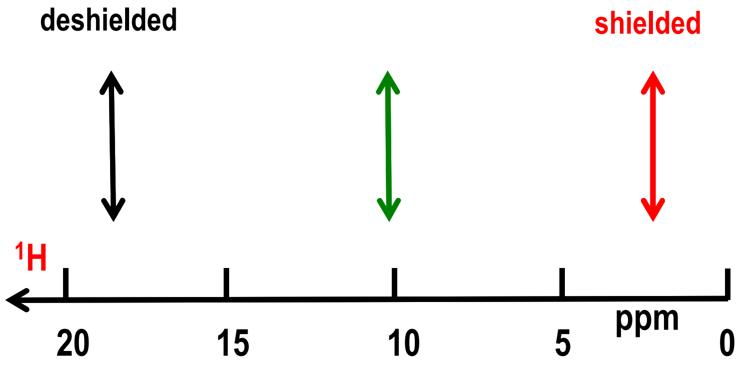
a. Chemical shift





- Nuclei are surrounded with electrons

  the local magnetic field at the nucleus is not the same as the external magnetic field

- Consider a CH bond: electron density in TMS is bigger than in chloroform

  stronger electron density diminishes the external field, external magnetic field is shielded

- Energy absorption takes place at effective magnetic field B_{eff} which is acting at the nucleus
- Different concentration different absorption
- Solvent environment change the B_{eff}

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a. Chemical shift

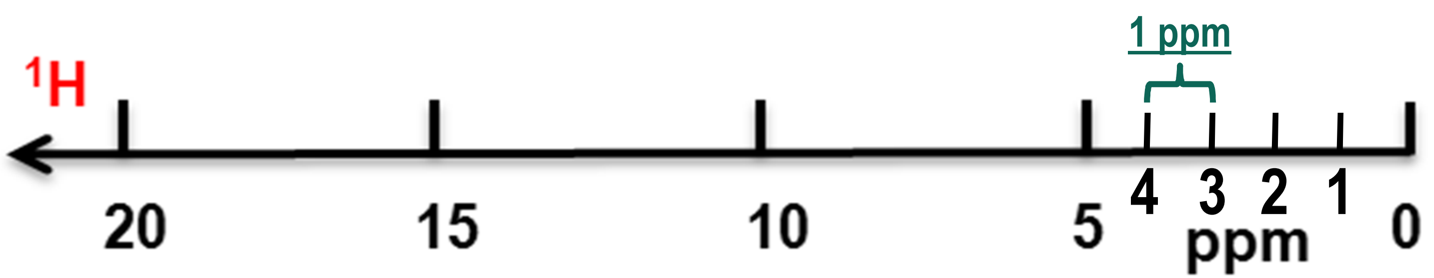
definition of the ppm versus Hz scale:

- **δ is independent of the magnetic field** $\delta = \frac{\nu_{Probe} - \nu_{Ref}}{\nu_{Ref}}$
- **Hz scale magnetic field dependent**

Example

1 ppm at 500 MHz (11,7 T) ^{1}H NMR = 500 MHz

1 ppm at 1000 MHz (23,4 T) ^{1}H NMR = 1000 MHz



example later why this part is important

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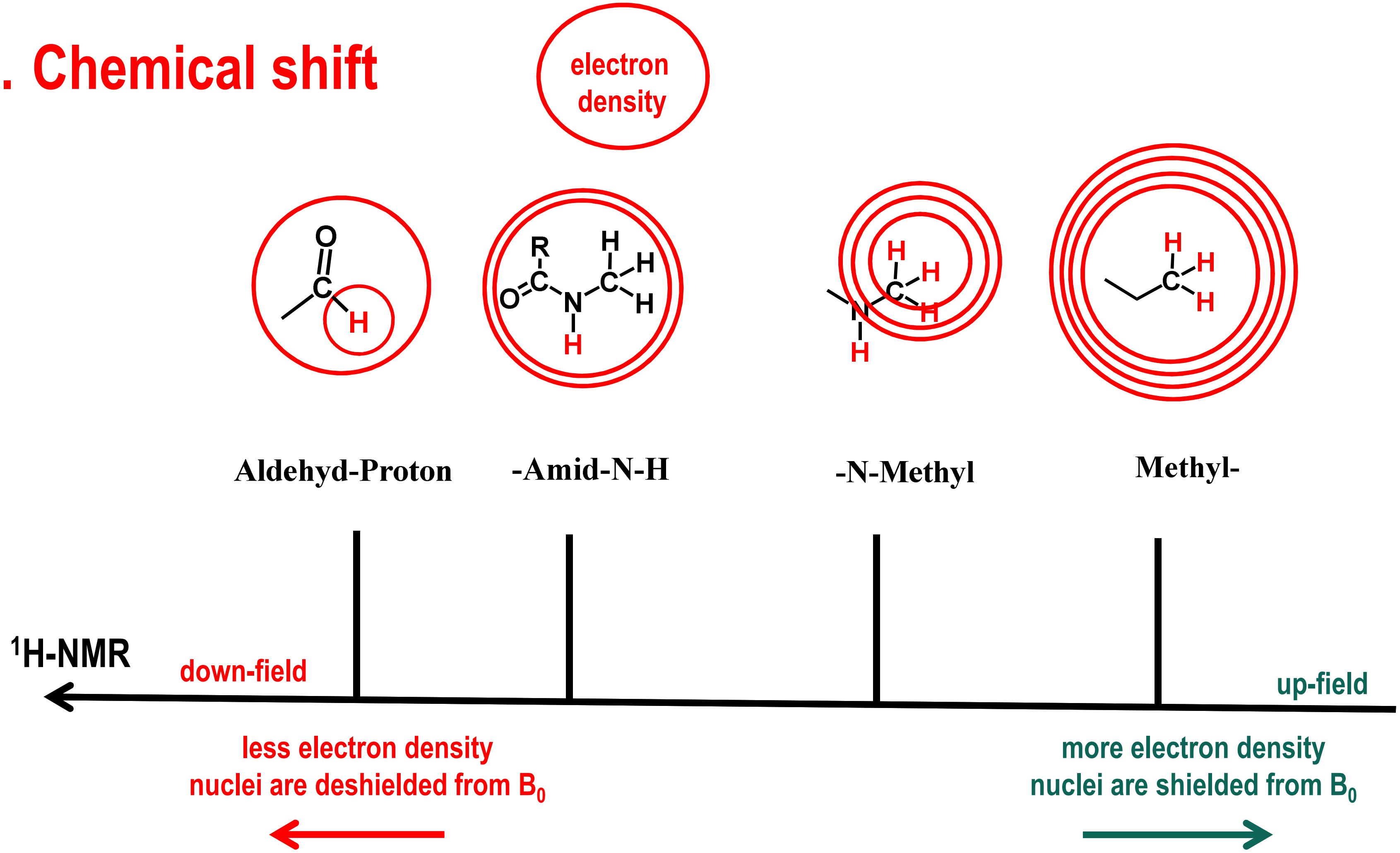
a. Chemical shift



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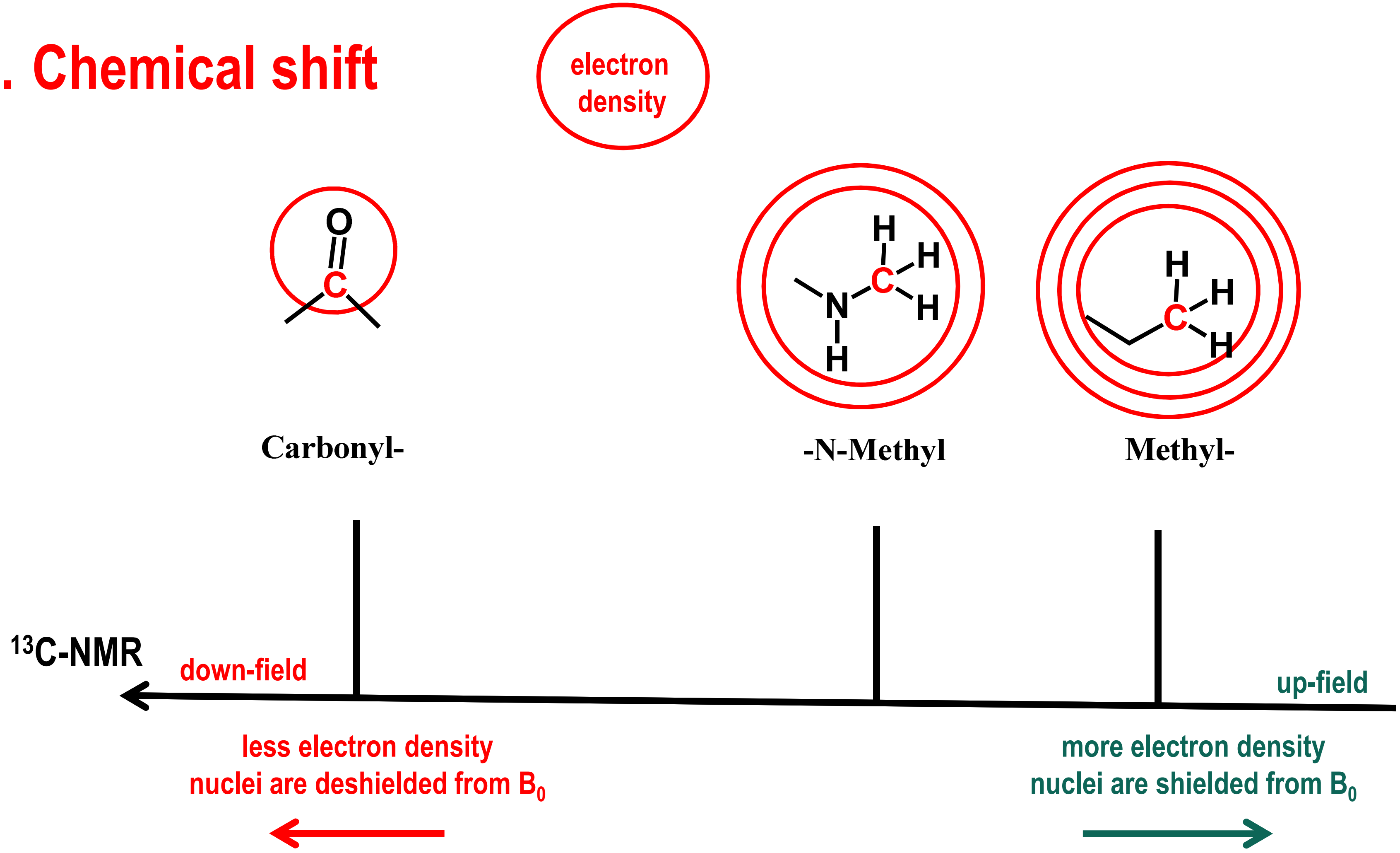
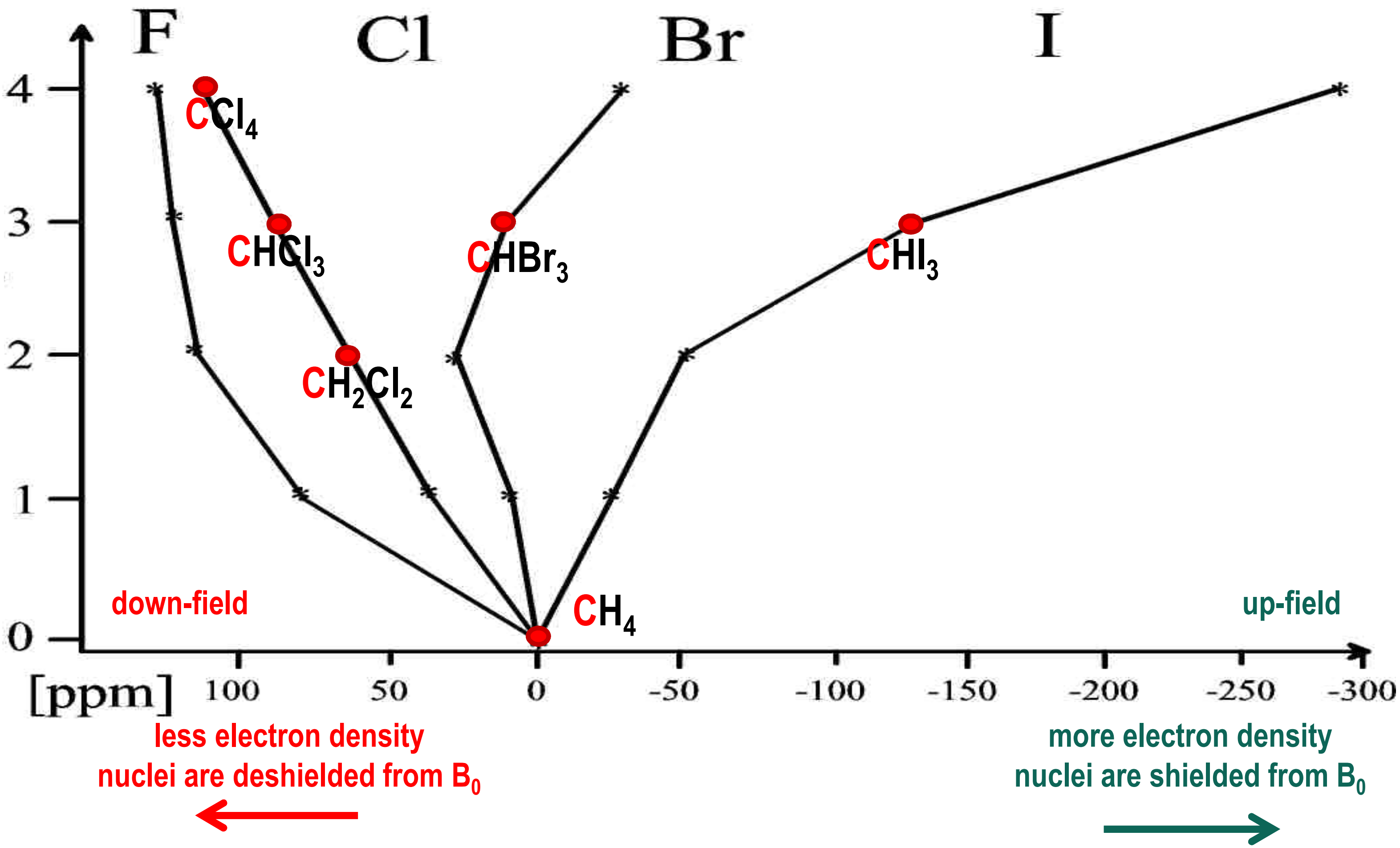
a. Chemical shift

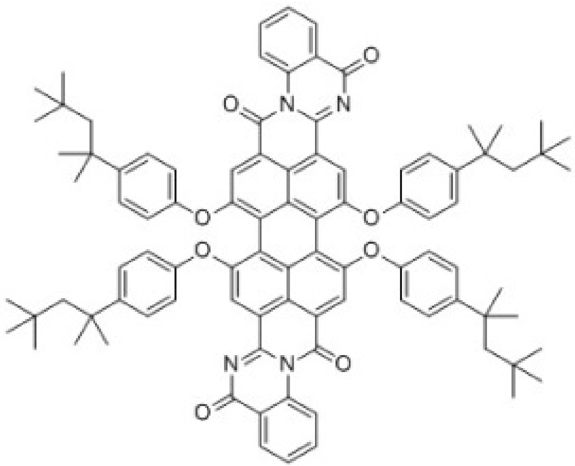


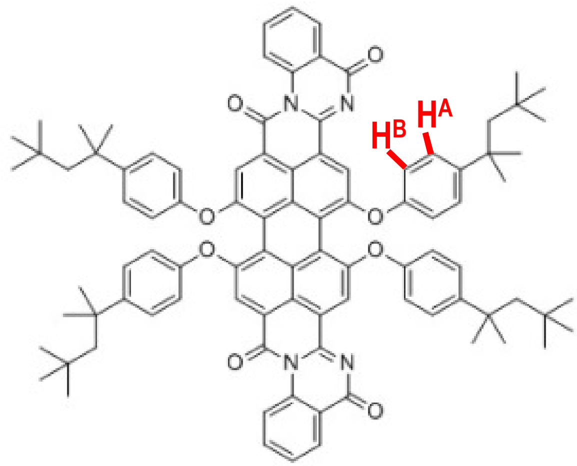
a. Chemical shift

^{13}C-NMR

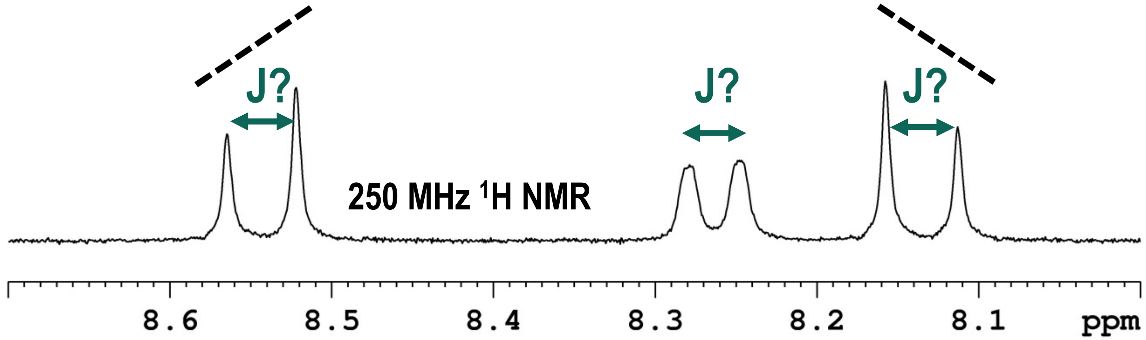


B-Field dependence of **coupling J** and **chemical shift δ**





only one doublet in the low field at 7 ppm



example chemical shift versus Hz

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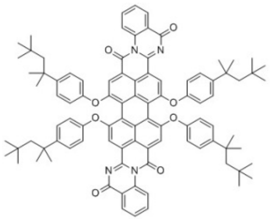
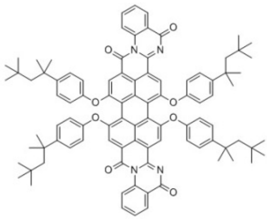
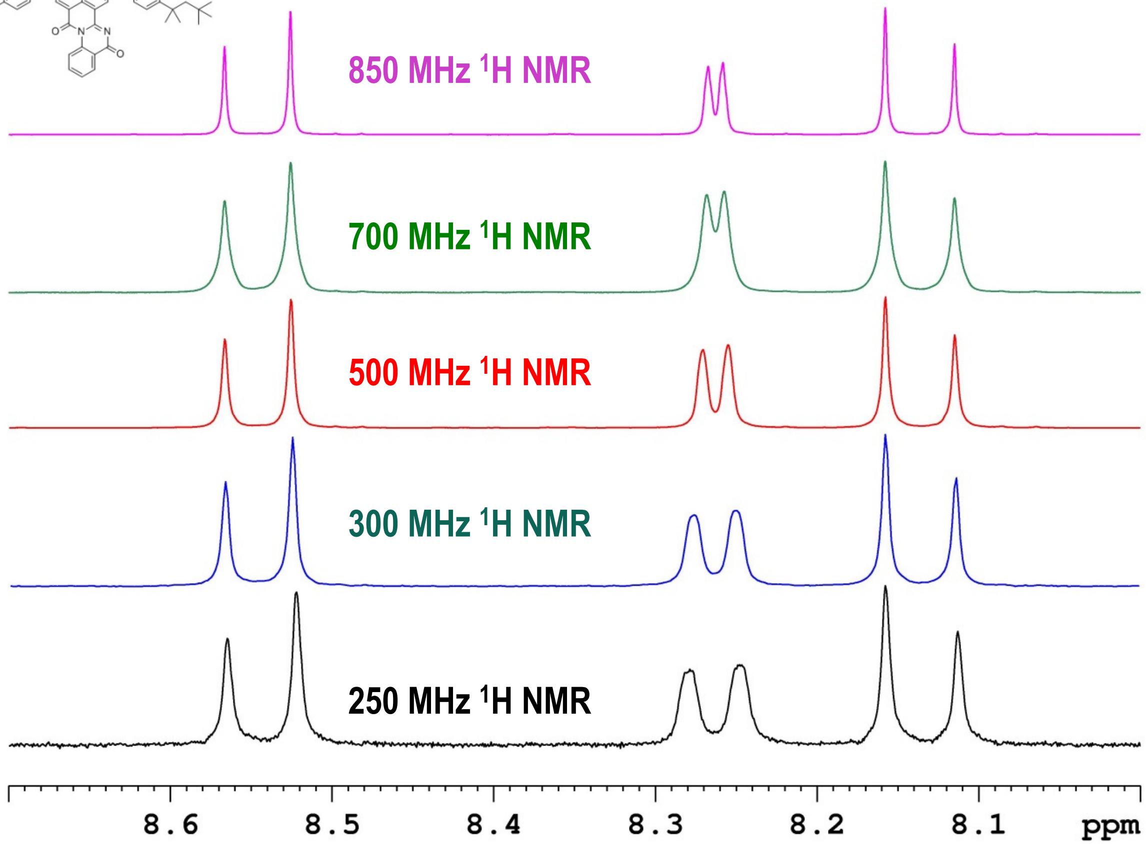




B-Field dependence of **coupling J** and **chemical shift δ**



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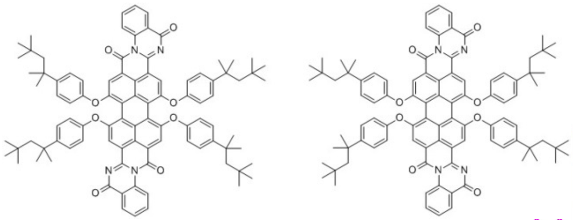
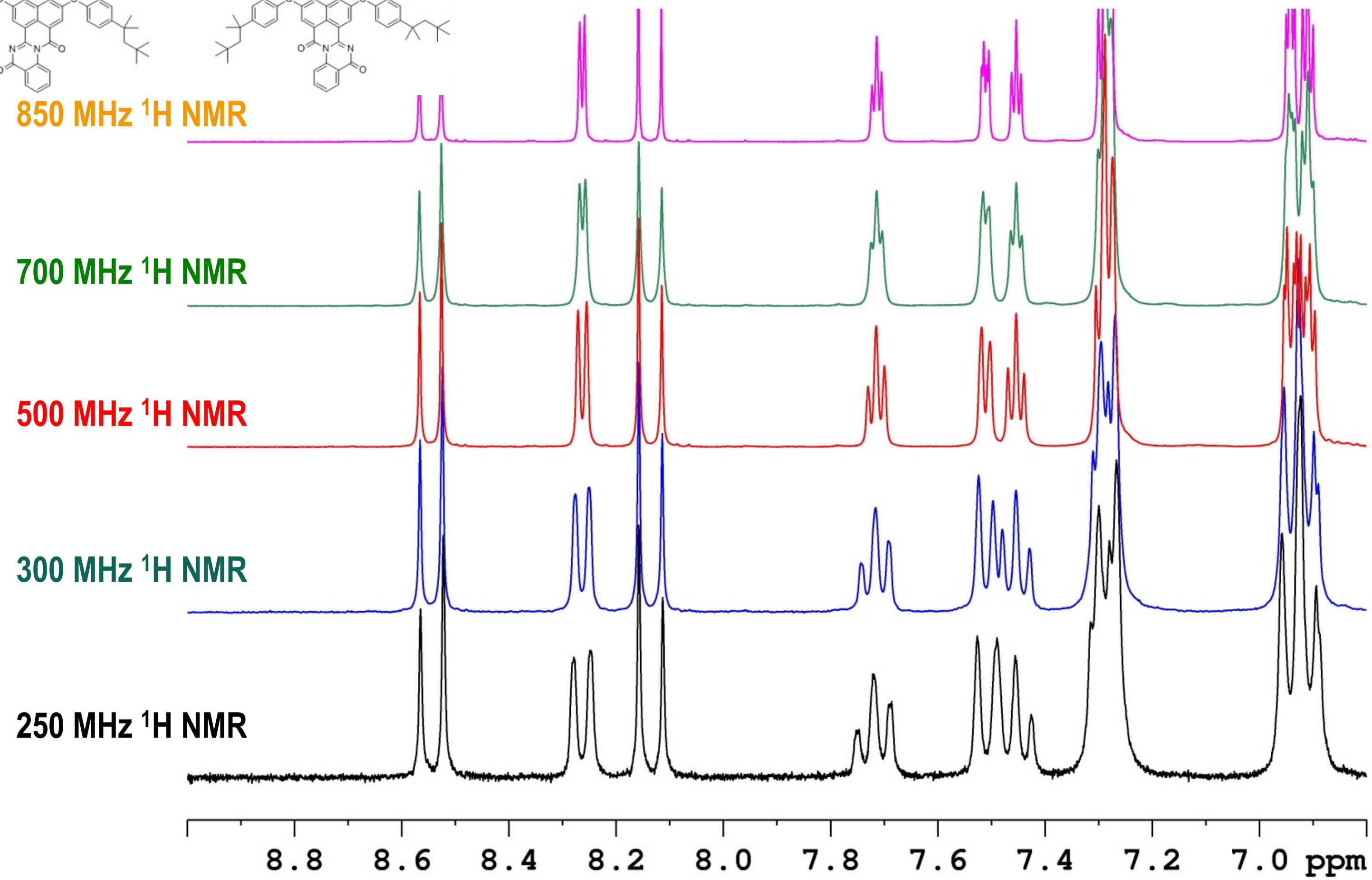


B-Field dependence of **coupling J** and **chemical shift δ**

a. Chemical shift
b. Indirect coupling



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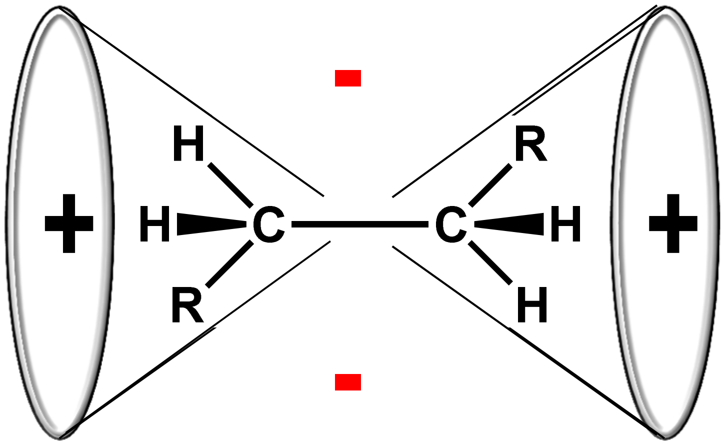
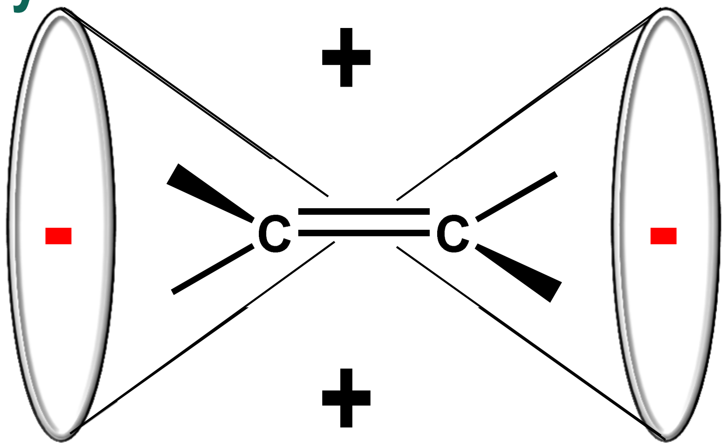
Keyword

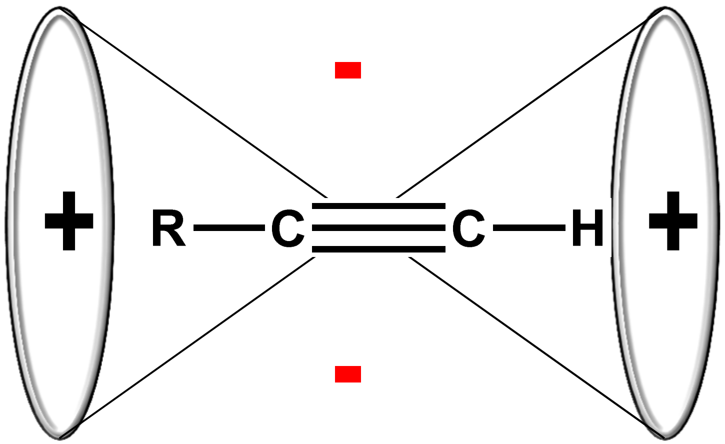
magnetic anisotropy

a. Chemical shift

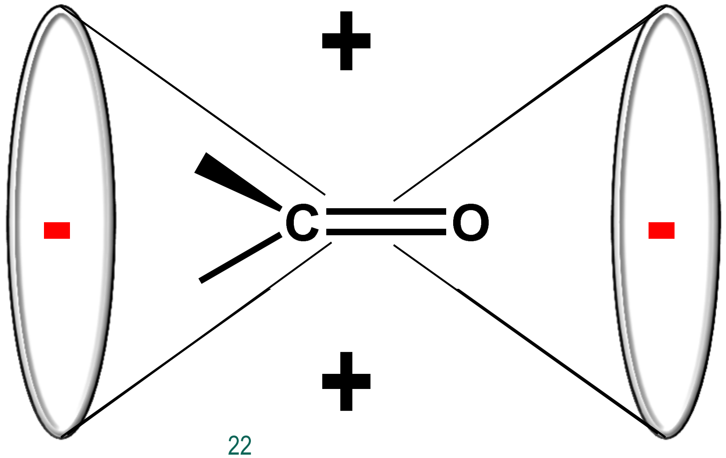




+ means high field shift, more shielding effect



- means low field shift, more deshielding effect



22

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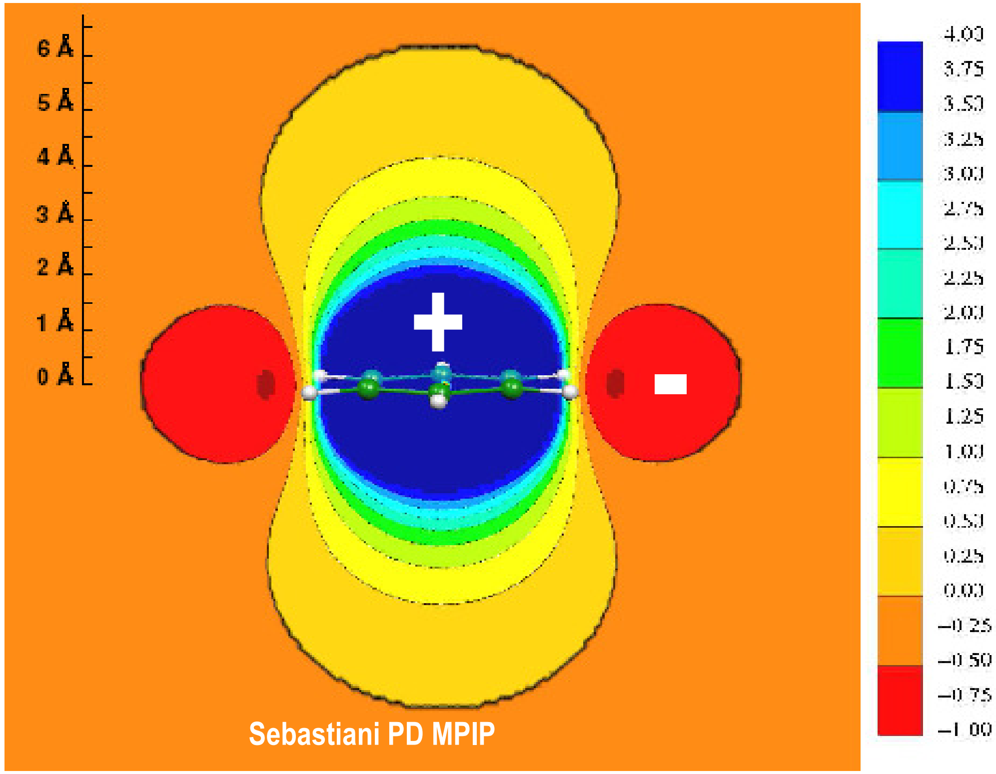
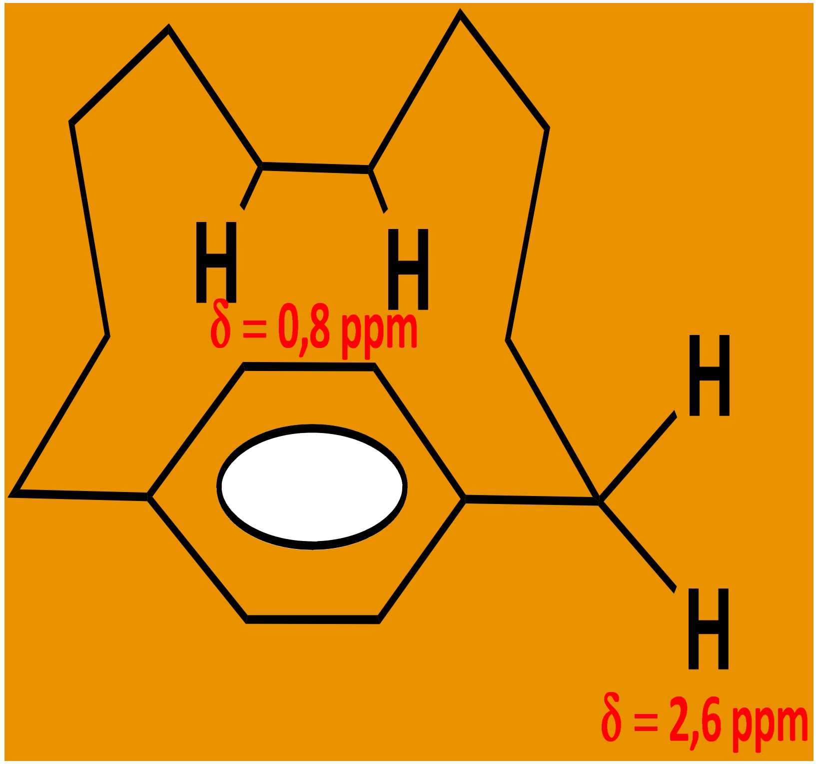
a. Chemical shift

Keyword
magnetic anisotropy





+ means high field shift, more shielding effect

- means low field shift, more deshielding effect

b. Indirect coupling

- H-nucleus has two orientations to the external magnetic field (parallel and anti parallel)
- **I = ½** => two additional fields can be observed at H‘ und H“
- Considering all nuclei in the sample: some of them are parallel and some anti parallel
- As a consequence: two lines of H‘ will be observed
- Both lines are caused by the same nucleus H
- They will be called „doublet“

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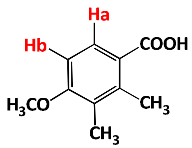
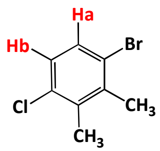
Spin-systems:

b. Indirect coupling

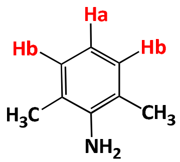
AX



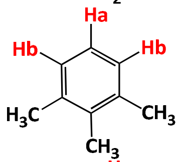
AB



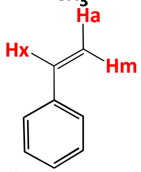
AX_2



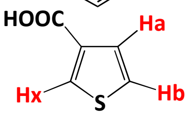
AB_2



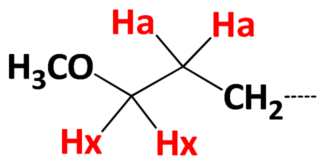
AMX



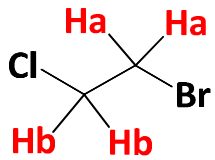
ABX



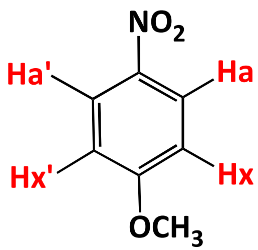
A_2X_2



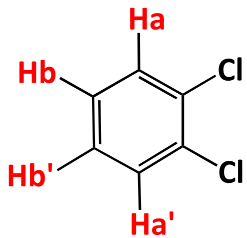
A_2B_2



AA'XX'



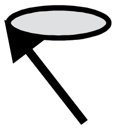
AA'BB'



b. Indirect coupling

H with I = 1/2

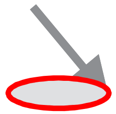
can be

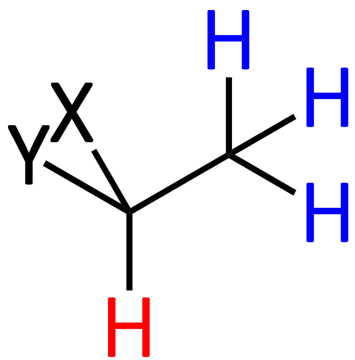


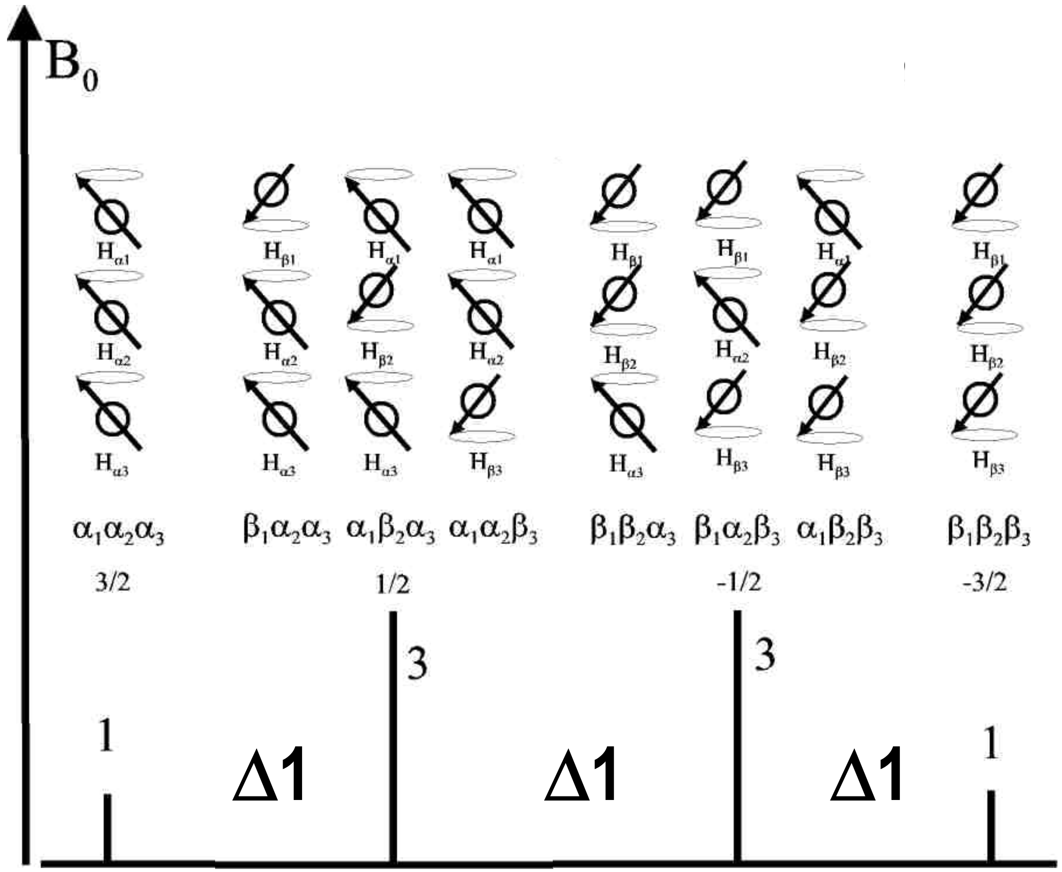
up

or

down







we are looking on the CH which has a coupling to the methyl group (CH_3)

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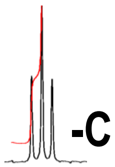
b. Indirect coupling

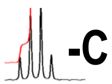
nuclear spin I = 1/2

neighbor	signal multiplication
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

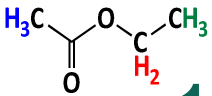
^1H NMR



$-CH_2CH_3$ → 1 2 1



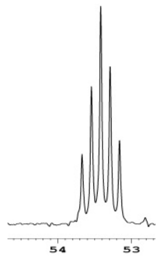
$-CH_2CH_3$ → 1 3 3 1



nuclear spin I = 1

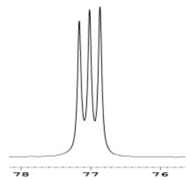
neighbor	signal multiplication
0	1
1	1 1 1
2	1 2 3 2 1
3	1 3 6 7 6 3 1
4	1 4 10 16 19 16 10 4 1
5	1 5 15 30 45 51 45 30 15 5 1

^{13}C NMR



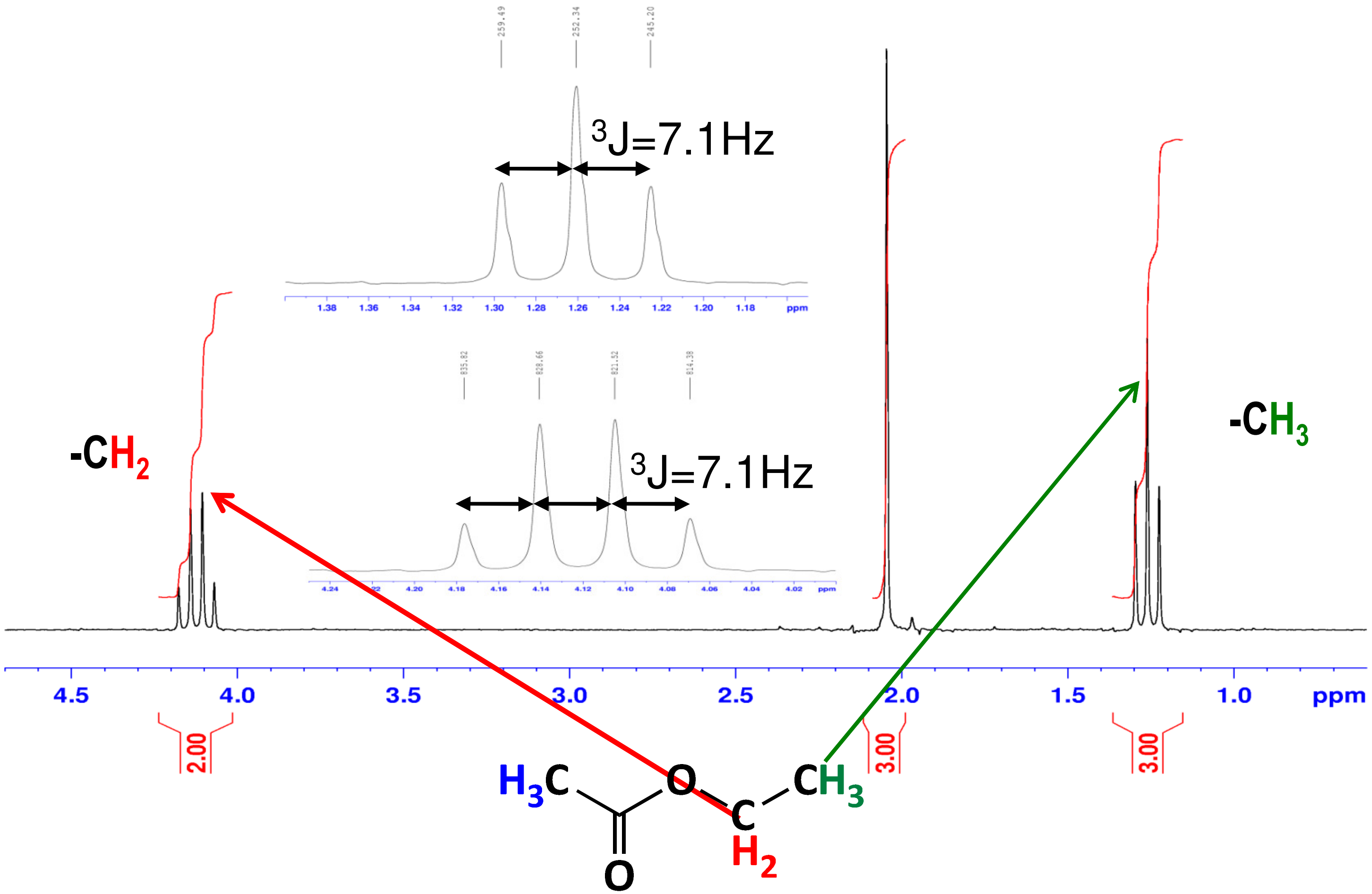
CD_2Cl_2 → 1 2 3 2 1

^{13}C NMR



$CDCl_3$ → 1 1 1

b. Indirect coupling



b. Indirect coupling

Responsible for the indirect spin-spin coupling is the contact-term:

direct interaction of nuclear magnetic moments and the binding electrons in the s-orbitals

(the electron in the 1s orbital has a infinite probability of presence in the die nucleus place)

it's understandable, why the coupling constants are strongly depending on:

- the hybridization
- valence and torsion angle
- lengths of the bonds
- π-bindings in the neighborhood
- effects of the bonded atoms in the neighborhood
- and many other effects

Keywords: Pauli-principles, binding electrons anti parallel and the moment of the nucleus anti parallel to the binding electrons

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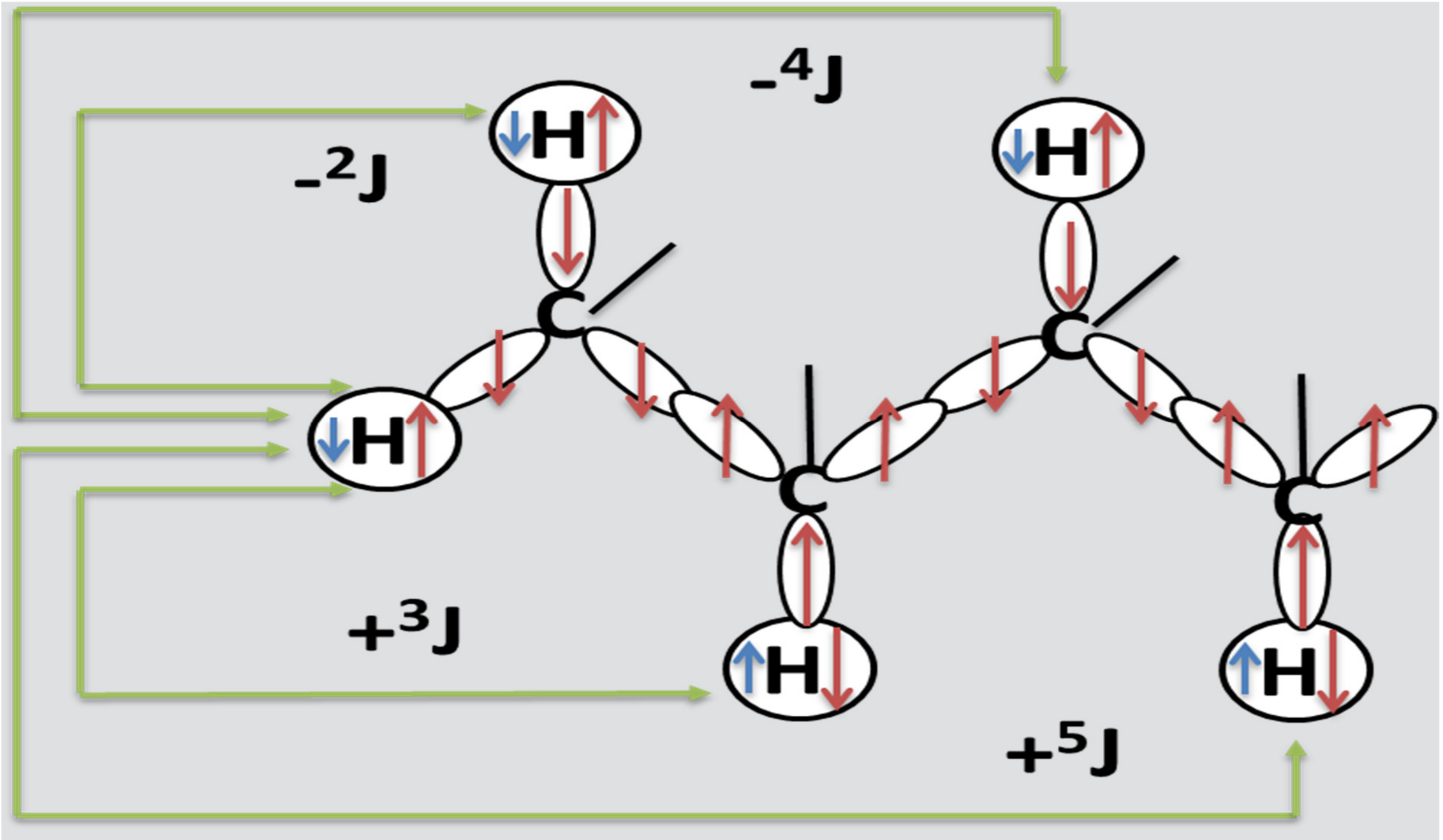
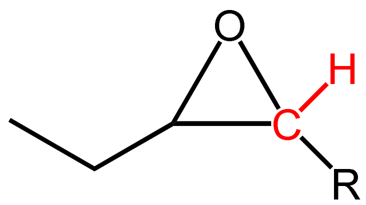
b. Indirect coupling

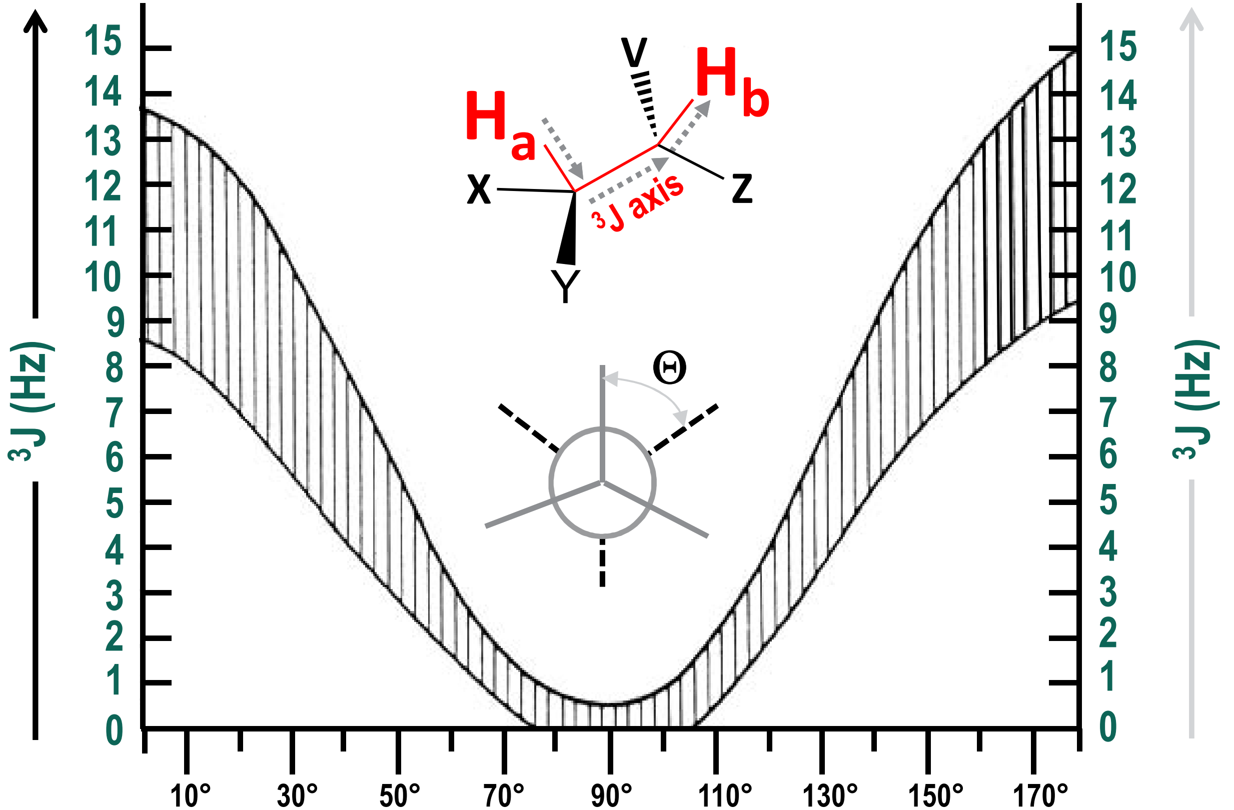


b. Indirect coupling

Carbon environment	$^1J_{CH}$-areas (Hz)
Aliphatic, CH_n-	120-135
Aliphatic, CH_n-X (X=N,O,S)	135-155
Alkene	155-170
Triple bond CH	240-250
Aromatic systems	155-170
Three member ring	170-190



Karplus Relation (1959)



$$^3J = \begin{cases} 8.5\cos^2\Theta - 0.28 \text{ for } 0 \leq \Theta \leq 90° \\ 9.5\cos^2\Theta - 0.28 \text{ for } 90° \leq \Theta \leq 180° \end{cases}$$

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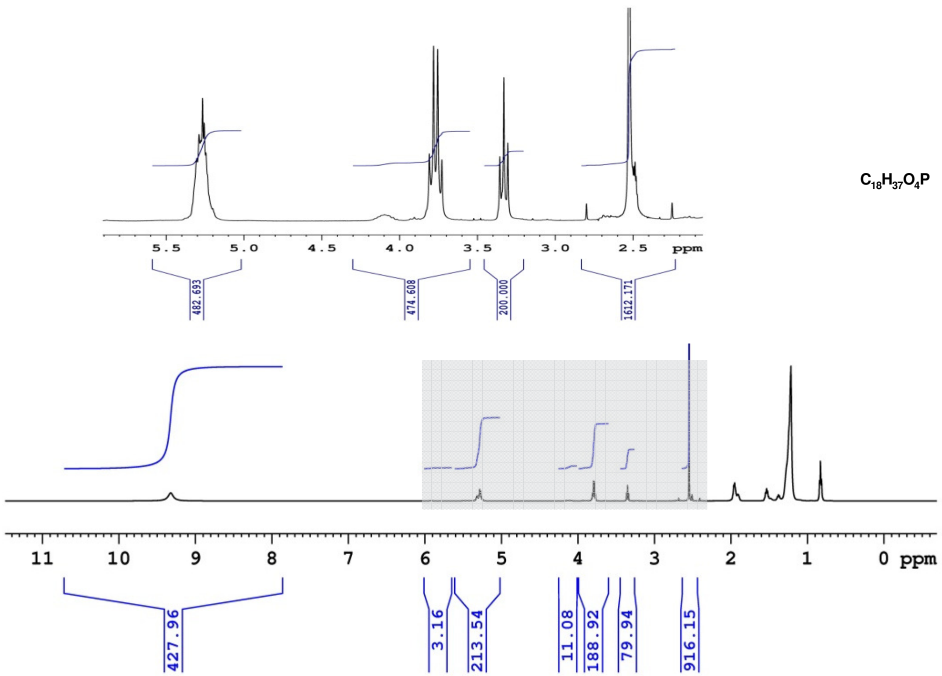
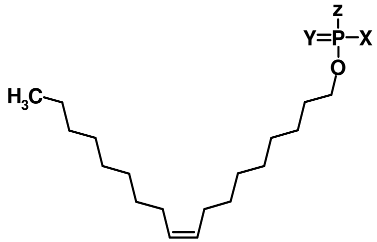
c. Integration

¹H NMR quantitative measurements

Probe 1 in DMSO bei 298K 120mg Oleylphosphat 803mg DMSO-d6 und 27,2mg DMSO





$C_{18}H_{37}O_4P$ Mol. Mass 348,47 C=62,04 %; H=10,70 %; O=18,37 %; P=8,89 %

c. Integration

Width of integrals

Lorentz-peaks

Integration width / **Half width** of the signal units are the half width of the signals	Percentage of the real **signal intensity** [%]
1,00	50,00
2,00	70,48
3,00	79,52
5,00	87,43
6,31	90,00
10,00	93,65
12,71	95,00
20,00	97,00
63,60	99,00
636,00	99,90
6366,00	99,99

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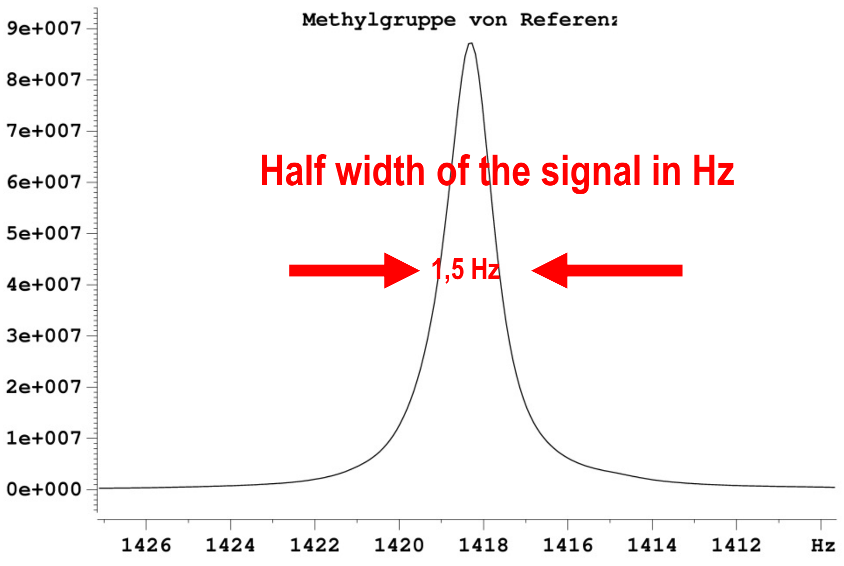
c. Integration

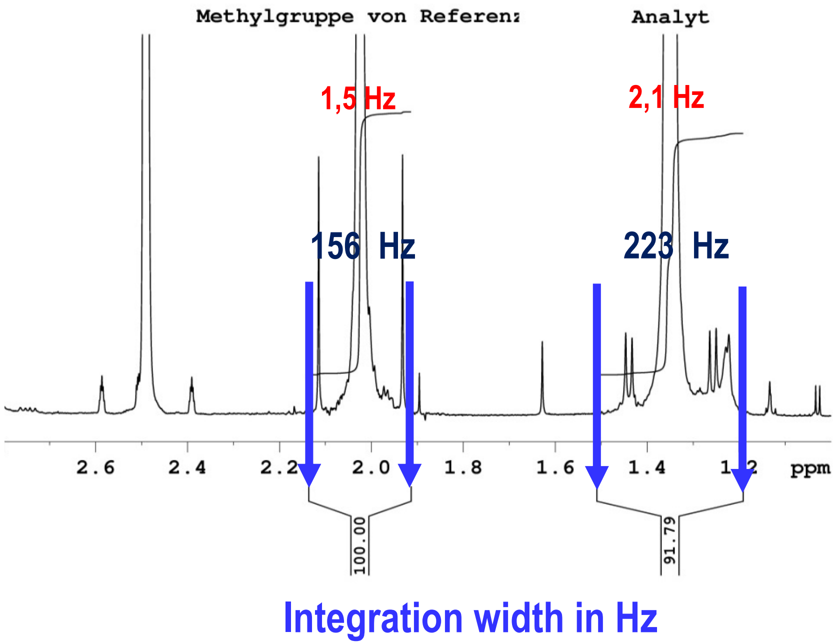
Factor = Integration width/Half width of the signal = 156/1,5 = 104

99,1 % of the signal is inside the integral!

Compared signals has the same factor!!

In this case 104!!





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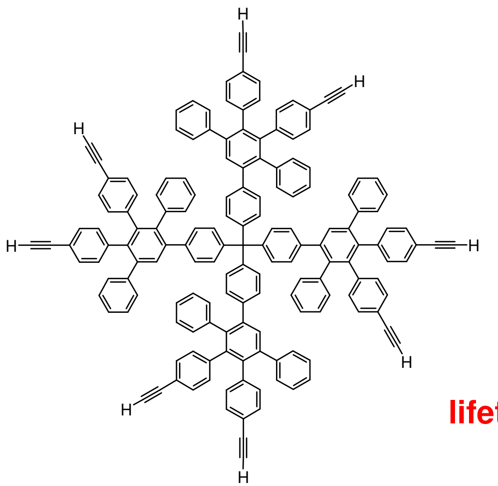
c. Integration

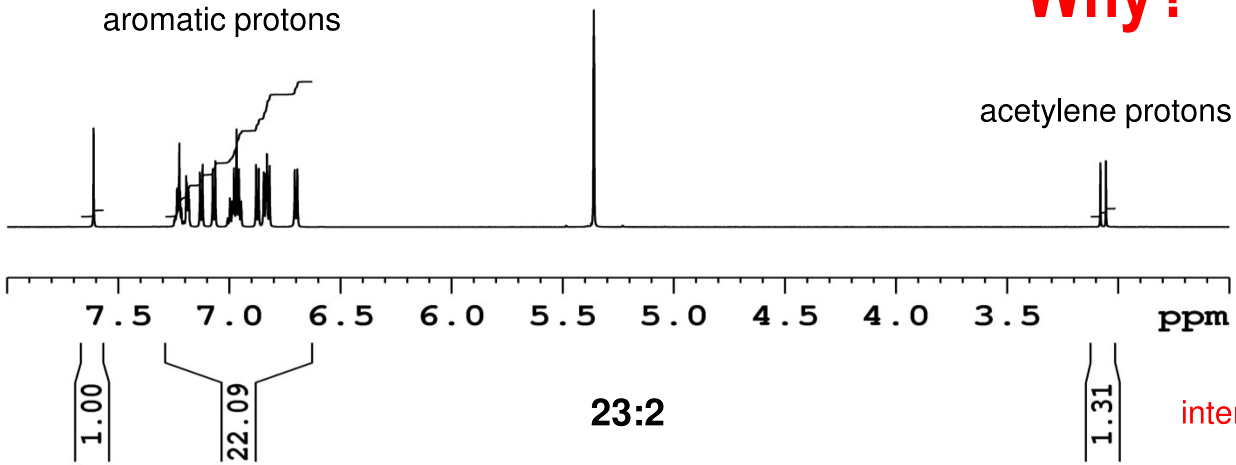


lifetime of acetylene protons T_1 can be minutes

Why?

aromatic protons

acetylene protons



23:2

intensity has to be 2 not 1.31

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2D "HOMO" NMR Experiment:

- Introduction and theory
- 2D Acronyms, what does that mean
- Example from our house

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Introduction and theory

- Correlation of nuclei from one species (e.g. ^{1}H)
- Via bond correlation
- Via space correlation (mainly dipole-dipole)

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Introduction and theory

Acronyms

COSY (Correlation SpectroscopY)

TOCSY (TOtal Correlation SpectroscopY)

NOESY (Nuclear Overhauser Effect SpectroscopY)

ROESY (Rotating Frame Overhauser Effect SpectroscopY)

INADEQUATE (Incredible Natural Abundance DoublE QUAntum Tranfer Experiment)

Have in mind that the first 4 Experiments exist also with solvent suppression

Nuclear Magnetic Resonance (NMR)

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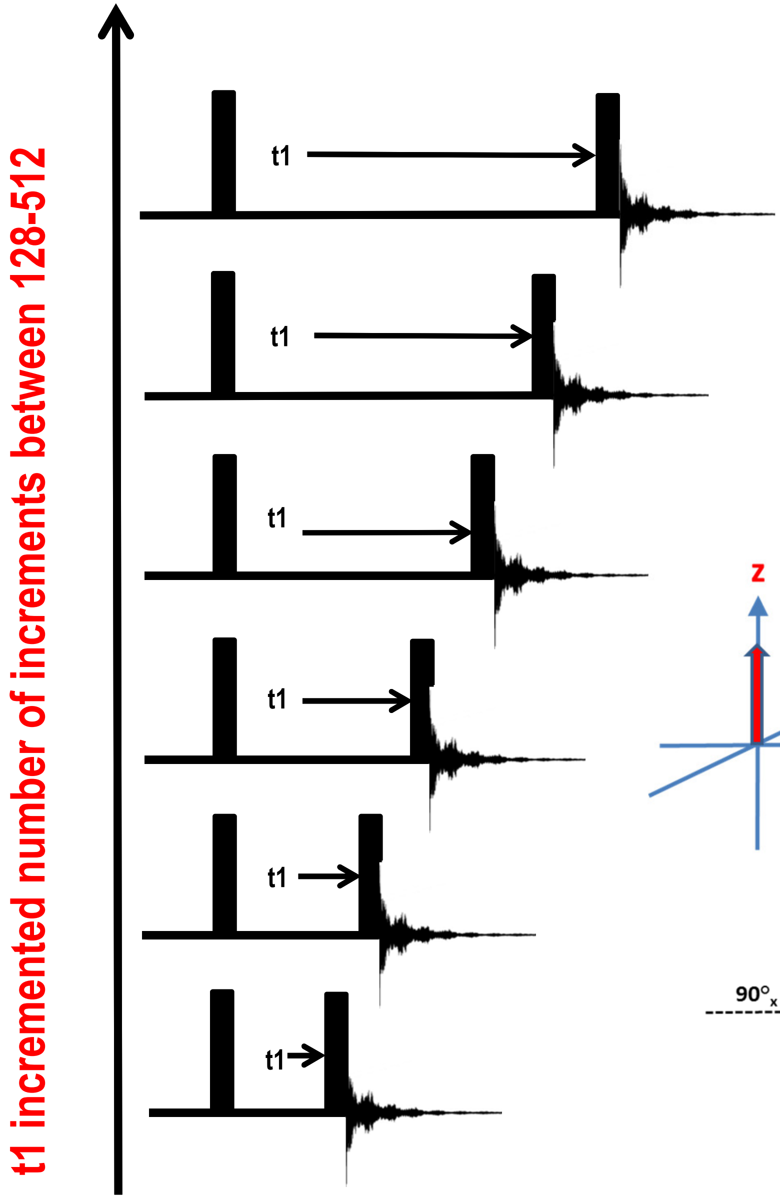
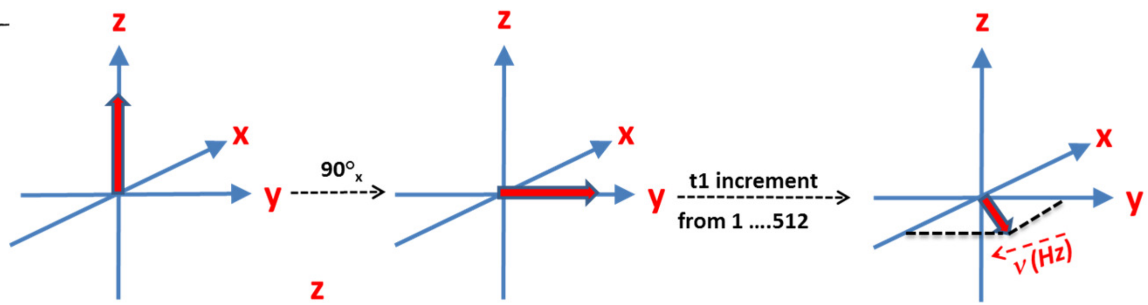
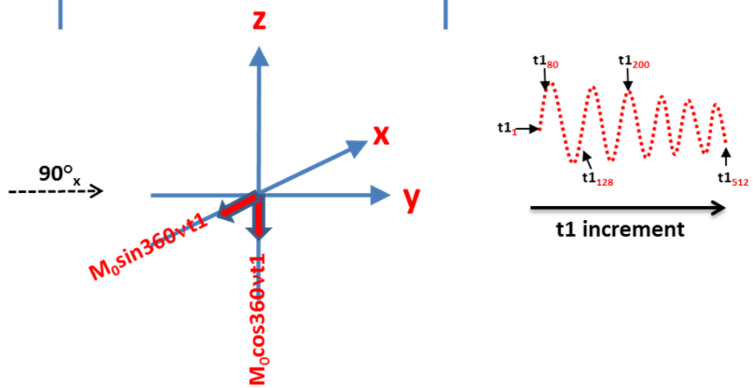


General:

- number of increments are number of experiments in one 2D measurement,
- every experiment has a number of scans (eg. 8 or less due to gradient techniques)





This modulation of the M_0 creates the correlation cross peaks, when there are e.g. J-coupling between nuclei

Nuclear Magnetic Resonance (NMR)

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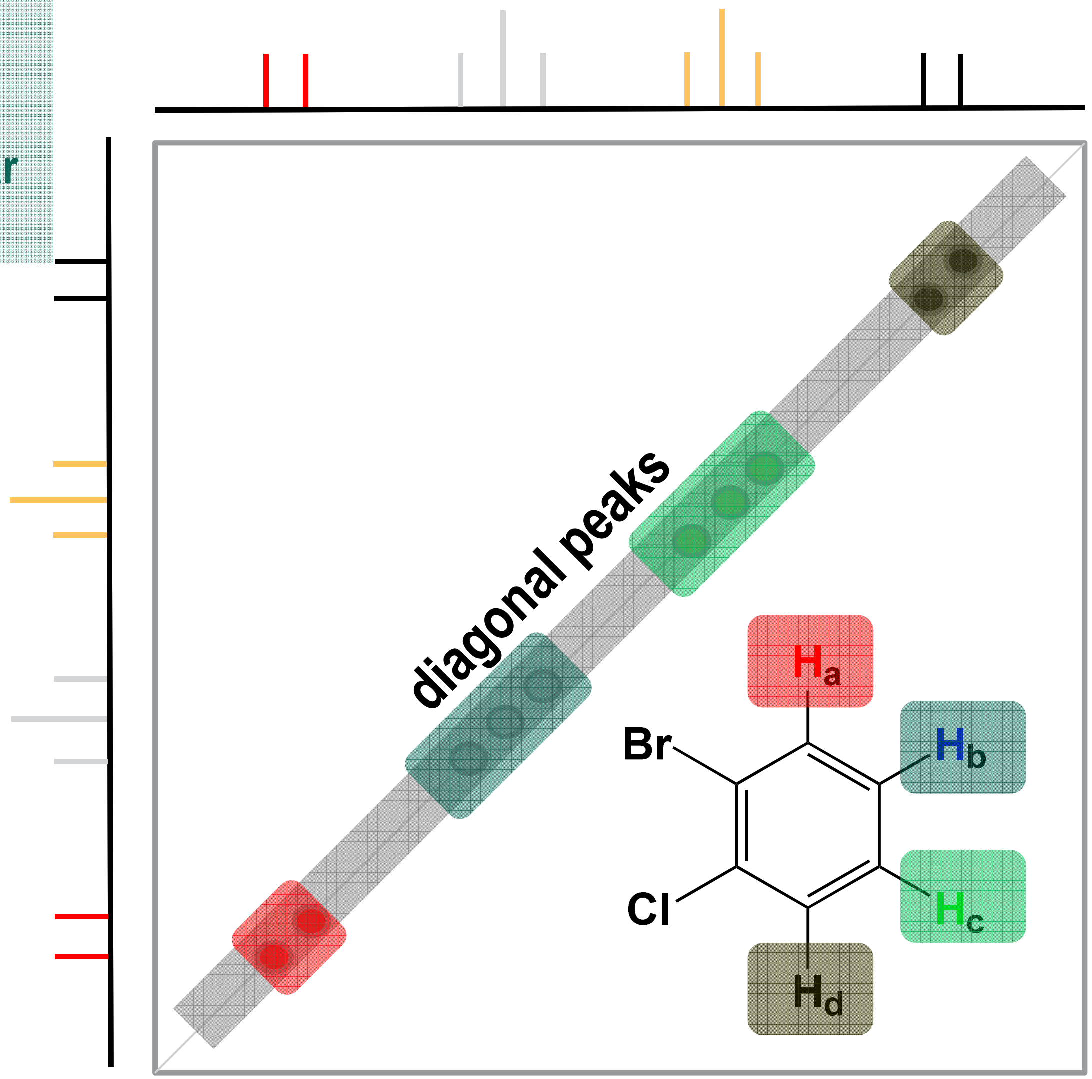
General Information about 2D Homonuclear spectra



diagonal peaks representing the chemical shifts of the protons

Nuclear Magnetic Resonance (NMR)

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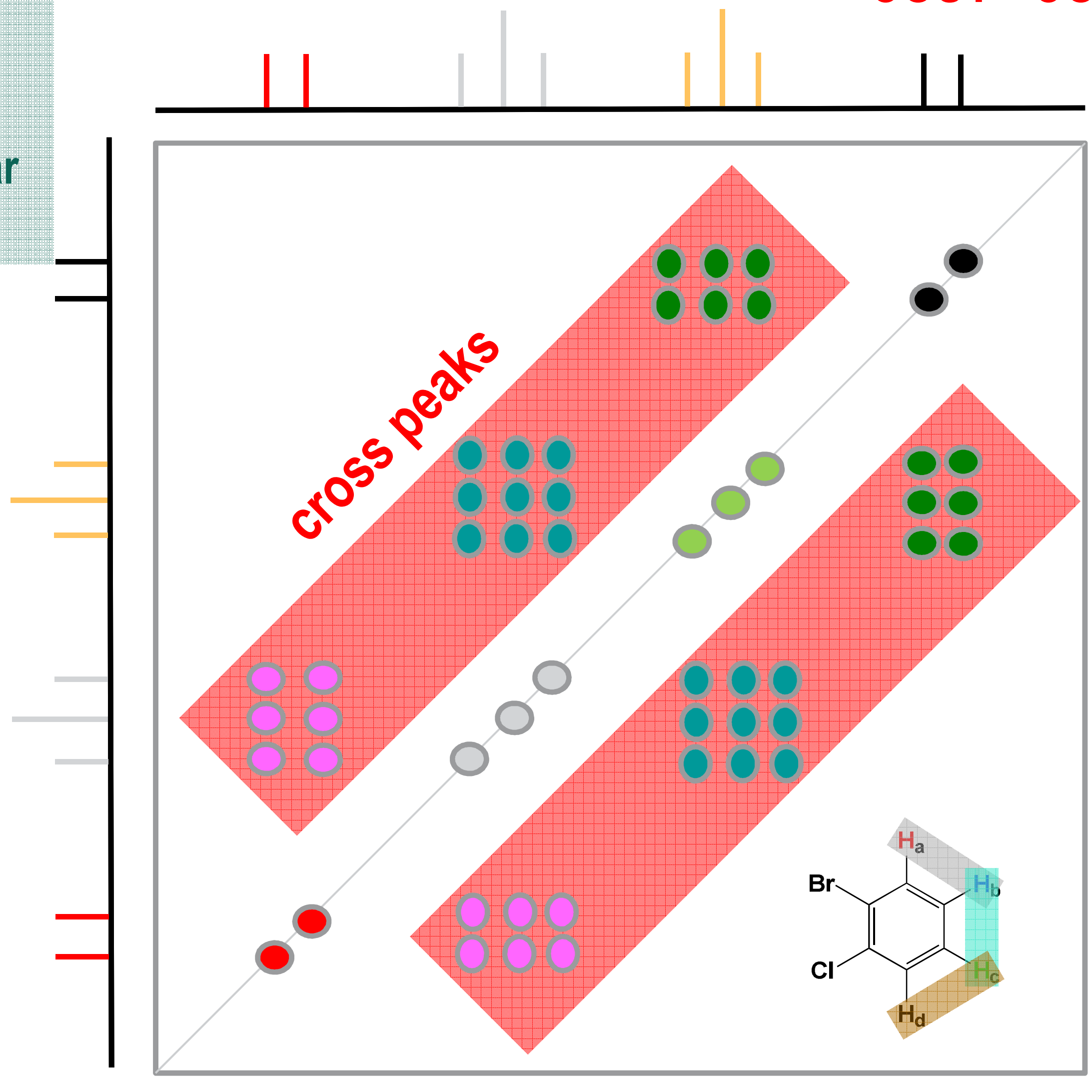
General Information about 2D Homonuclear spectra

COSY - COrrelation SpectroscopY

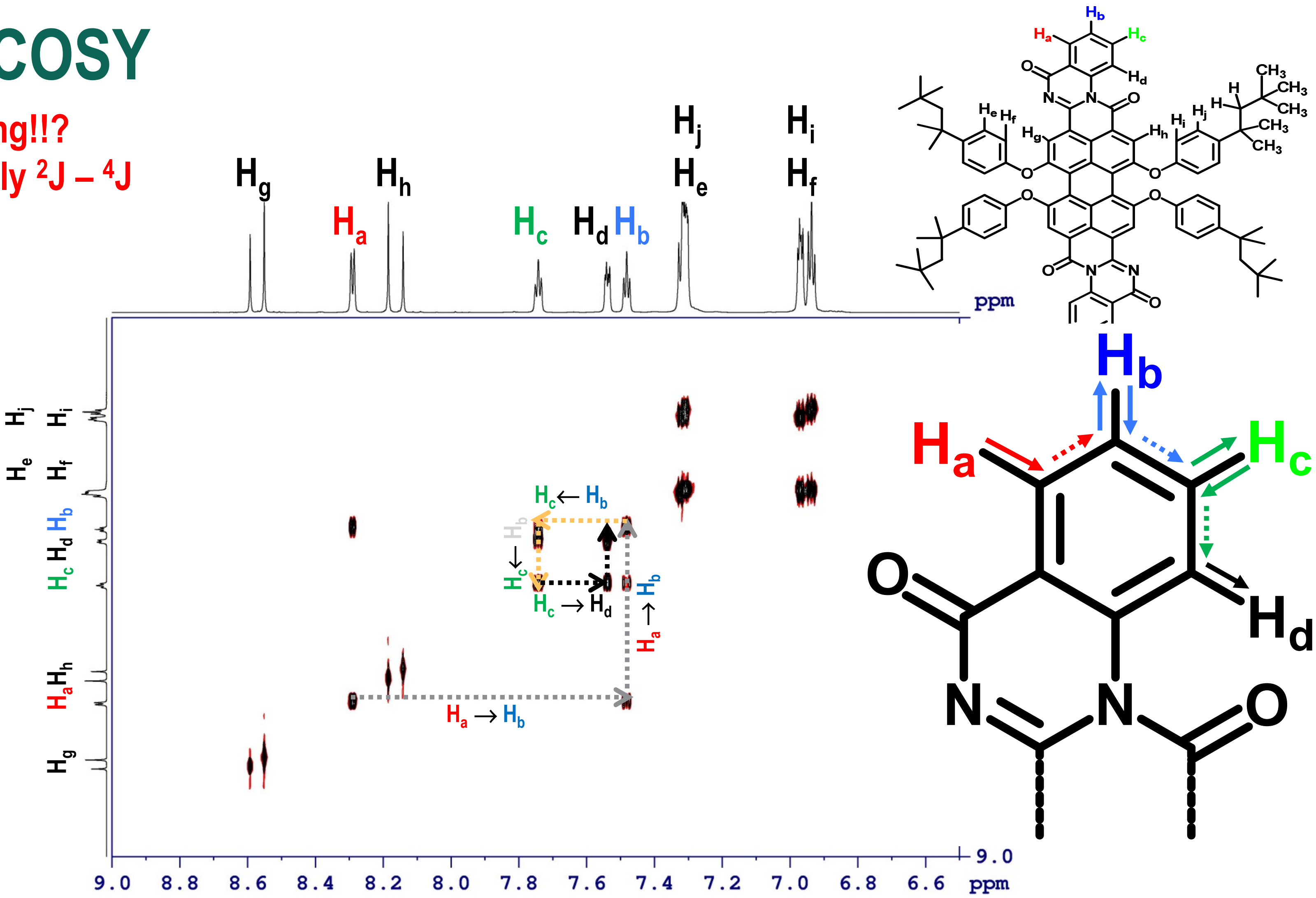


cross peaks representing the chemical shifts of the protons

2D H,H-COSY

^3J coupling!!?

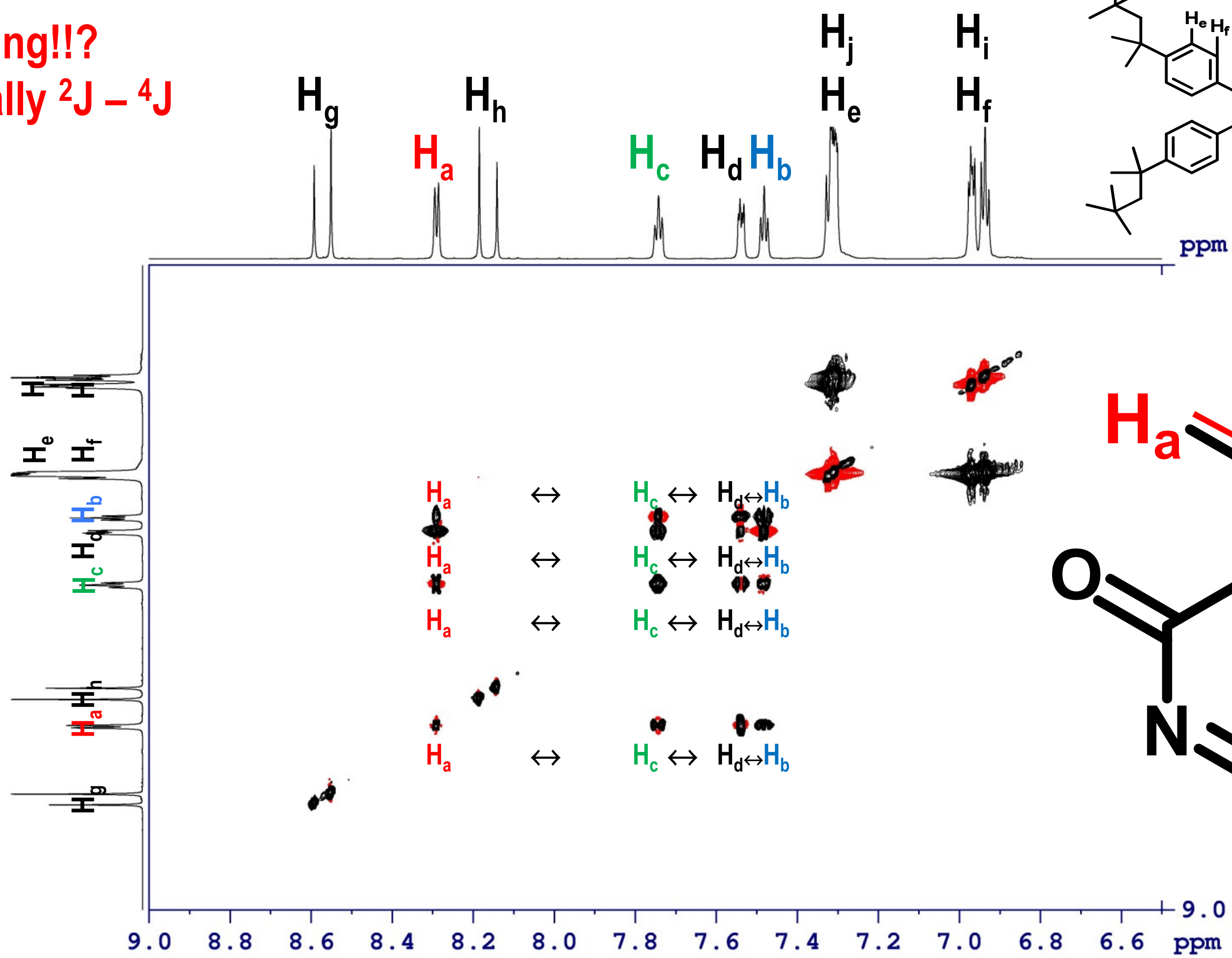
COSY normally ^2J – ^4J

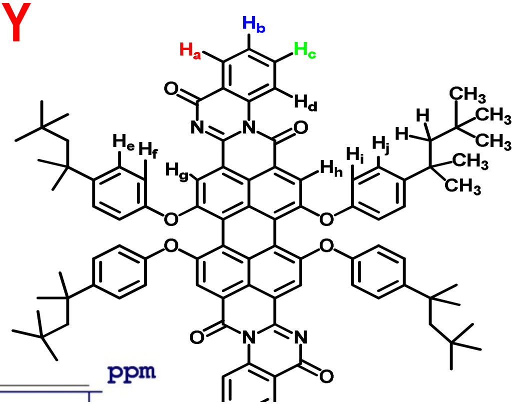


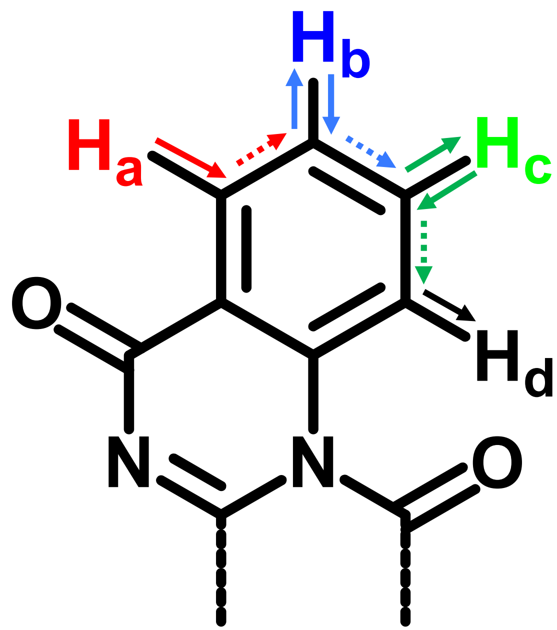
2D H,H-TOCSY

TOCSY - TOtal Correlation SpectroscopY

^{3}J coupling!!?
COSY normally ^{2}J – ^{4}J







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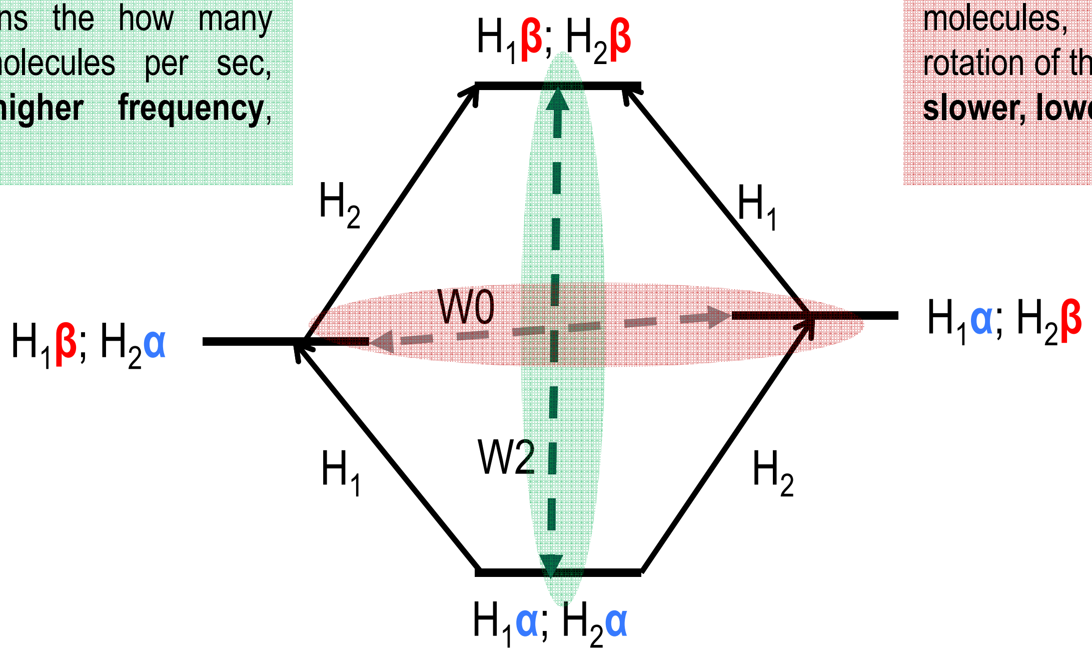
2D H,H-NOESY

NOESY - Nuclear Overhauser Enhanced SpectroscopY

W2 has more probabilities with smaller molecules, τ_c means the how many rotation of the molecules per sec, **smaller faster, higher frequency**, ββ⇒αα

W0 has more probabilities with larger molecules, τ_c means the how many rotation of the molecules per sec, **larger slower, lower frequency**, αβ⇒βα



W2: ββ⇒αα, -1 to +1
Double Quantum Transition, **positiv signal**

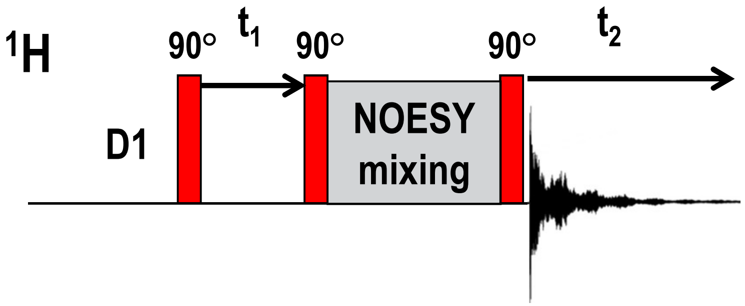
W0: αβ⇒βα, 0 to 0
Zero Quantum Transition, **negative signal**

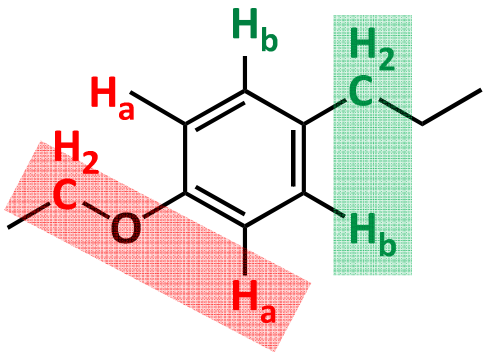
isotropic rotational correlation time: ⇒ $\tau_c = 4\pi\eta_W r^3_H/(3k_BT)$

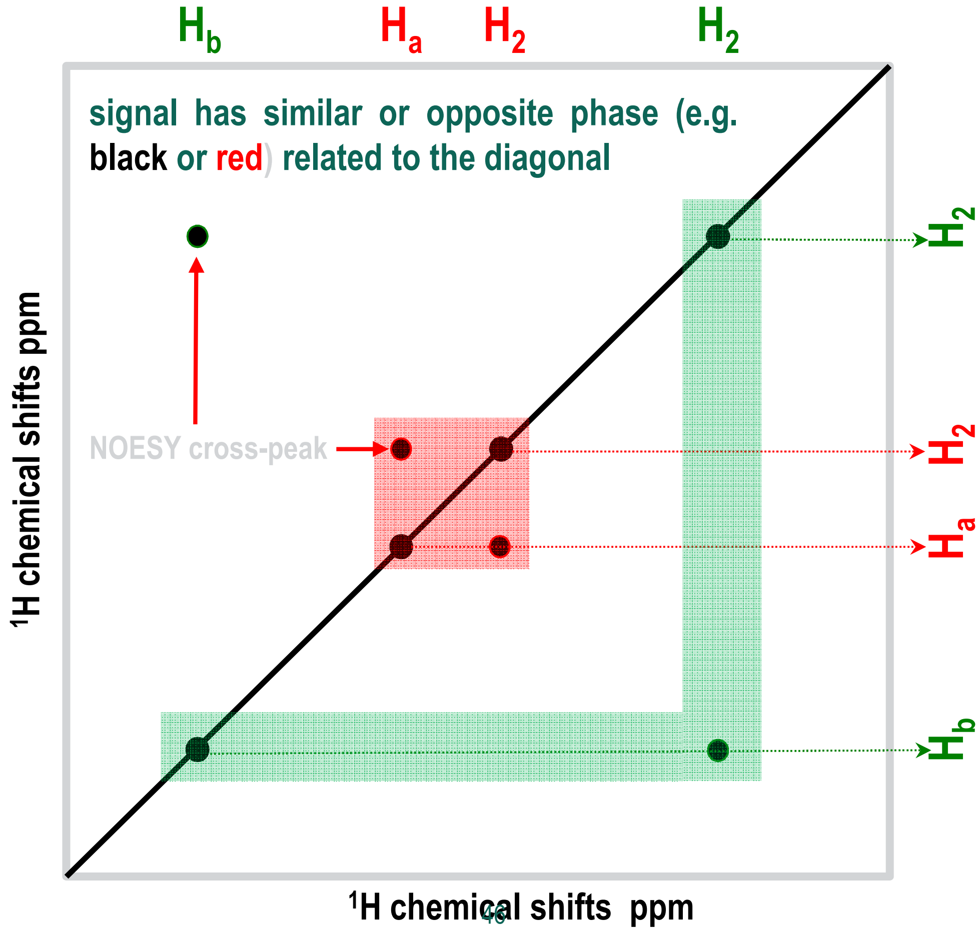
NOESY - Nuclear Overhauser Enhanced SpectroscopY

protons close in space (<5 Å) or in chemical exchange

noe=f(1/r⁶)







Nuclear Magnetic Resonance (NMR)

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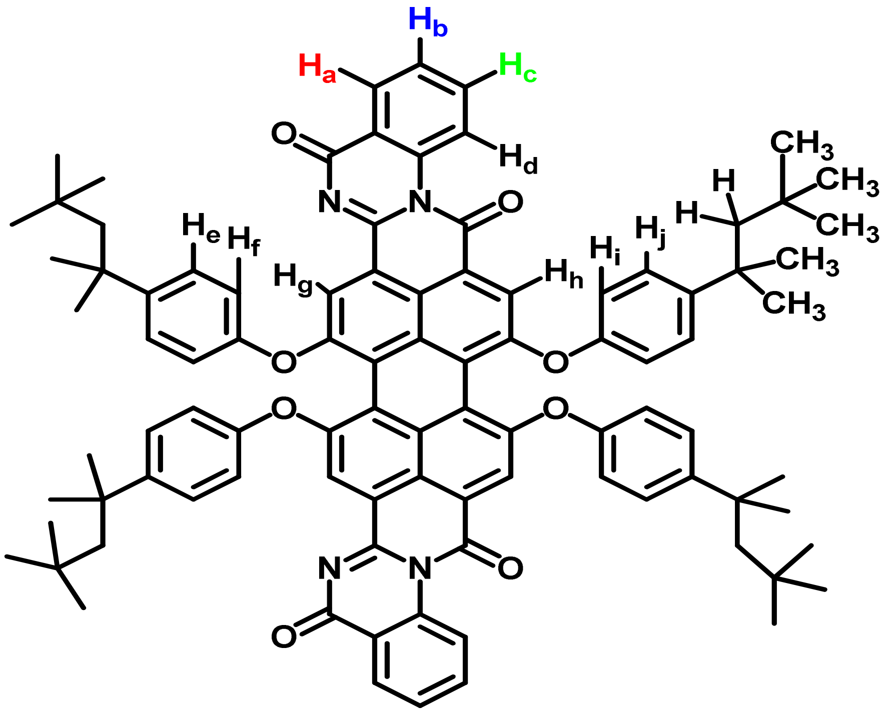
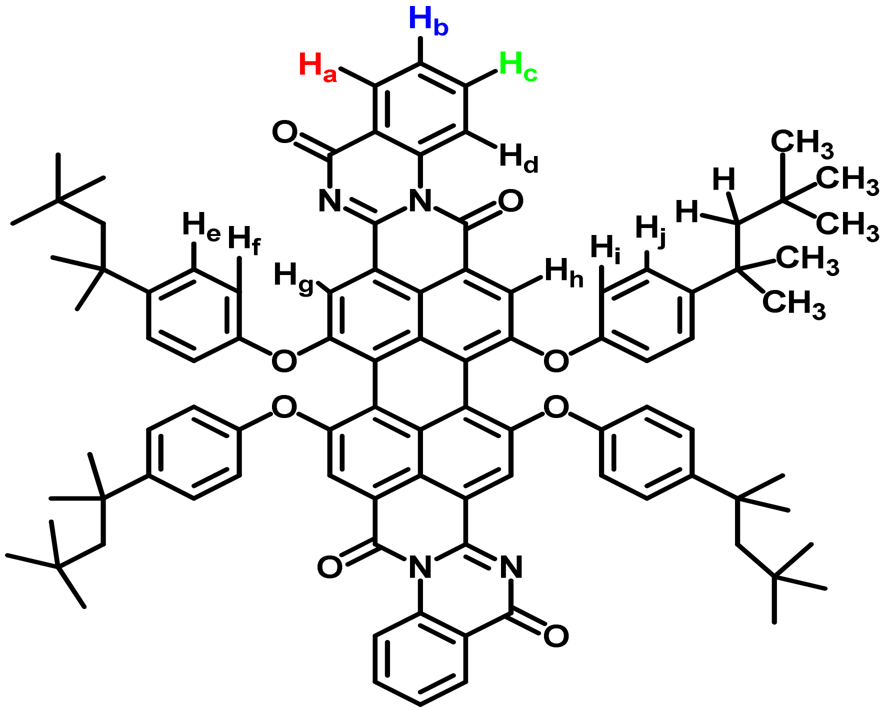
Dye compound from AK Müllen





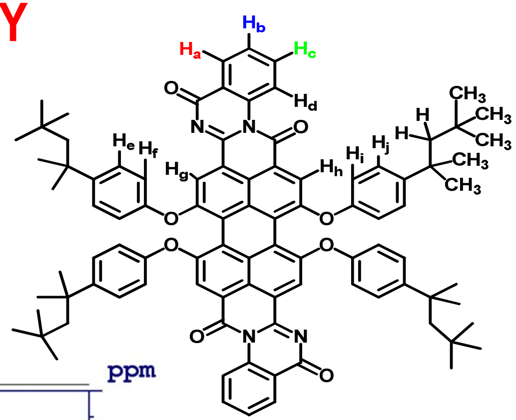
two isomers

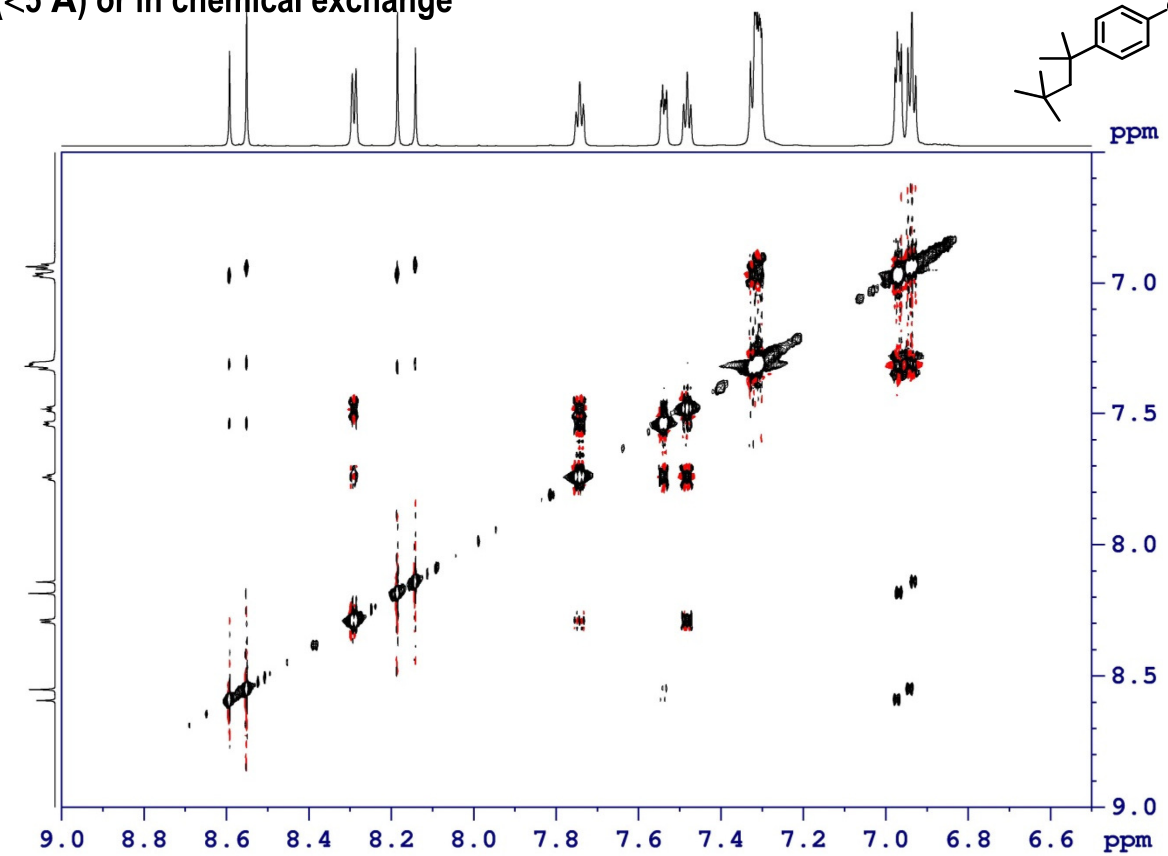
Compound in Phd work from S. Stappert (currently)

NOESY - Nuclear Overhauser Enhanced SpectroscopY

2D H,H-NOESY

protons close in space (<5 Å) or in chemical exchange

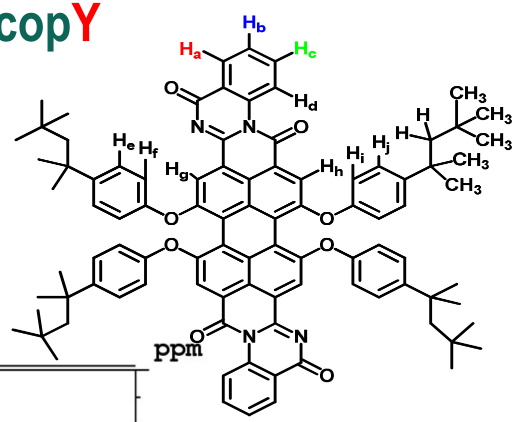


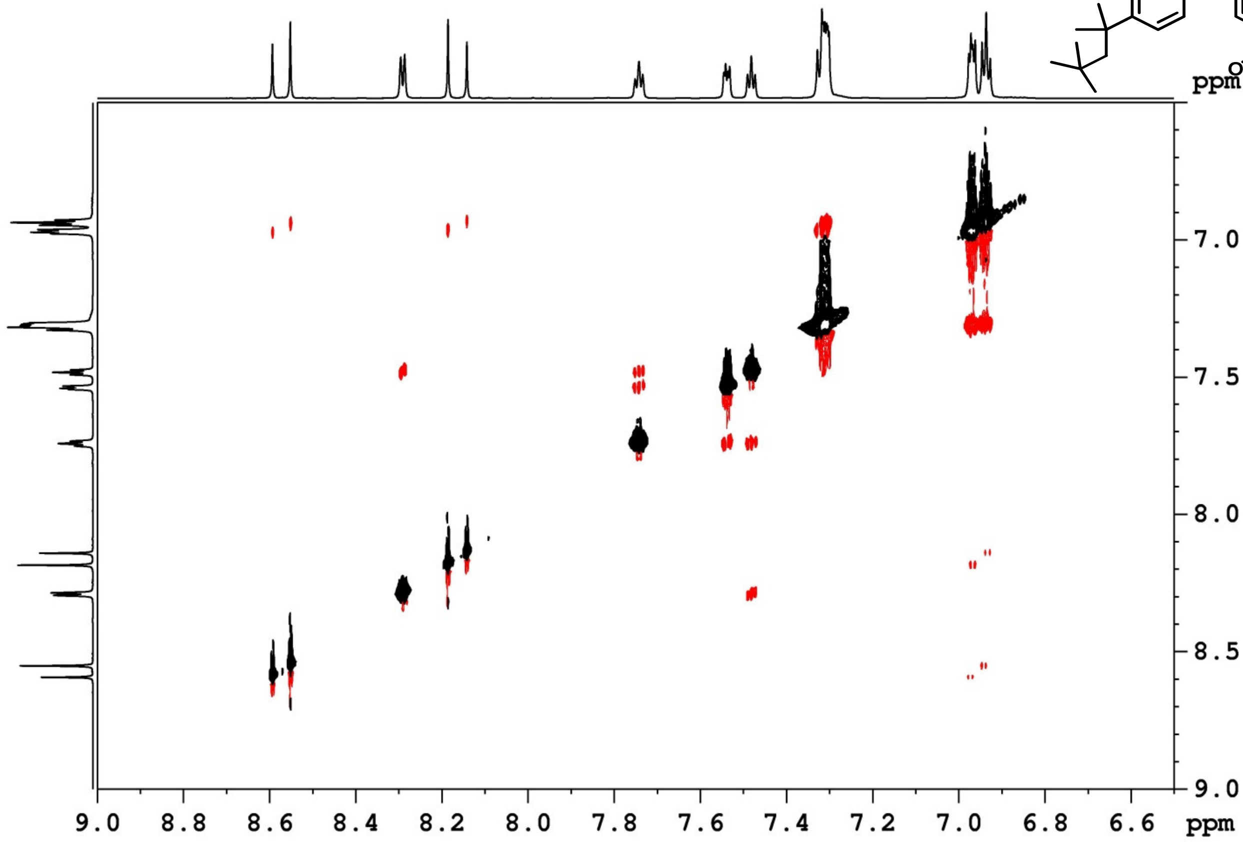


Nuclear Magnetic Resonance (NMR)

ROESY - Rotating Frame Overhauser Effect SpectroscopY

2D H,H-ROESY





Nuclear Magnetic Resonance (NMR)

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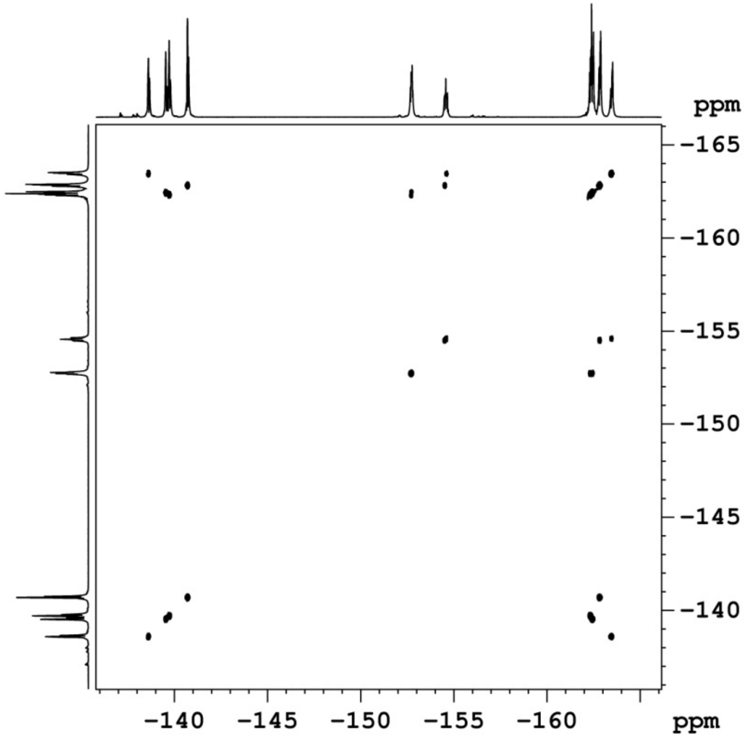
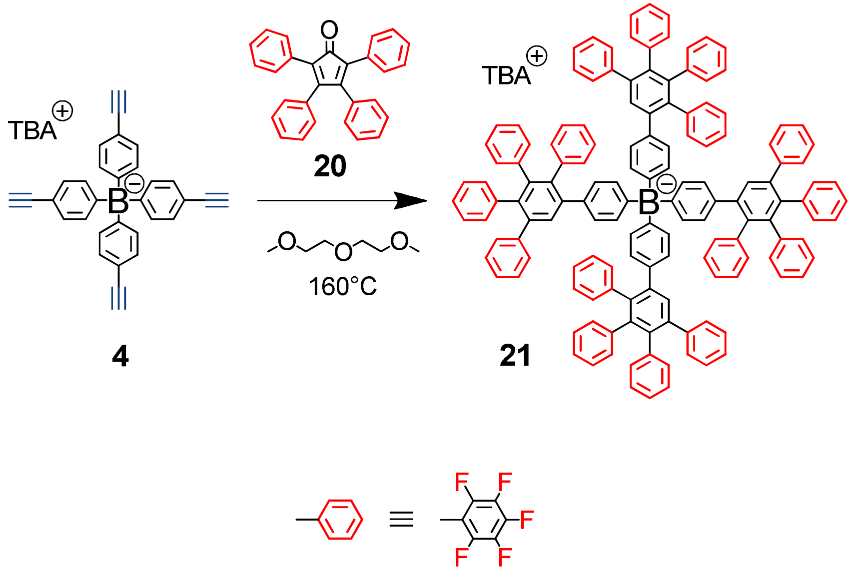
2D ^{19}F COSY 2D correlation

also possible with ^{31}P





Ph. D work D. Türp measured 470 MHz

Nuclear Magnetic Resonance (NMR)

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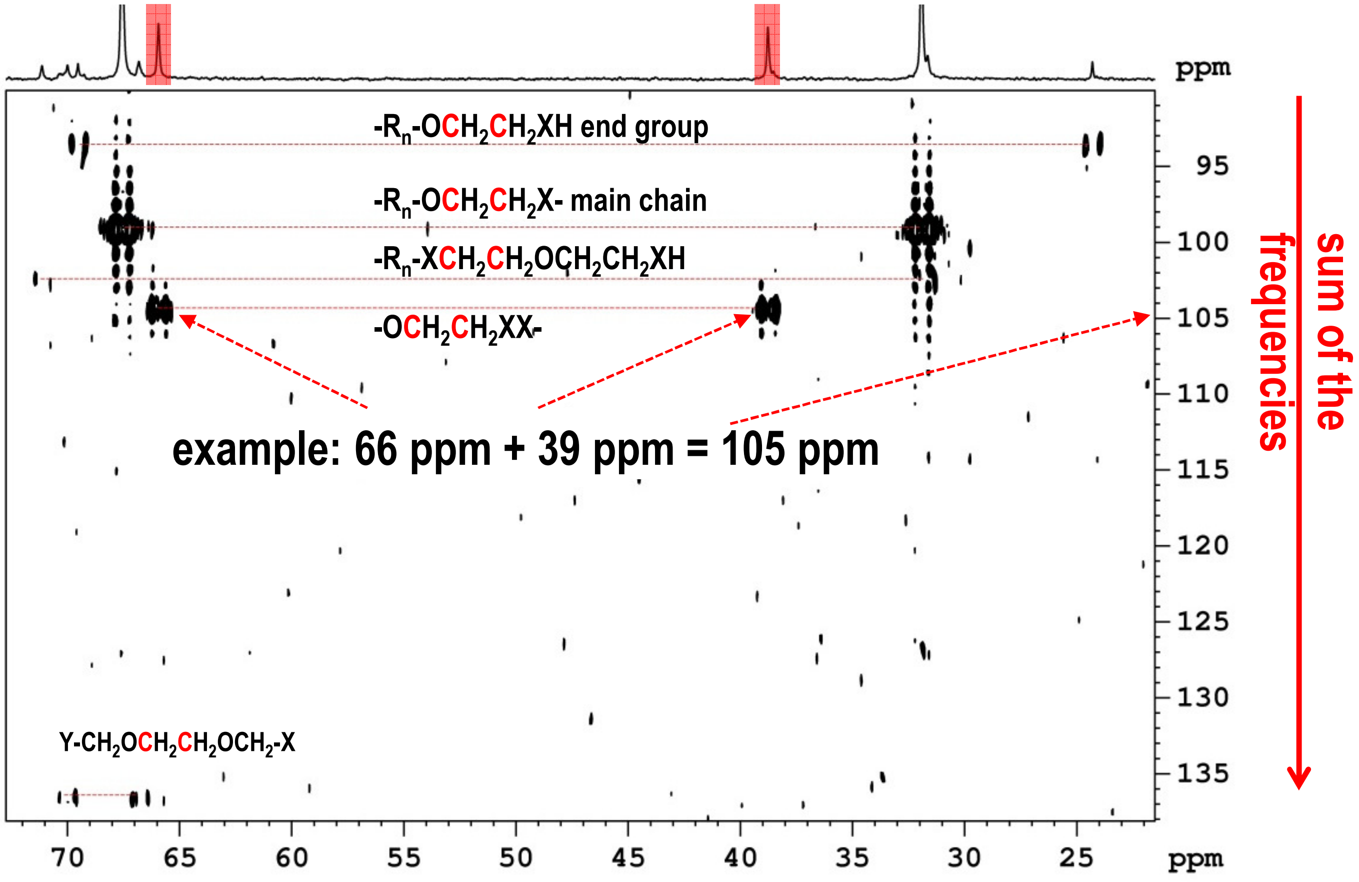
2D C,C-INADEQUATE
(Incredible Natural Abundance DoublE QUAntum Tranfer Experiment)



Example of a liquid sealant

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➢ 2D "HETERO" Experiments

- ➢ **Introduction and theory (Acronyms?)**
- ➢ **Example from our house**

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Introduction and theory

Correlation of nuclei from different species

via bond correlation

via space correlation (mainly dipole-dipole)

Nuclear Magnetic Resonance (NMR)

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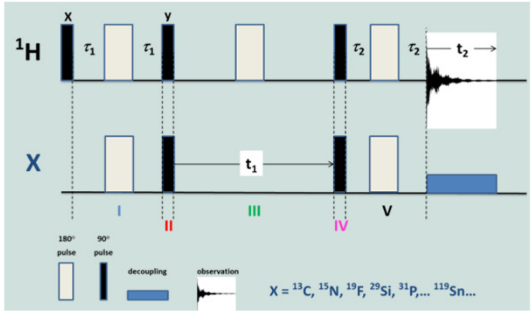
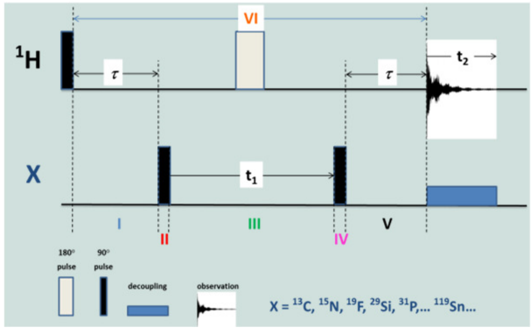
Acronyms

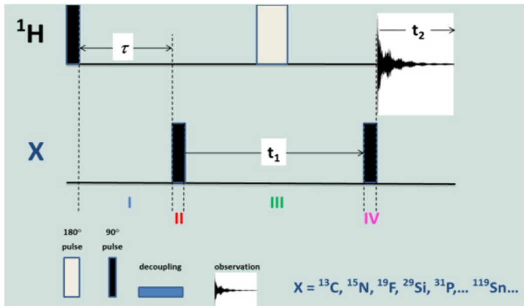
HSQC (Hetero-Single-Quant-Correlation)



HMQC (Hetero-Multiple-Quant-Correlation)



HMBC (Hetero-Multiple-Bond-Correlation)



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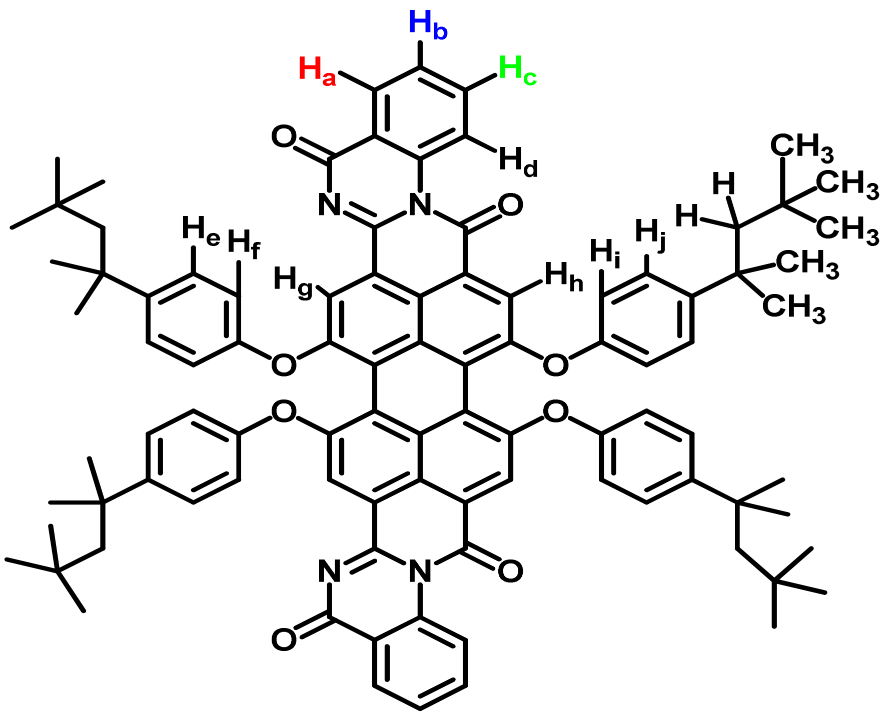
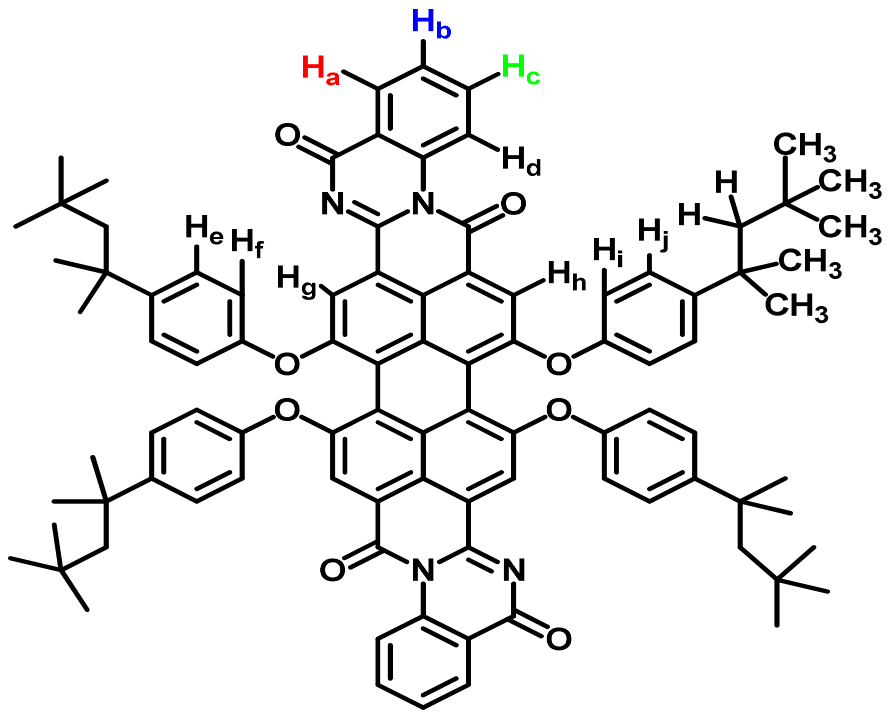
Dye compound from AK Müllen





two isomers

Compound in Phd work from S. Stappert (currently synthesized)

Nuclear Magnetic Resonance (NMR)

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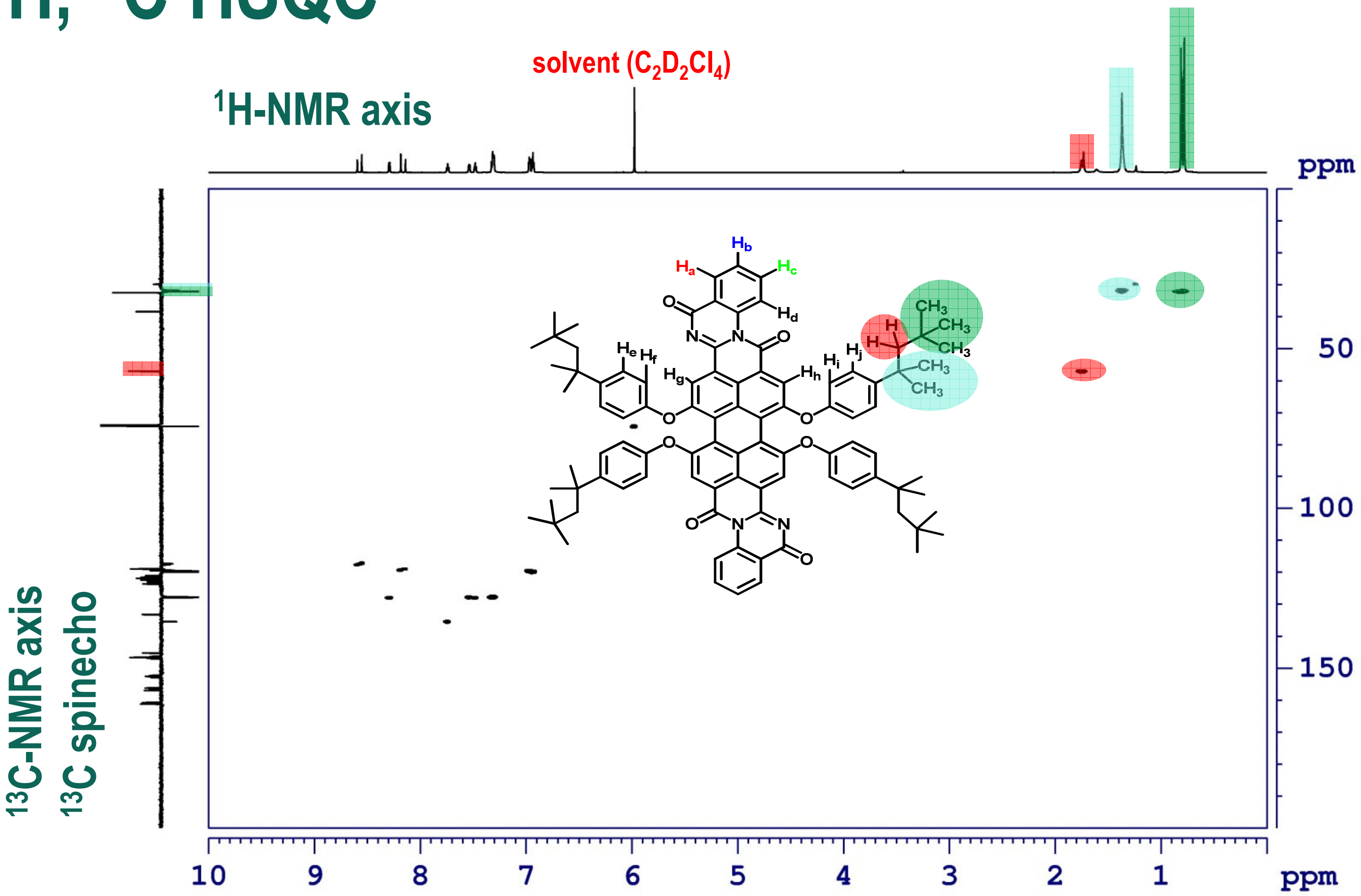
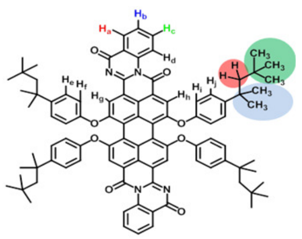
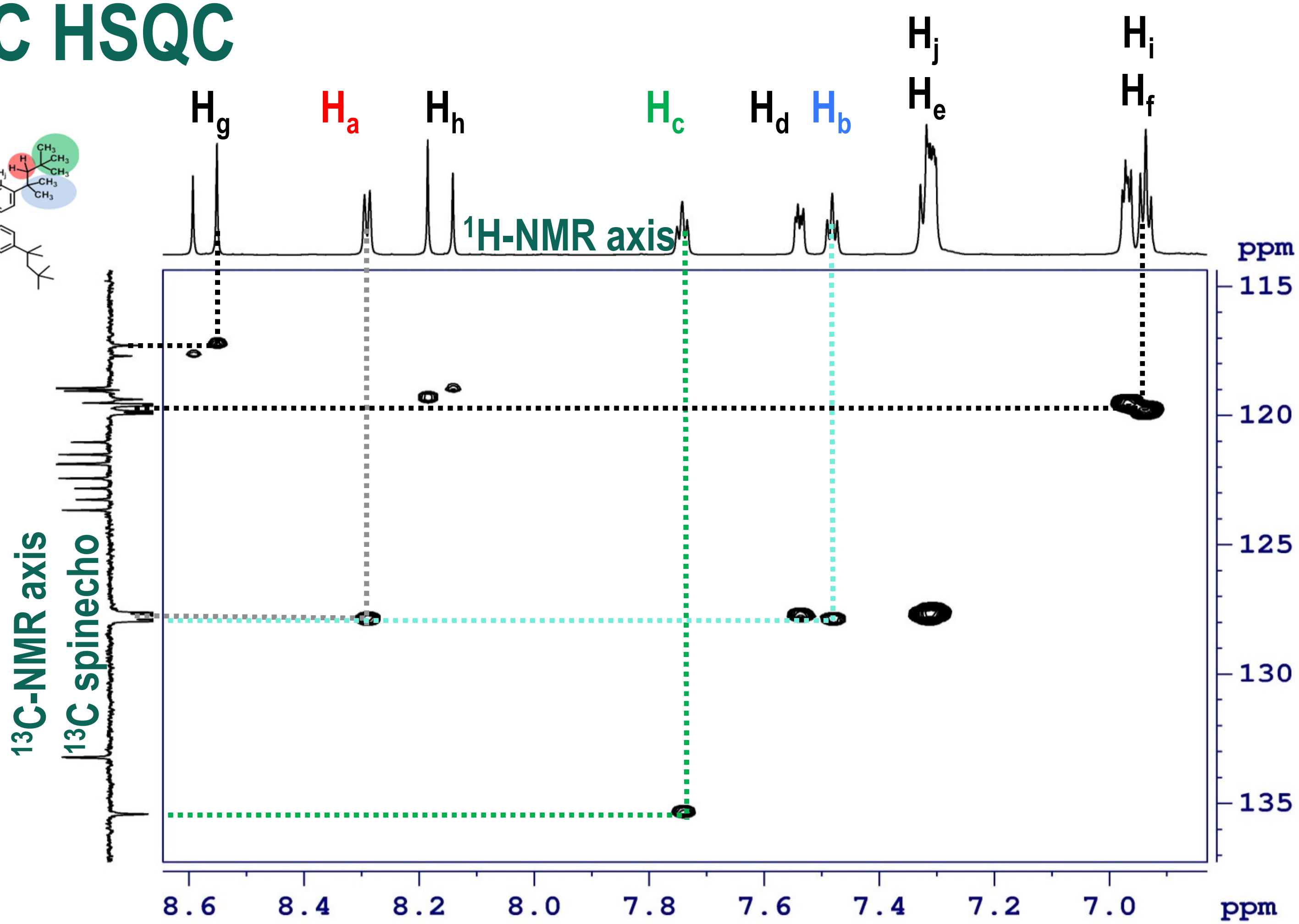
2D $^1H,^{13}C$ HSQC

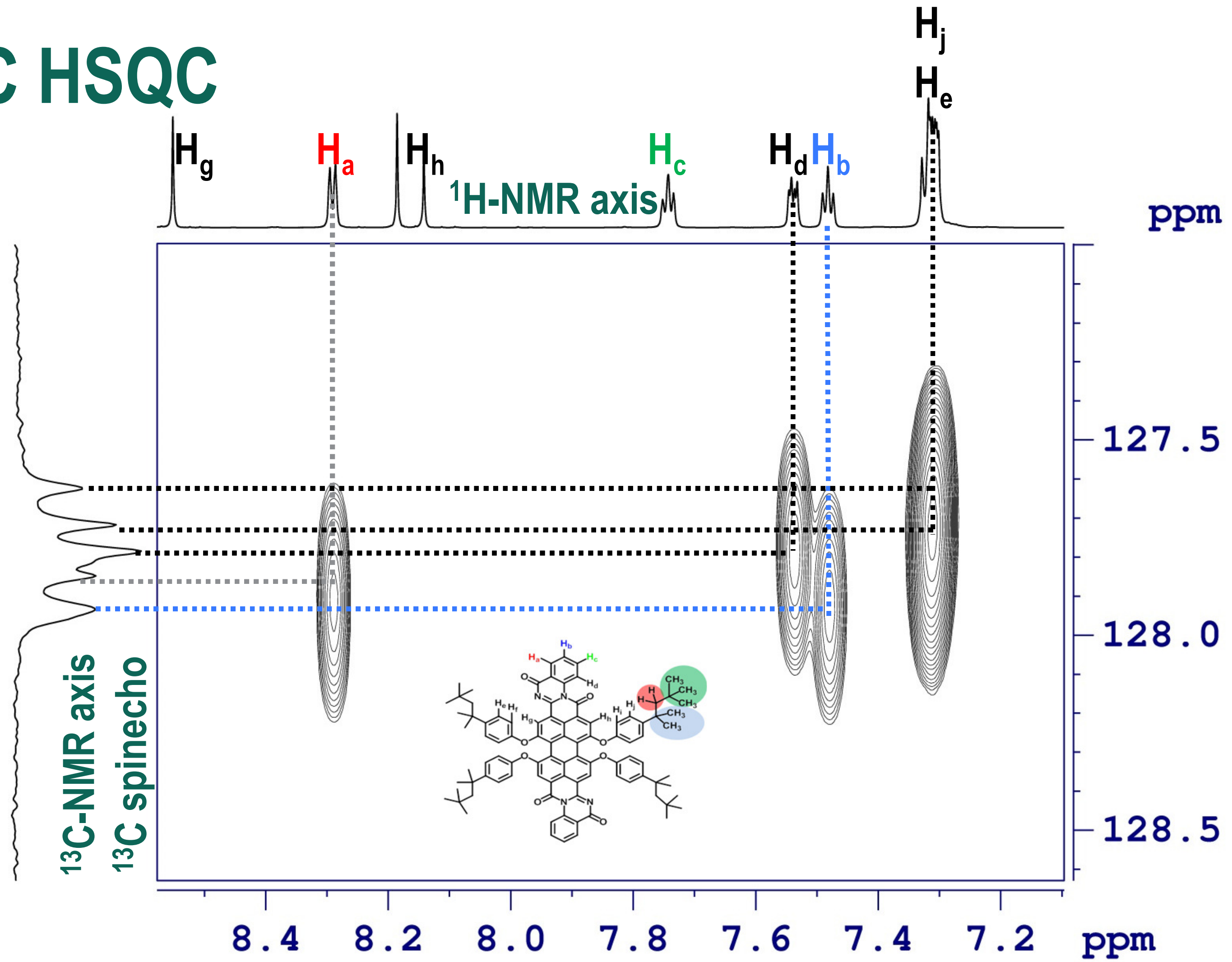


2D $^{1}H,^{13}C$ HSQC





2D $^{1}H,^{13}C$ HSQC



Nuclear Magnetic Resonance (NMR)

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2D $^1H,^{13}C$ HSQC

even and odd:

(CH; CH_2; CH_3) same color

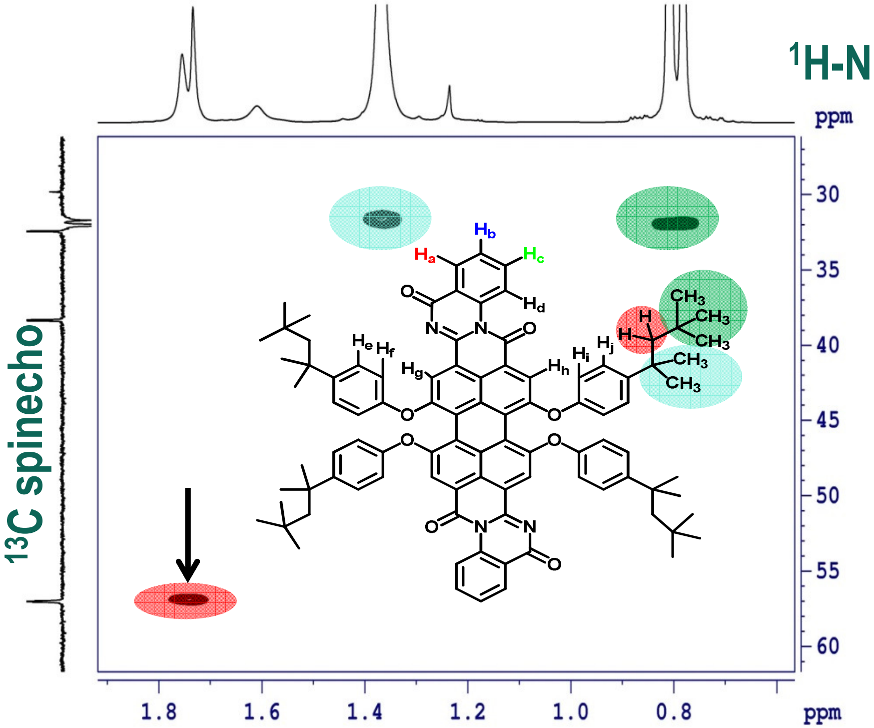
2D $^1H,^{13}C$ HSQC-edit

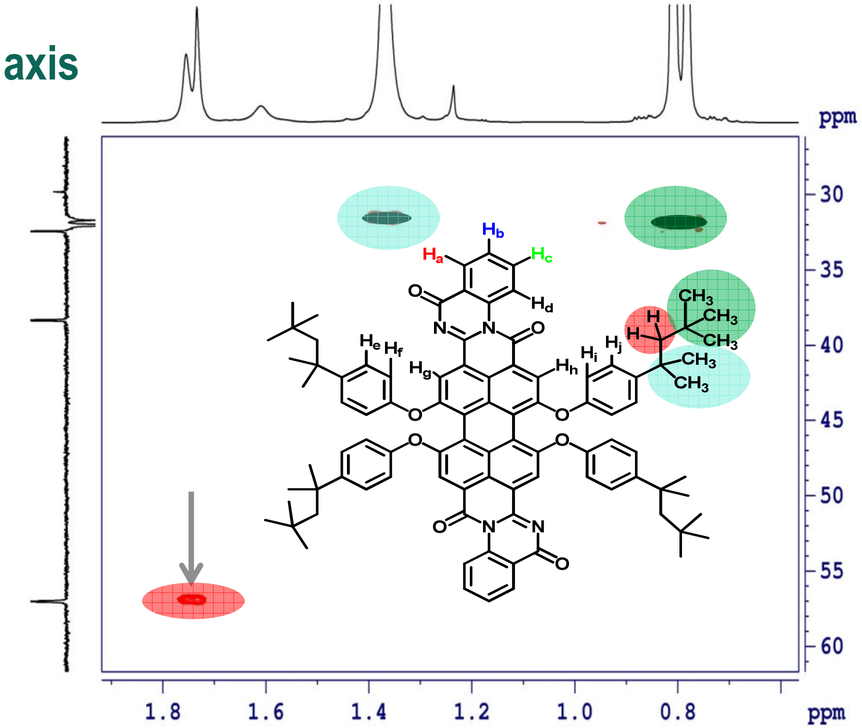
odd:	**black**	**(CH; CH_3)**
even:	**red**	**(CH_2)**

^1H-NMR axis

^{13}C-NMR axis

^{13}C spinecho





^1J: C-H (125 -145Hz)

Nuclear Magnetic Resonance (NMR)

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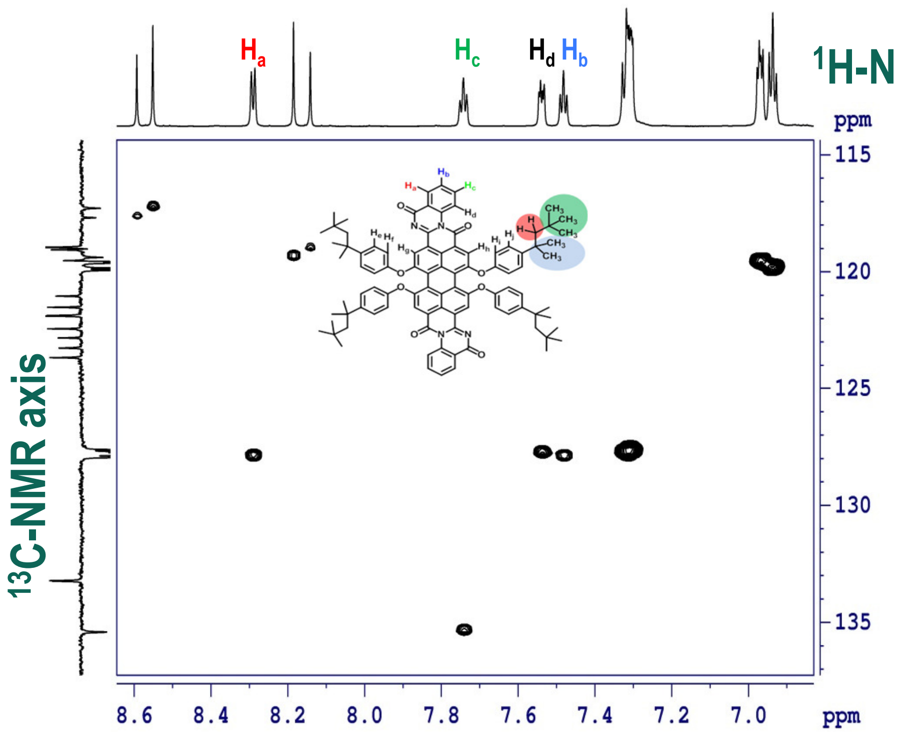
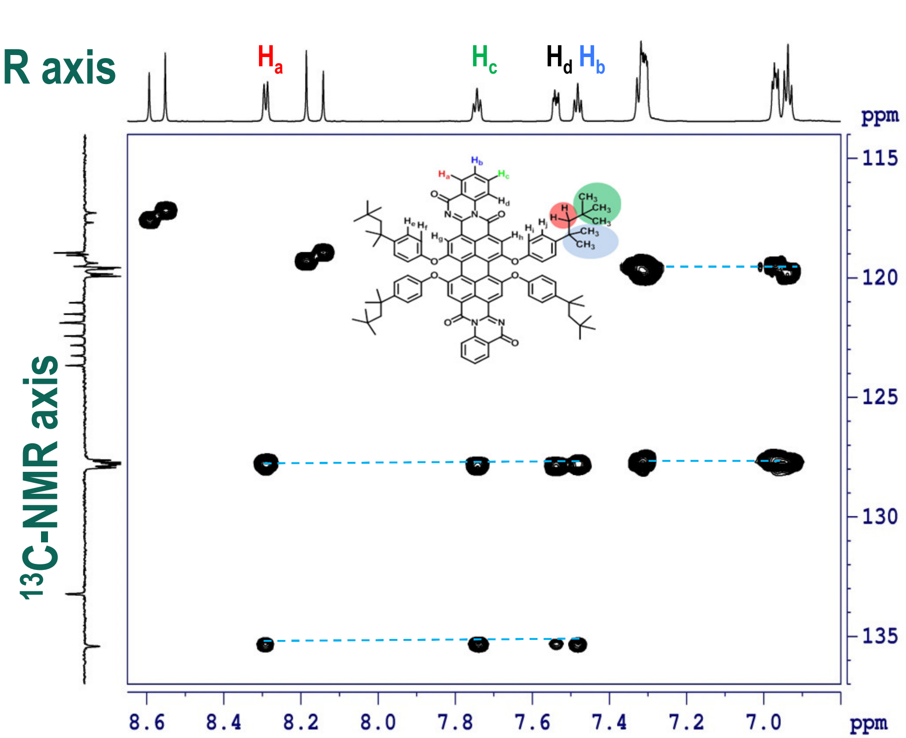
2D $^1H,^{13}C$ HSQC

2D $^1H,^{13}C$ HSQC-TOCSY





^1J: C-H (125 -145Hz);

TOCSY mixing time 80ms

Nuclear Magnetic Resonance (NMR)

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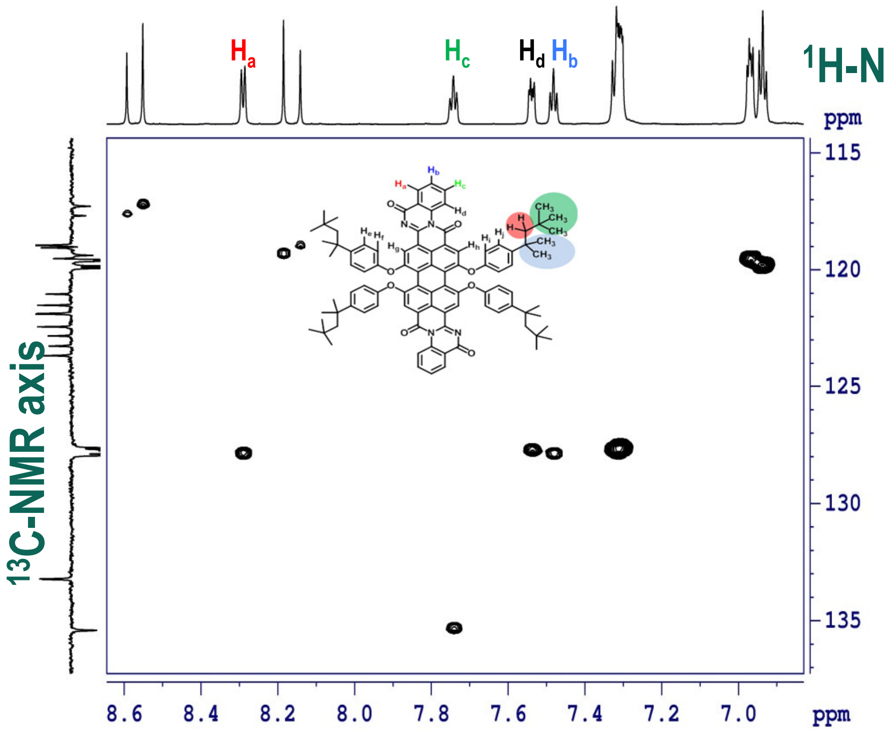
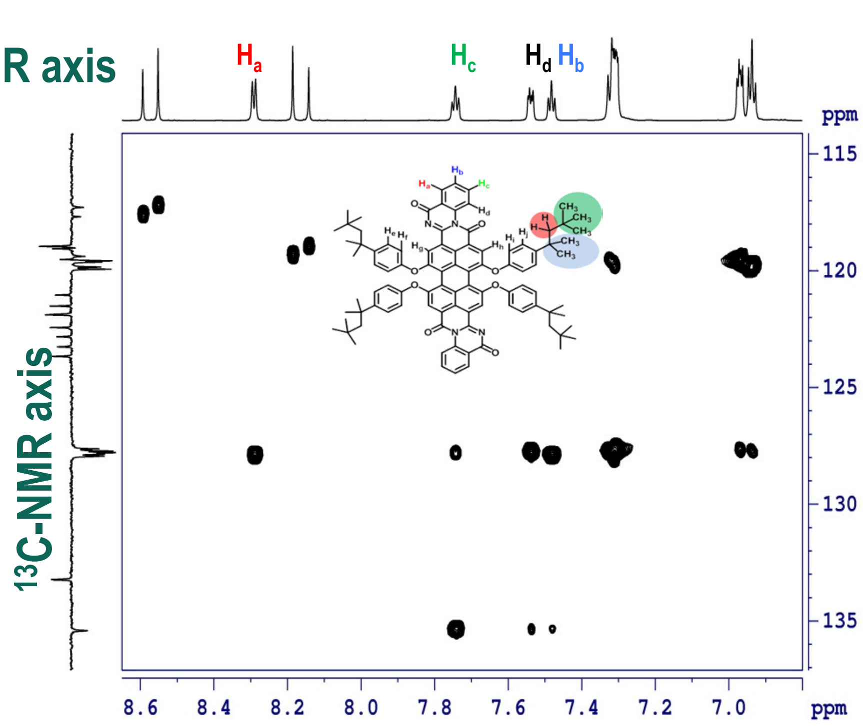
2D $^1H,^{13}C$ HSQC

2D $^1H,^{13}C$ HSQC-NOESY



1H-NMR axis



^1J: C-H (125 -145Hz);

NOESY mixing time 300ms

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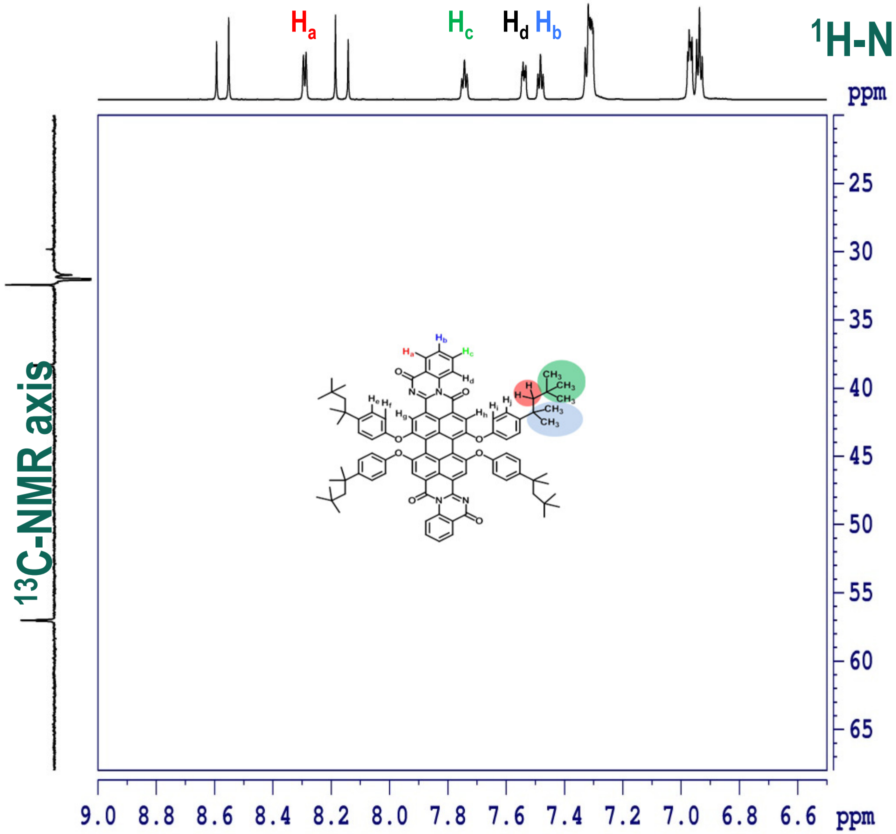
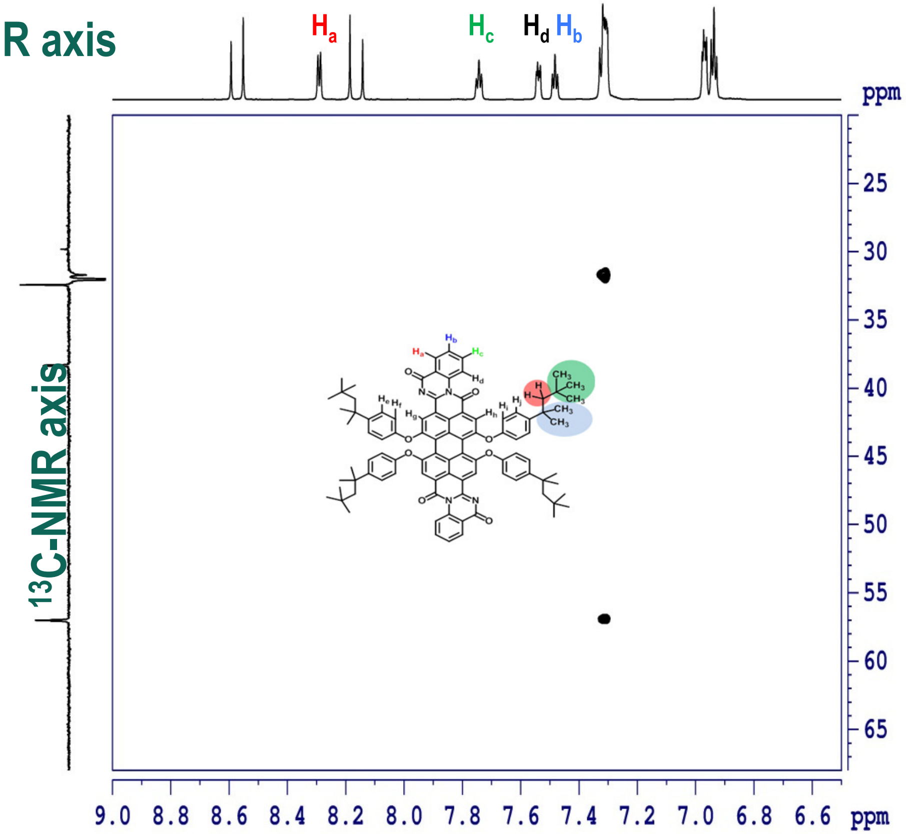
2D $^{1}H,^{13}C$ HSQC-TOCSY

2D $^{1}H,^{13}C$ HSQC-NOESY



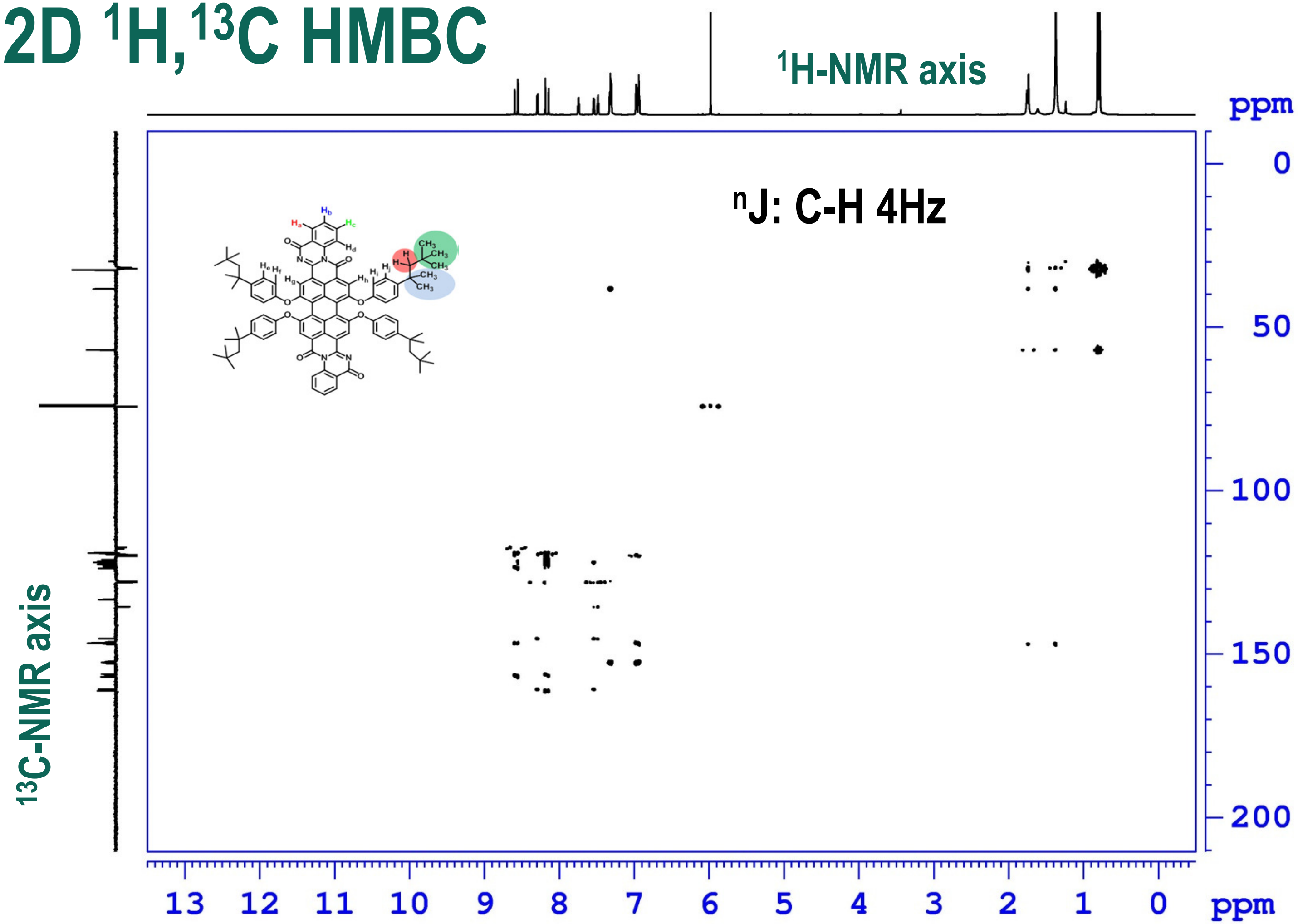
TOCSY mixing time 80ms



NOESY mixing time 300ms

Nuclear Magnetic Resonance (NMR)

2D $^{1}H,^{13}C$ HMBC



Nuclear Magnetic Resonance (NMR)

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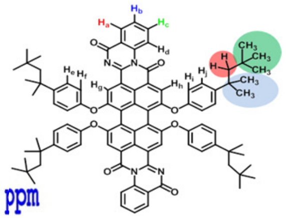
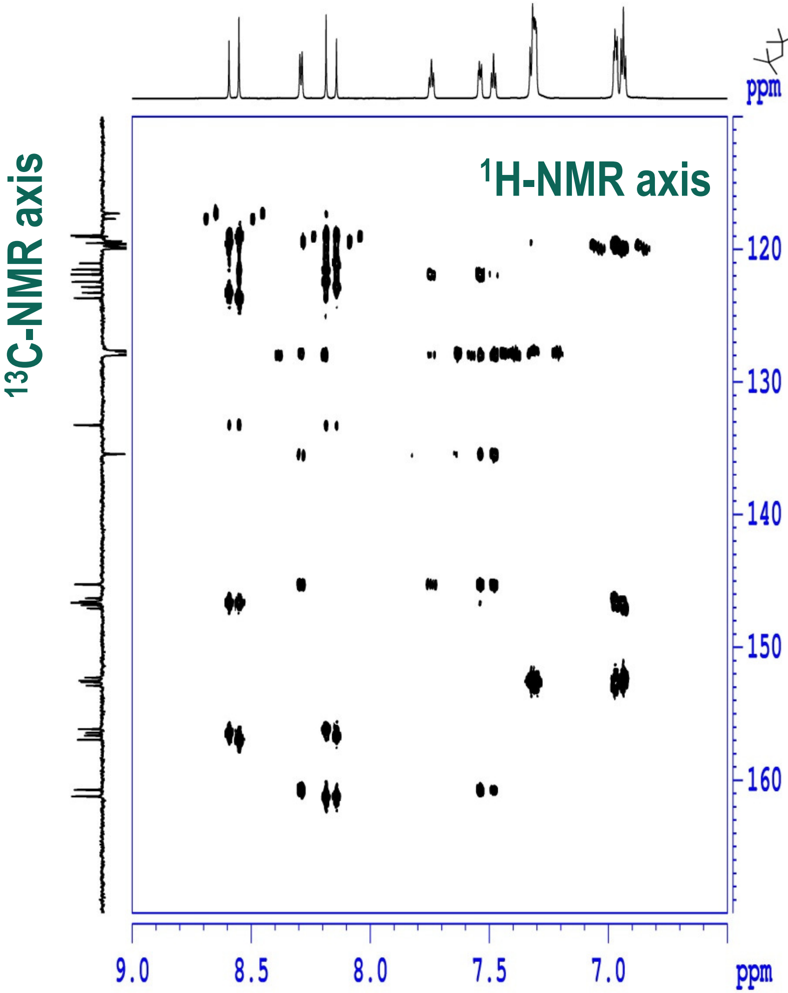
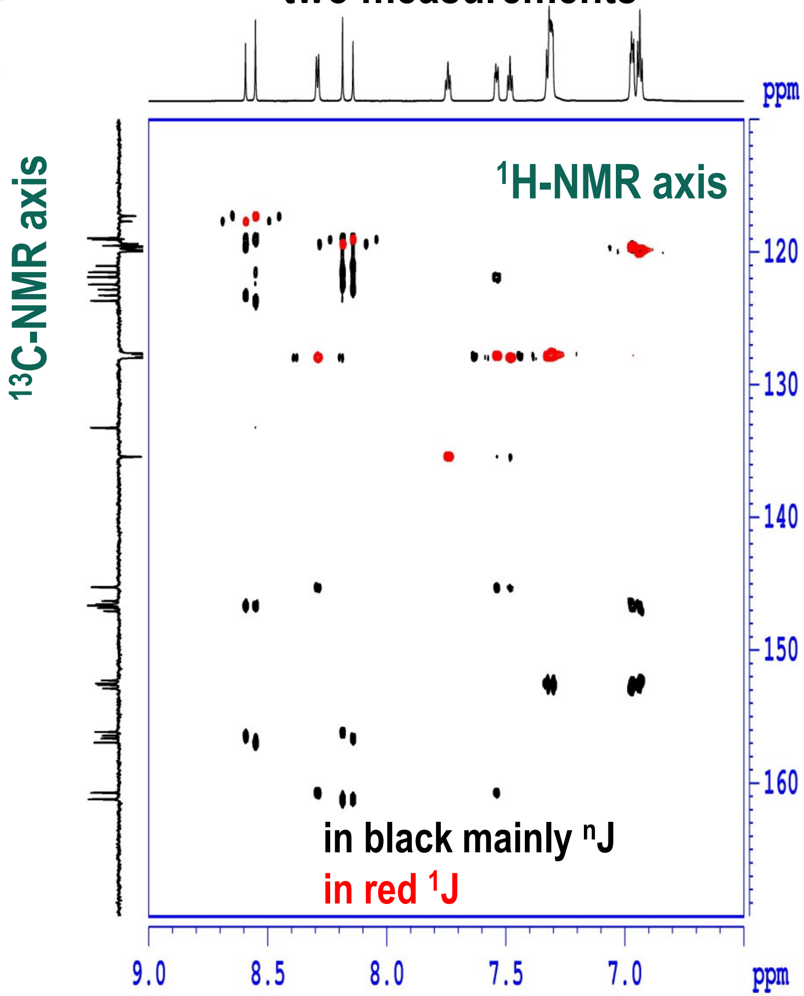
2D $^{1}H,^{13}C$ HMBC





2D: $^{1}H,^{13}C$ HMBC + HSQC

two measurements



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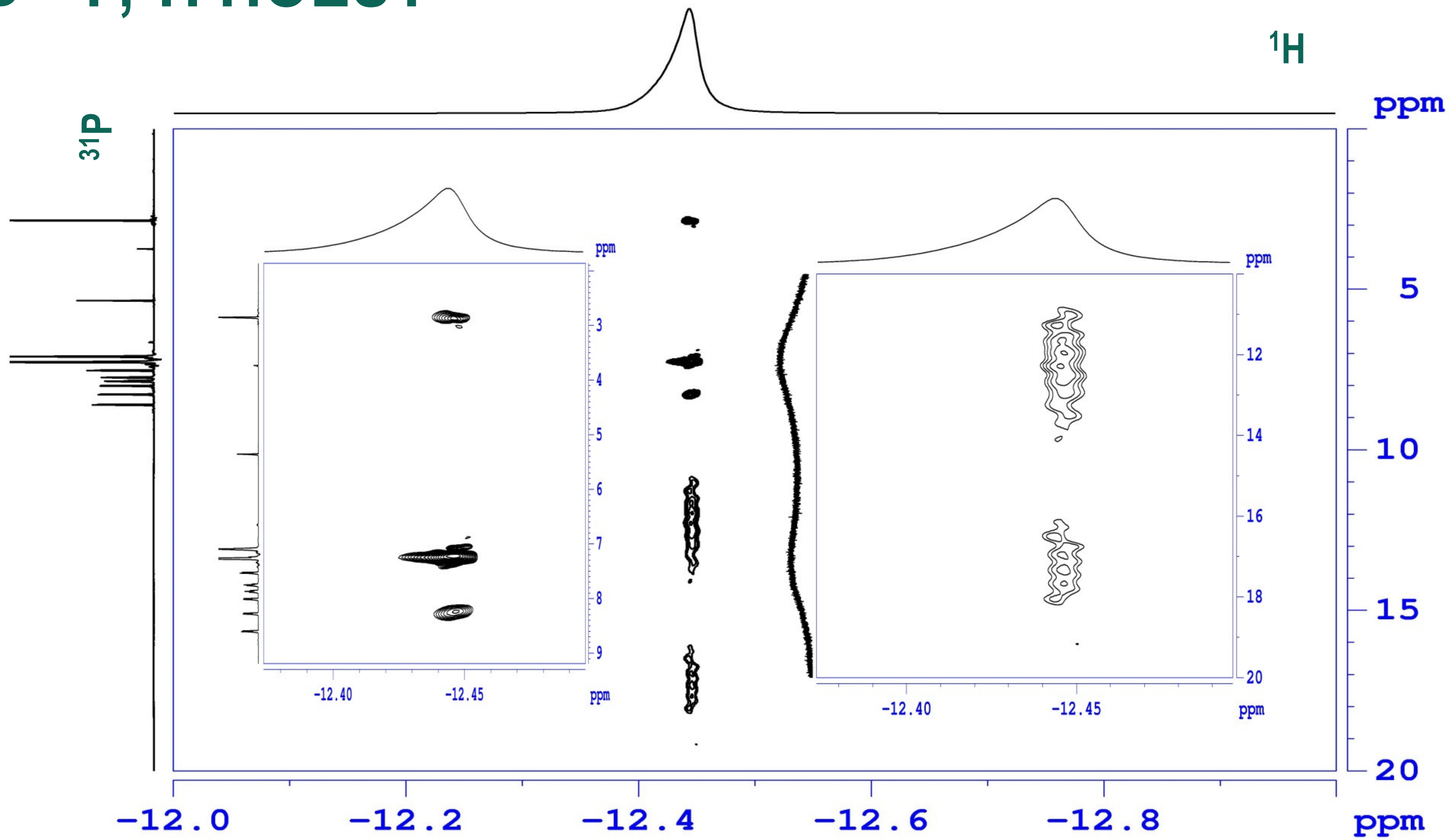
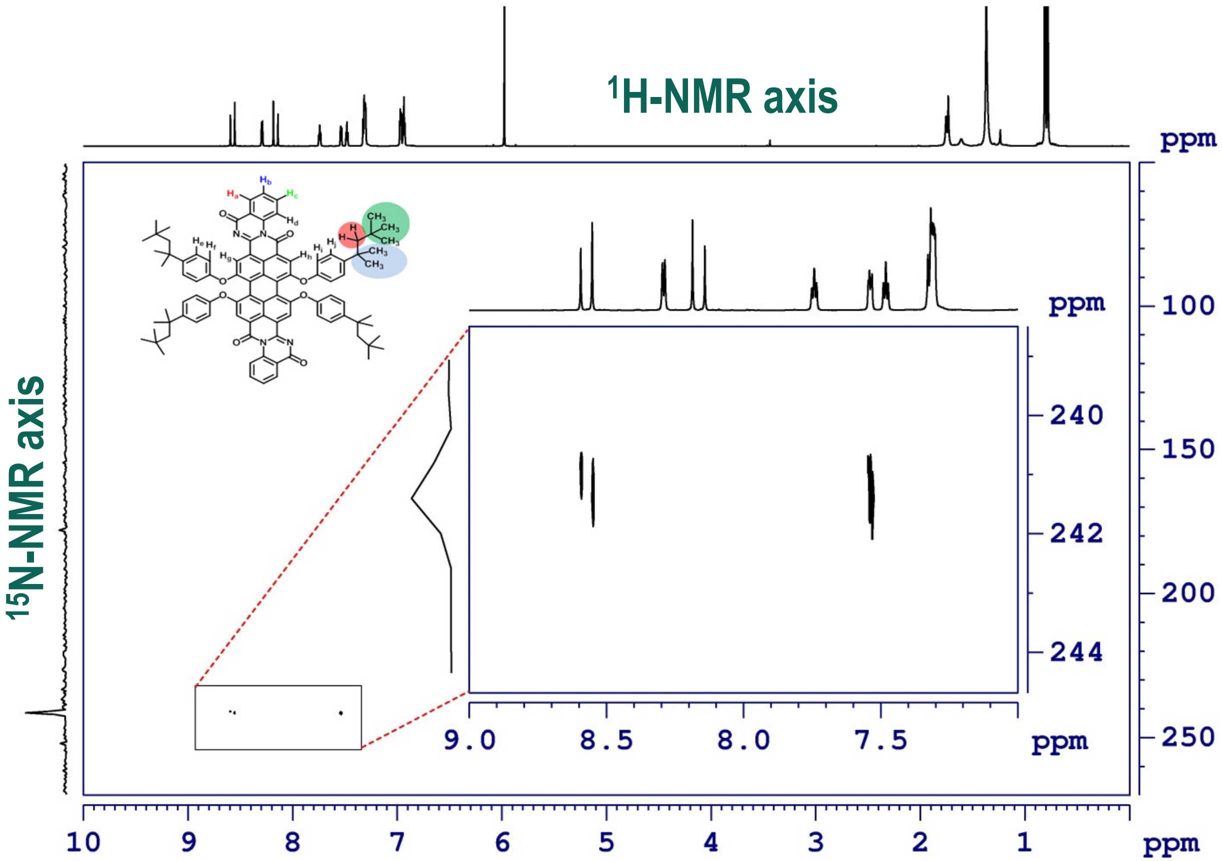
2D $^{31}P,^{1}H$ HOESY



2D ^{1}H,^{15}N HMBC



Nuclear Magnetic Resonance (NMR)

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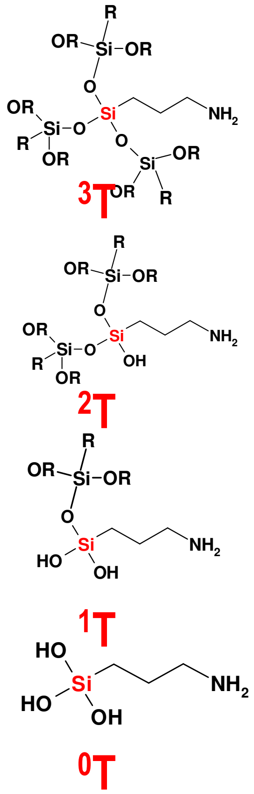
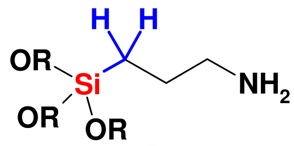
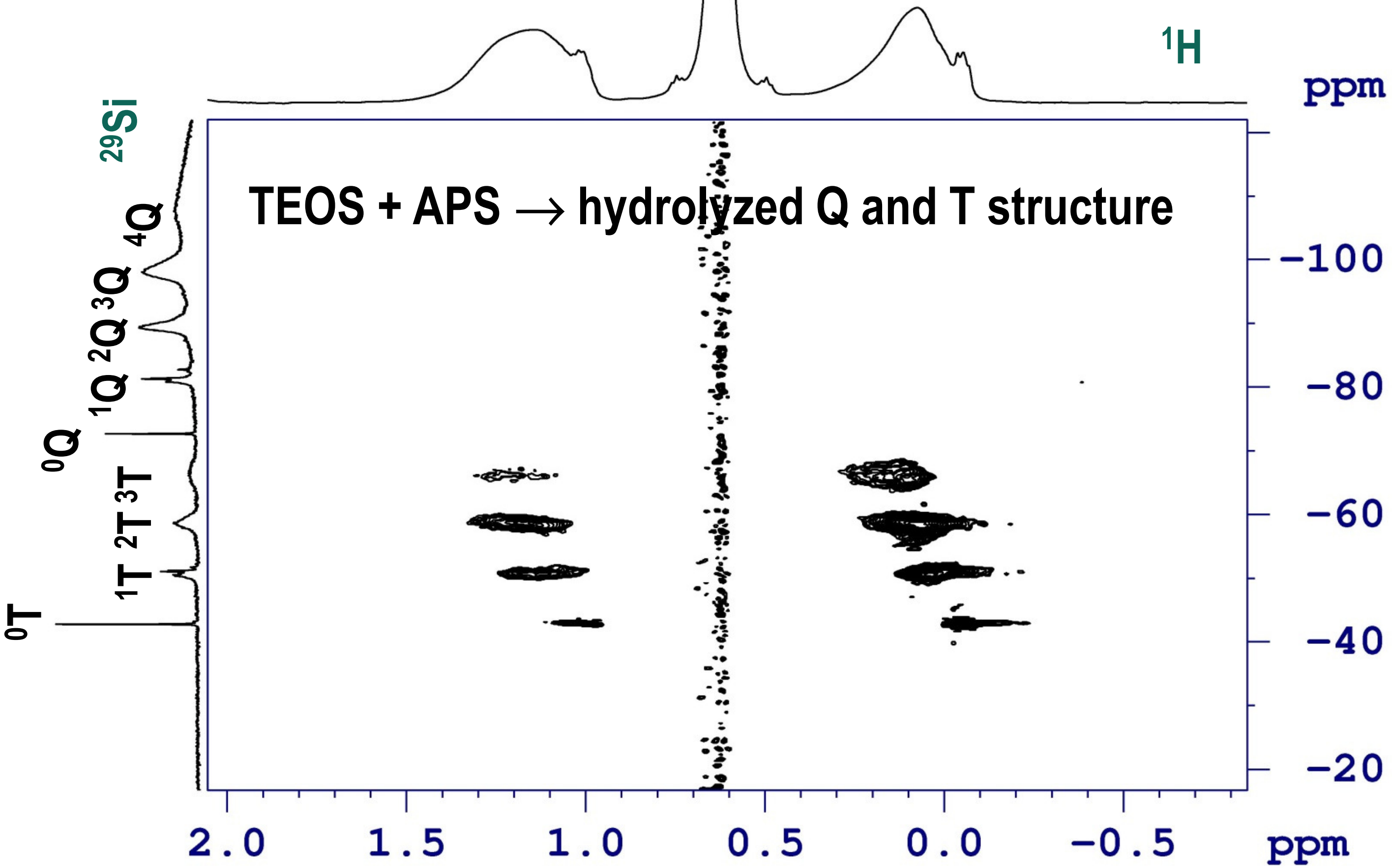
^{1}J 20Hz

2D $^{1}H,^{29}Si$ HSQC or HMBC







Nuclear Magnetic Resonance (NMR)

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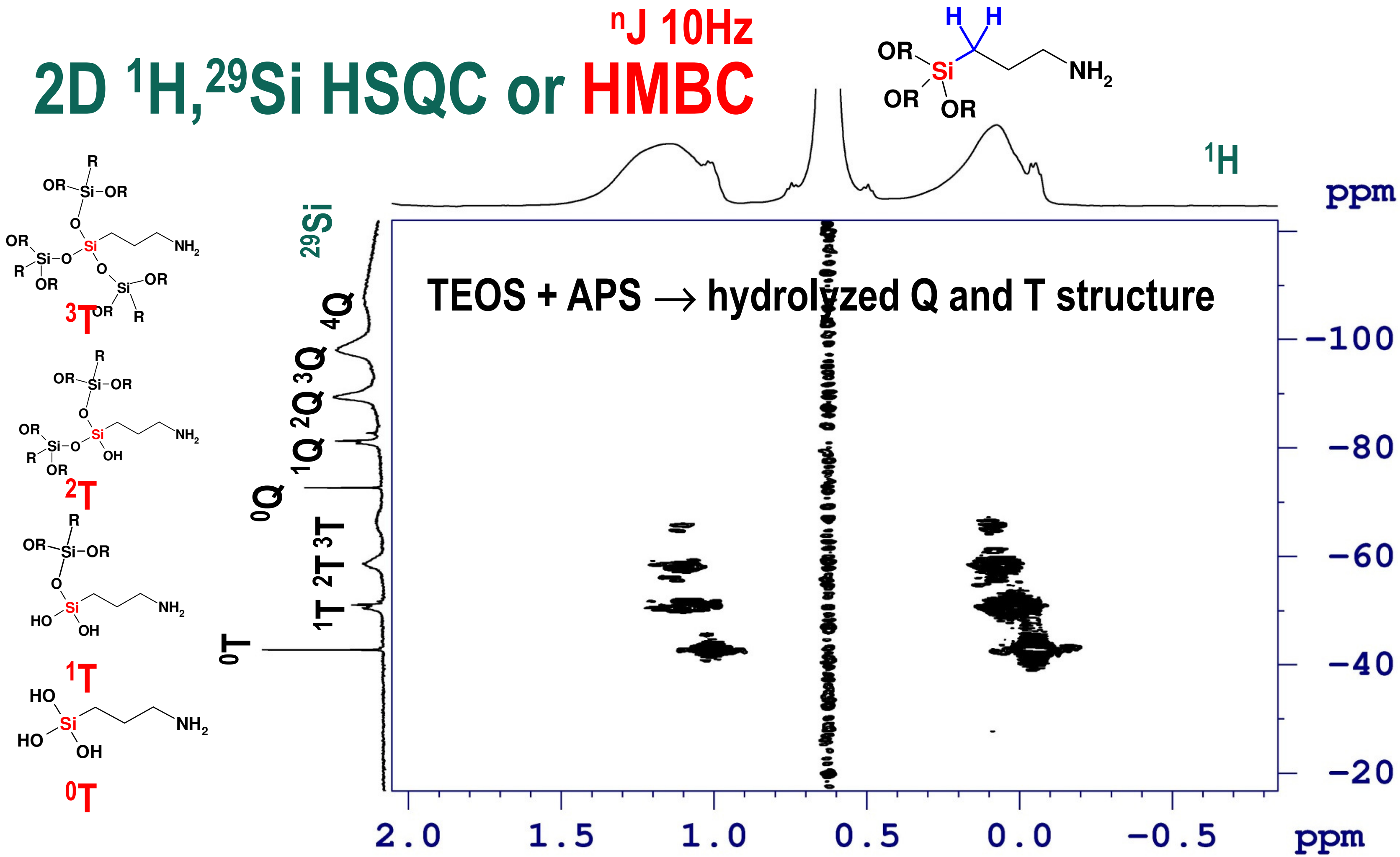
2D ^{1}H,^{29}Si HSQC or HMBC

^{n}J 10Hz



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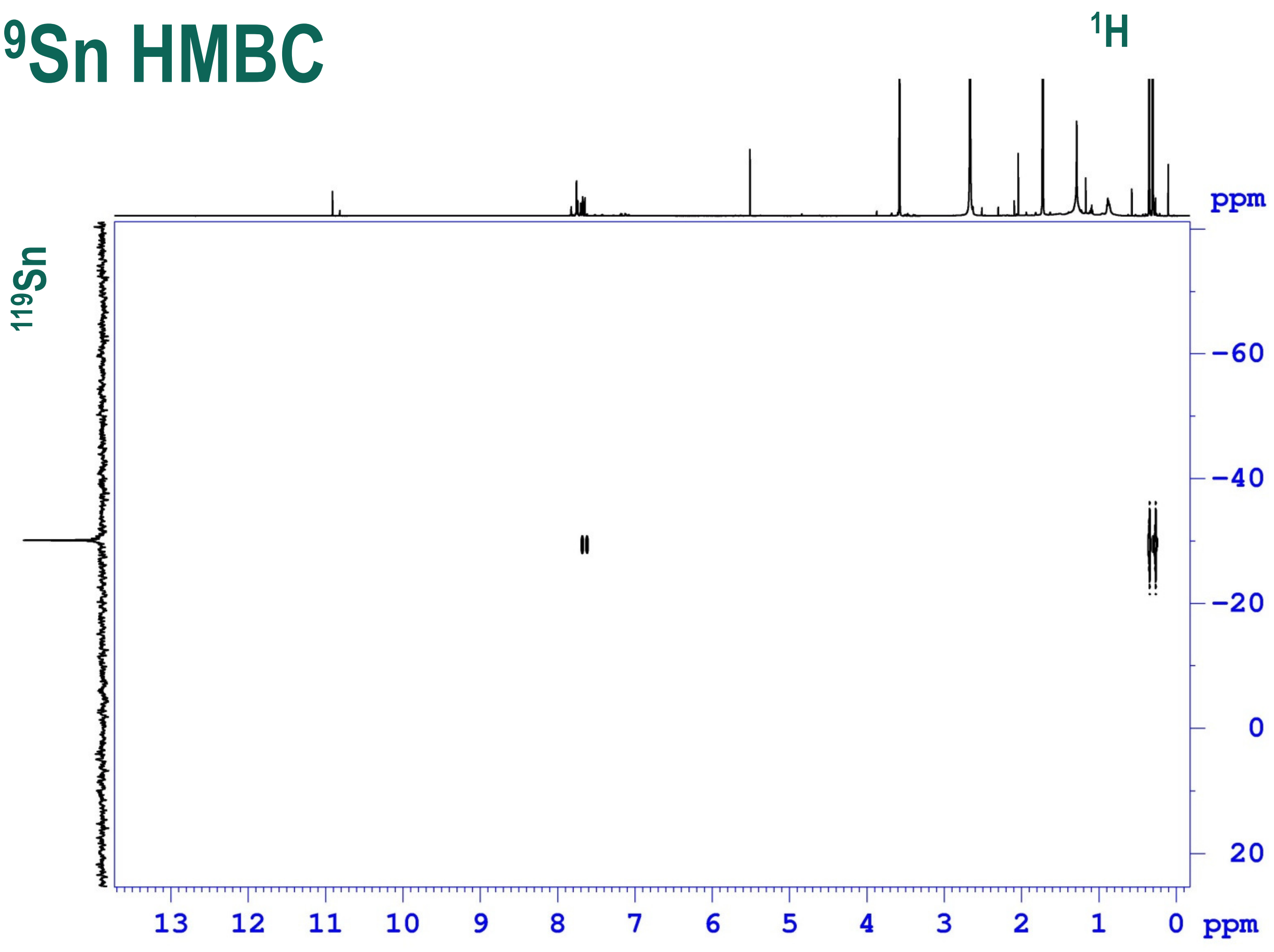
2D ^{1}H,^{119}Sn HMBC



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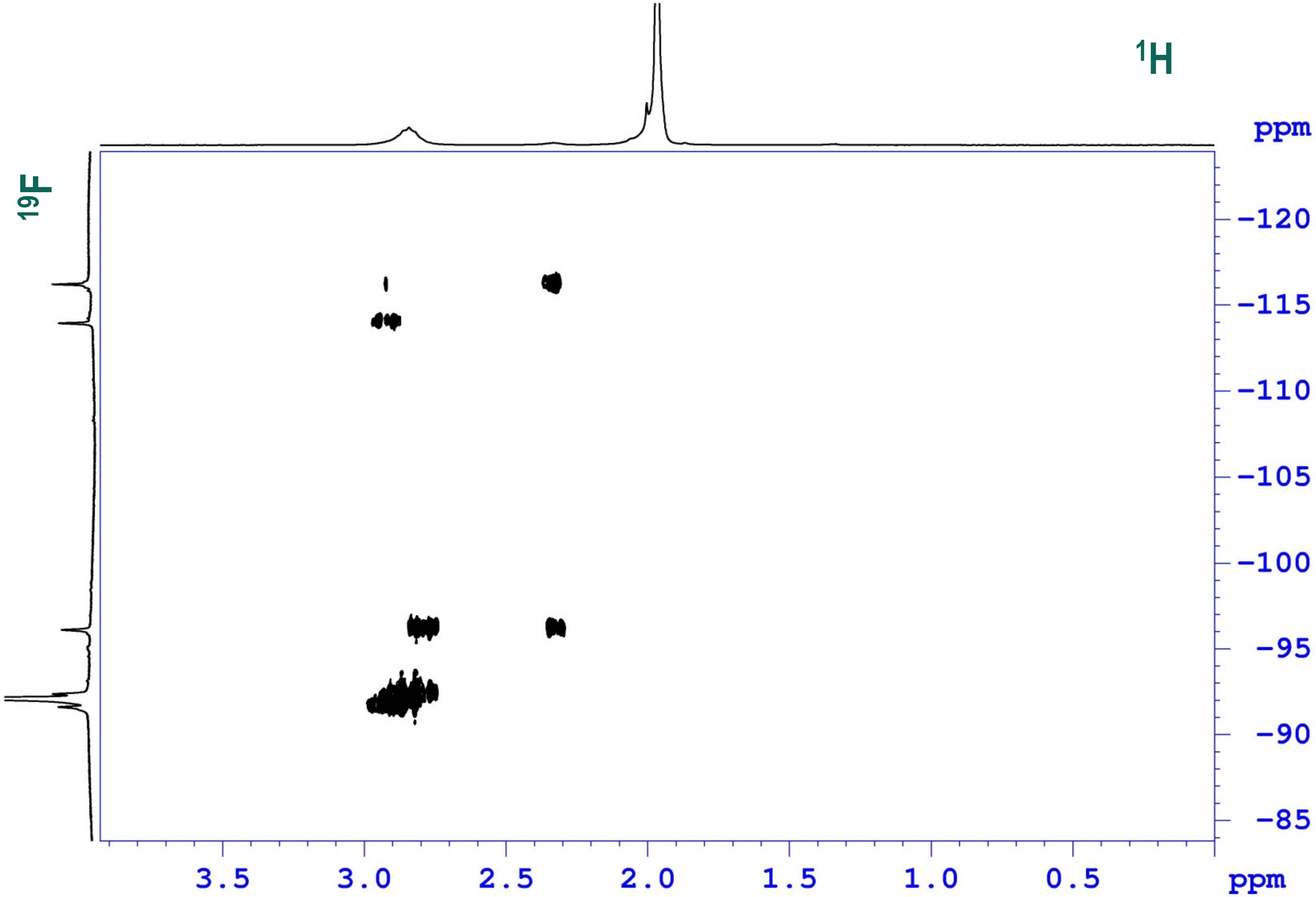
2D $^{1}H,^{19}F$ HMBC



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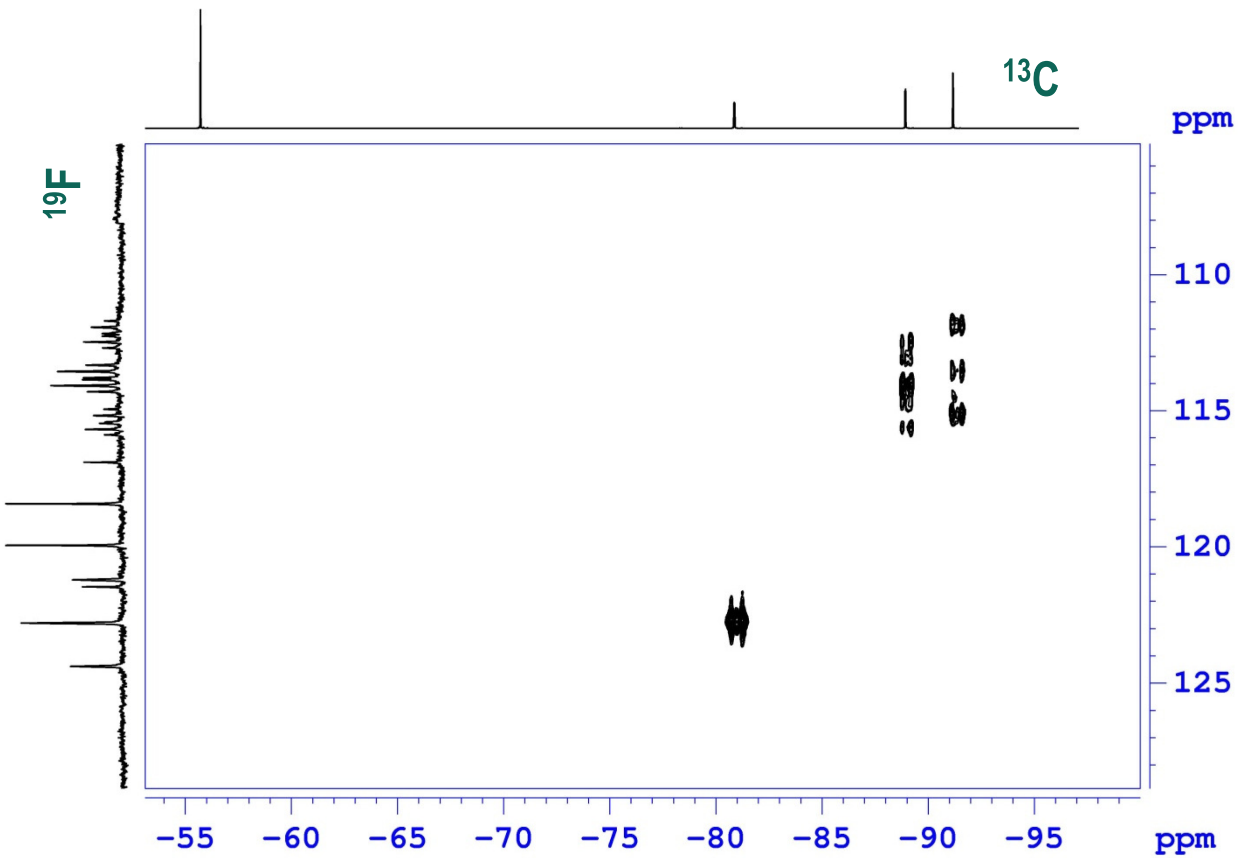
2D $^{19}F,^{13}C$ HSQC



Manfred Wagner

Nuclear Magnetic Resonance (NMR)

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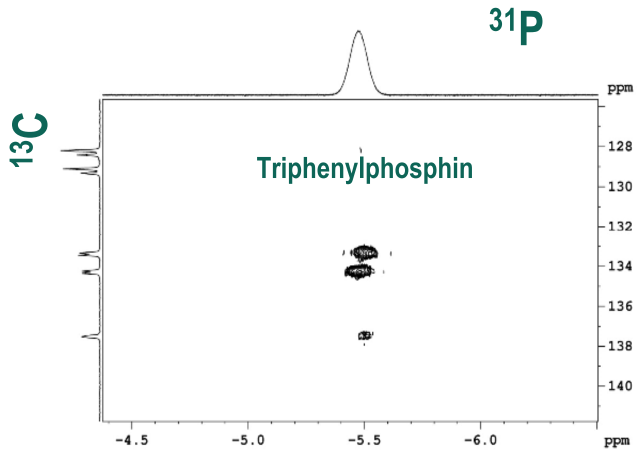
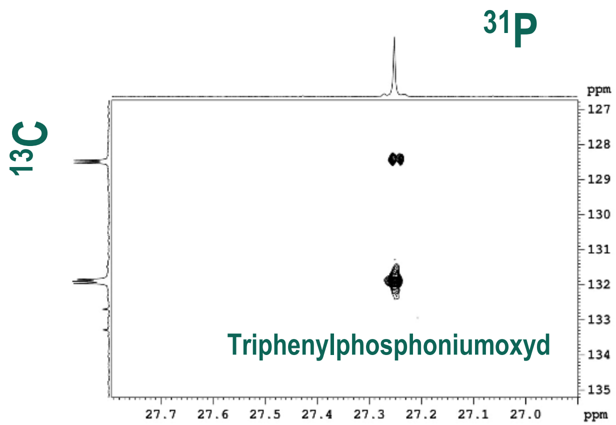
2D X-Y correlation (e.g. ^{13}C-^{31}P) of charged and dendritic structures

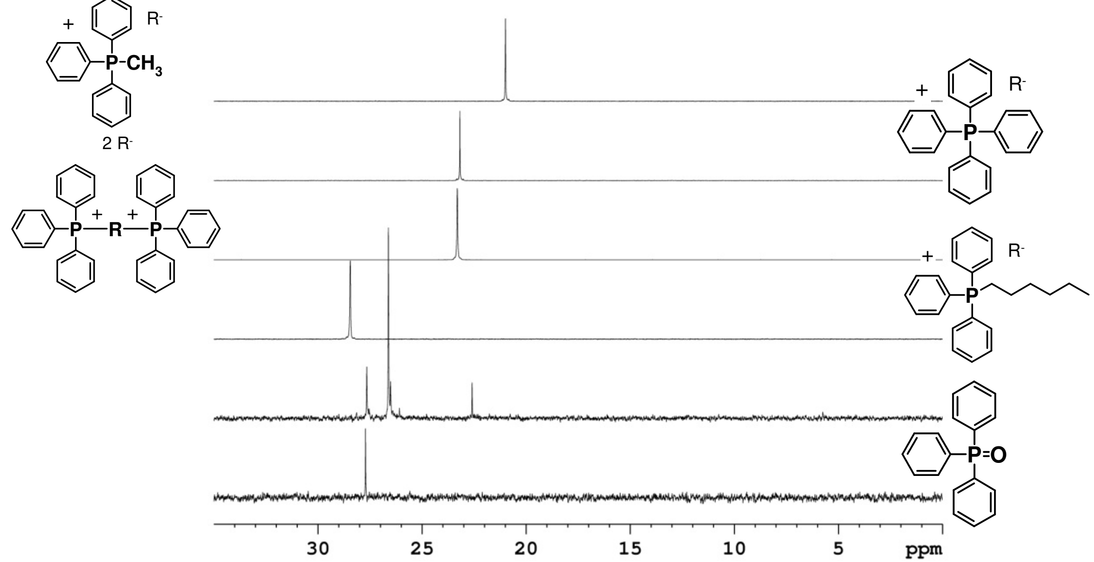
^{31}P NMR





2D- ^{31}P,^{13}C NMR HSQC 2D correlation with ^{1}H decoupling

^{31}P 1D measurement



Differentiation of the cations based on different phosphoric-structures:

- ^{13}C-^{31}P 2D/3D-correlation,
- ^{31}P NMR Chemical Shift,
- T_1-Relaxationrate as a function of the symmetry of the different Phosphor compound,
- Mol mass calibration against a standard system with quant. ^{31}P-NMR!

700 MHz Magnet TXI Probe (^{1}H/^{31}P/X/^{2}H) with z-gradient

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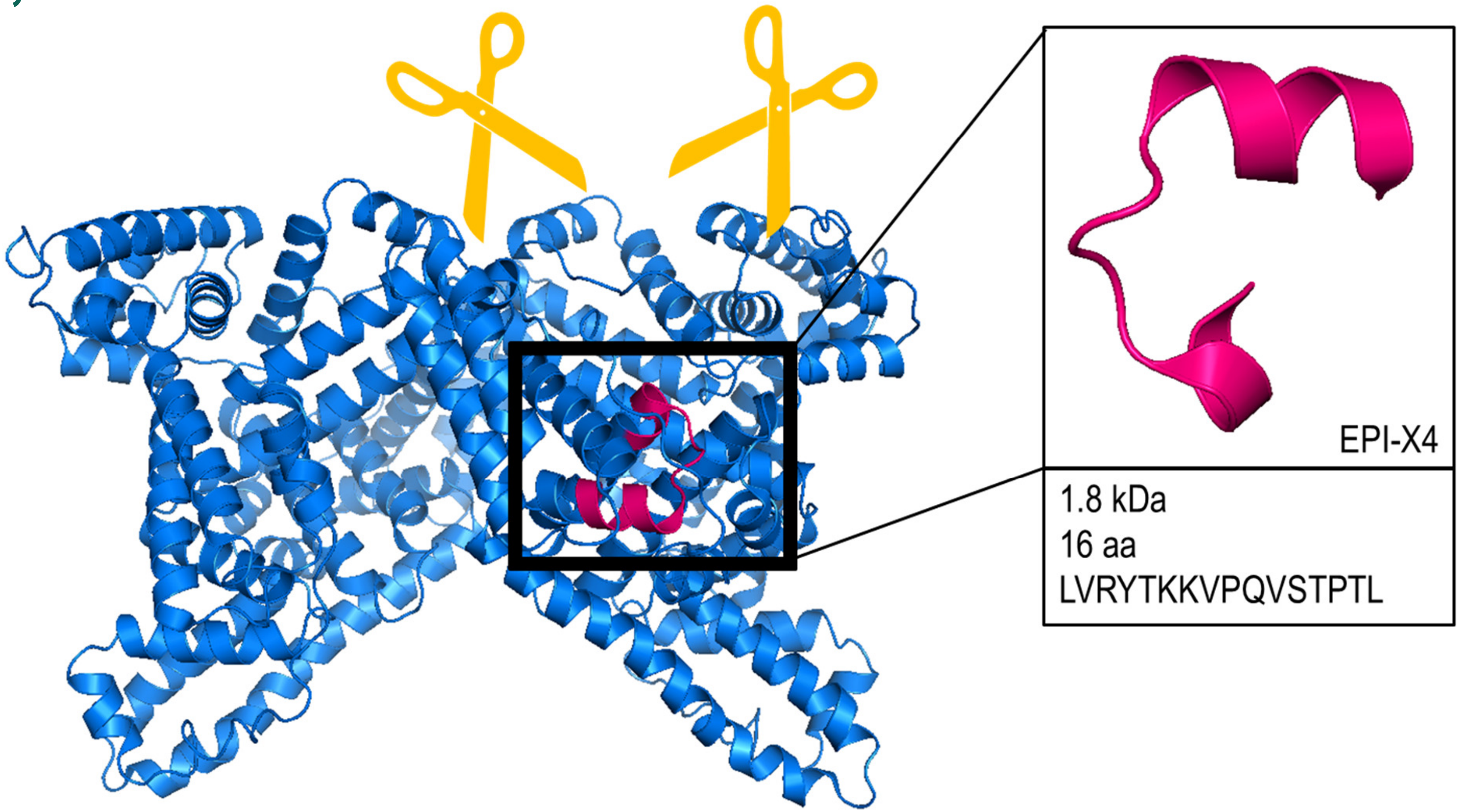
NOESY - Nuclear Overhauser Enhanced SpectroscopY

2D H,H-NOESY   Structure calculation



Current work from Dr. Gönül Kizilsavas (SFB Project 1279)

Nuclear Magnetic Resonance (NMR)

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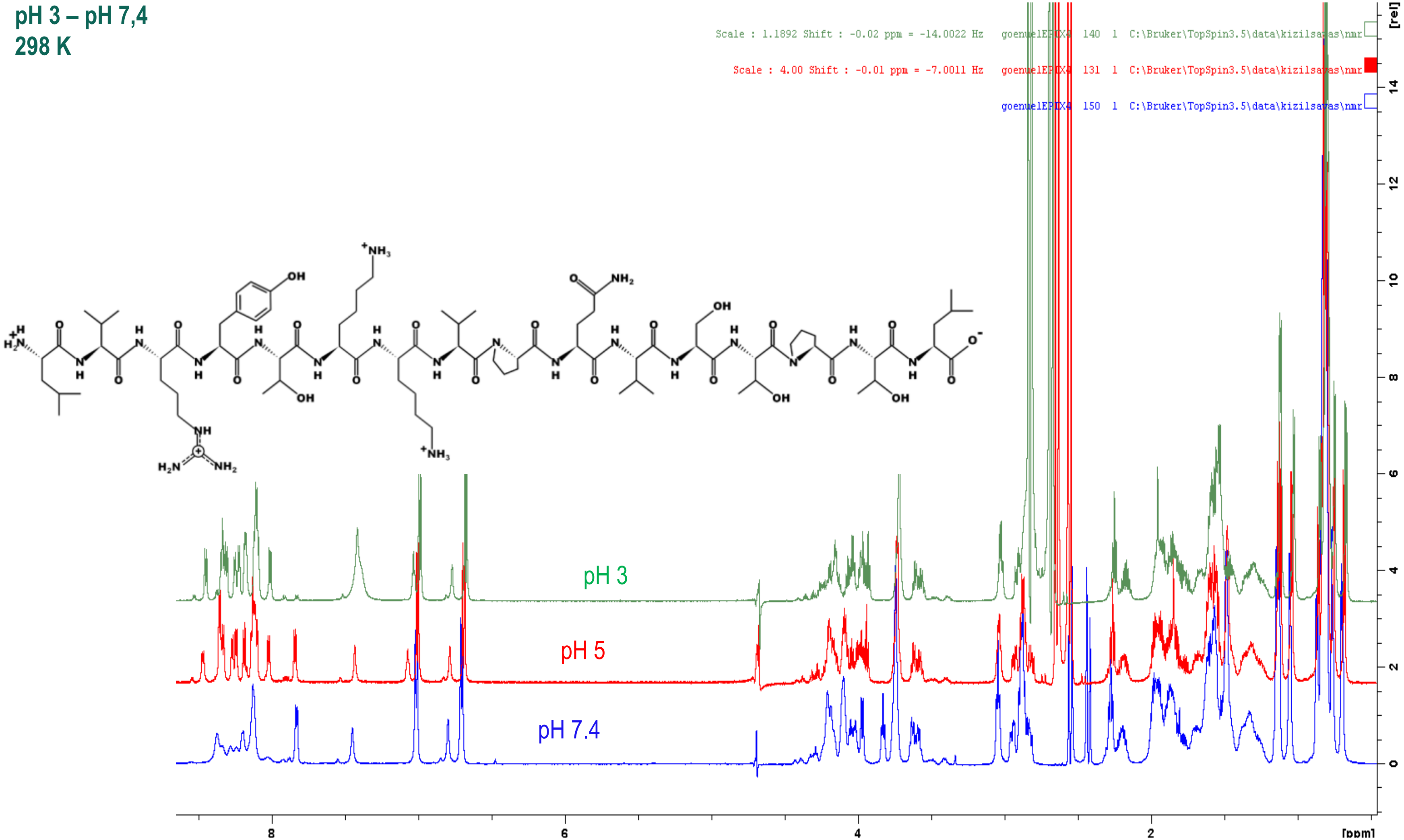
- In NaP/Citric acid buffer
- pH 3 – pH 7,4
- 298 K



Current work from Dr. Gönül Kizilsavas (SFB Project 1279)

74

Nuclear Magnetic Resonance (NMR)

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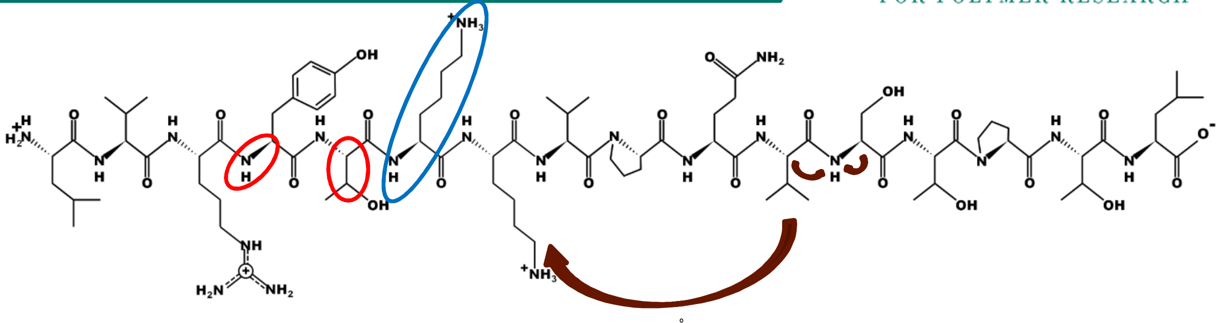
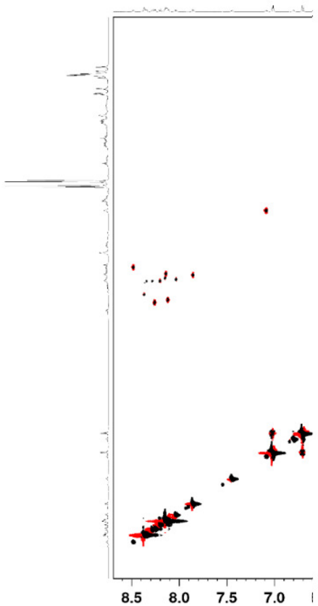
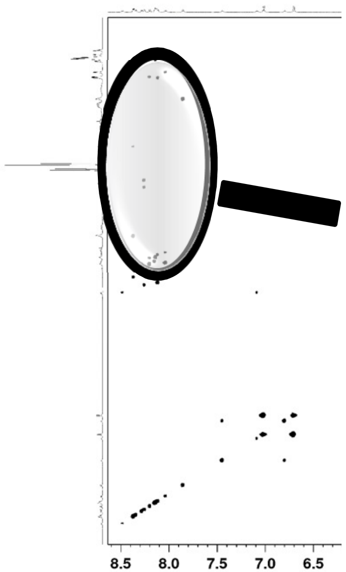
- In NaP/Citric acid buffer
- pH 3 – pH 7,4
- 298 K



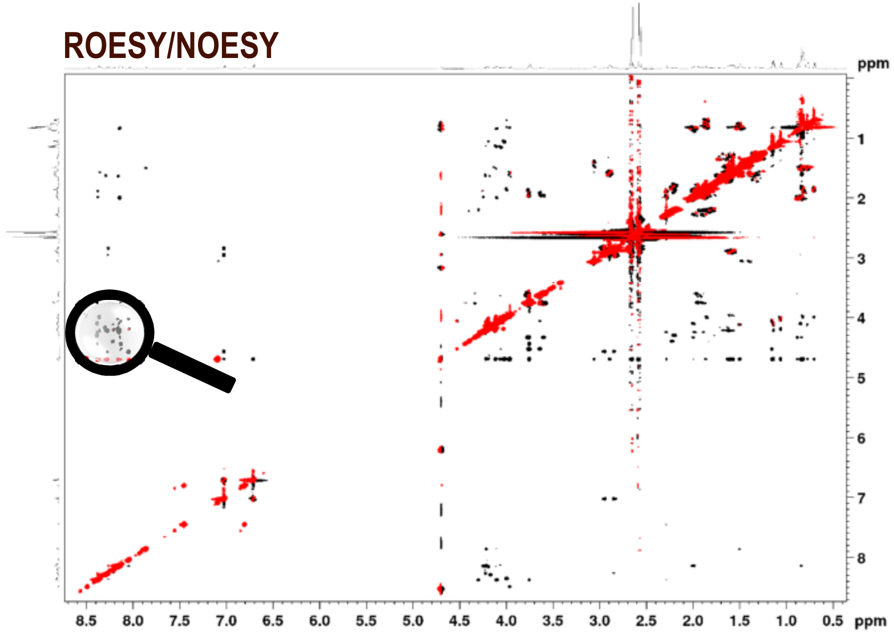
COSY



TOCSY



ROESY/NOESY



Current work from Dr. Gönül Kizilsavas (SFB Project 1279)

Nuclear Magnetic Resonance (NMR)

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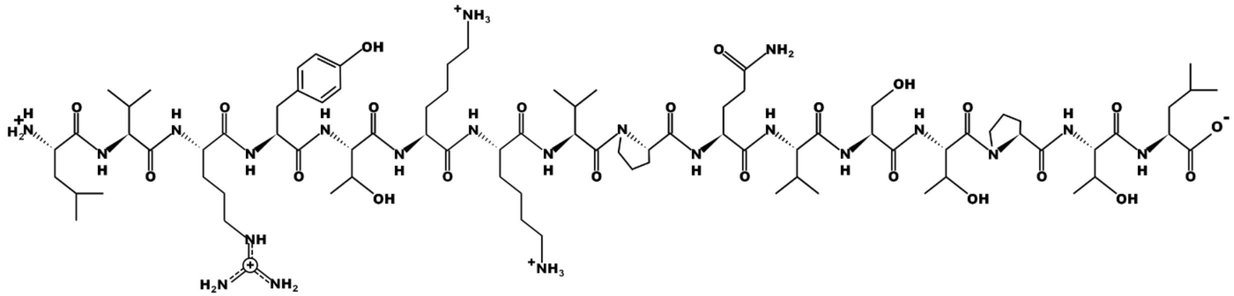
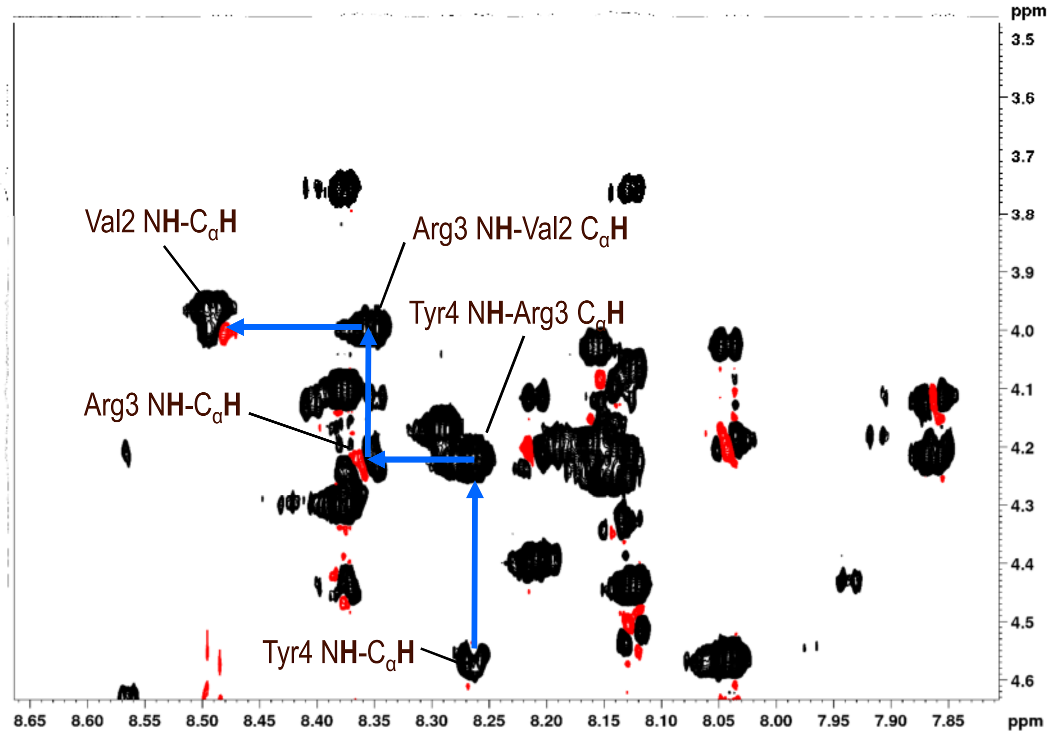
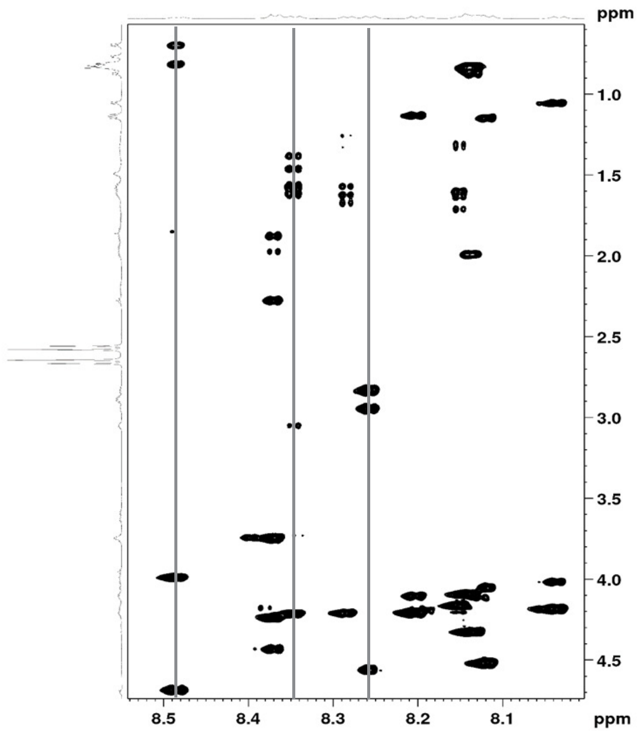


ROESY of EPI-X4 at pH5



TOCSY of EPI-X4 at pH5



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Nuclear Magnetic Resonance (NMR)

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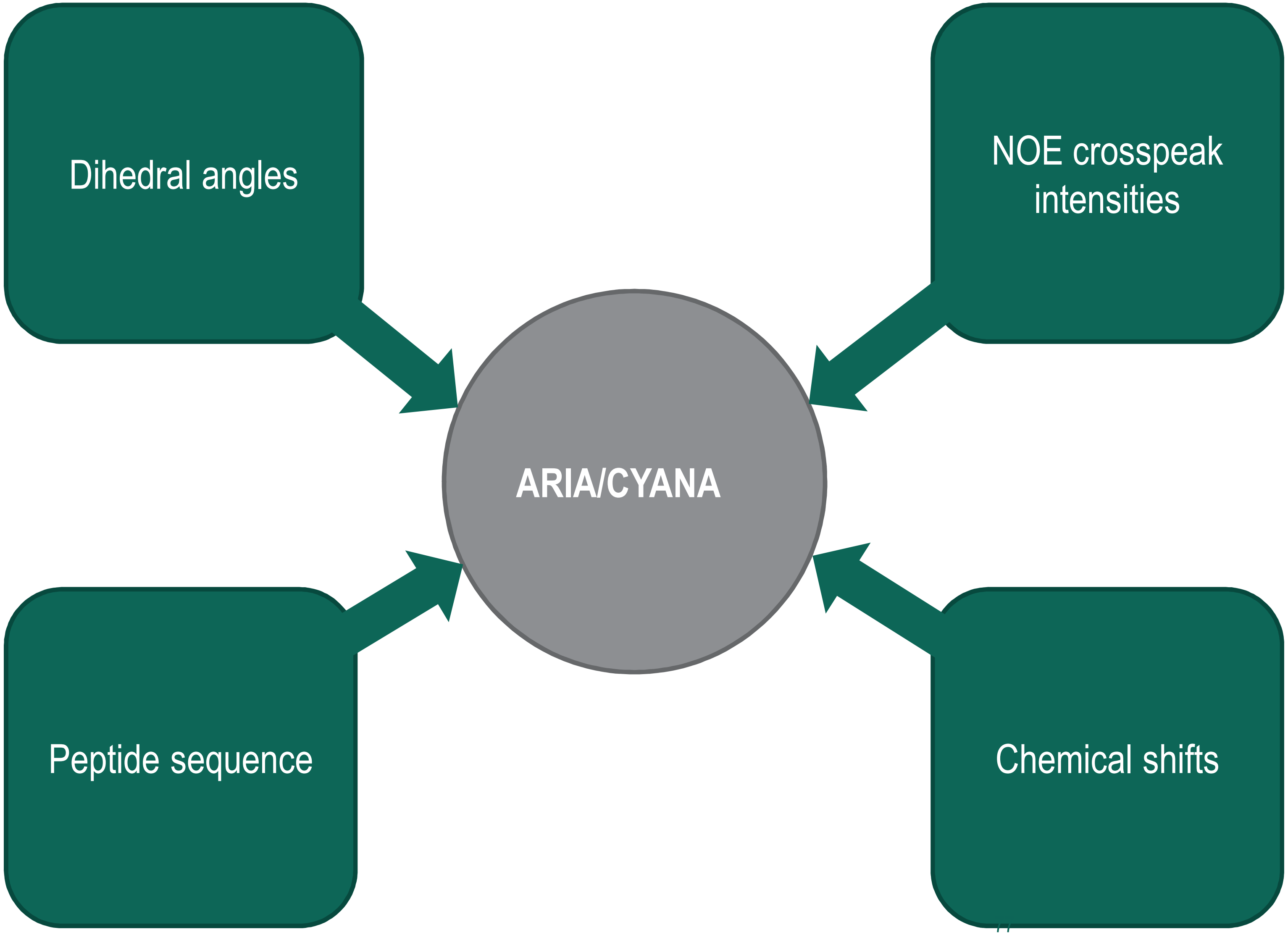

Nuclear Magnetic Resonance (NMR)

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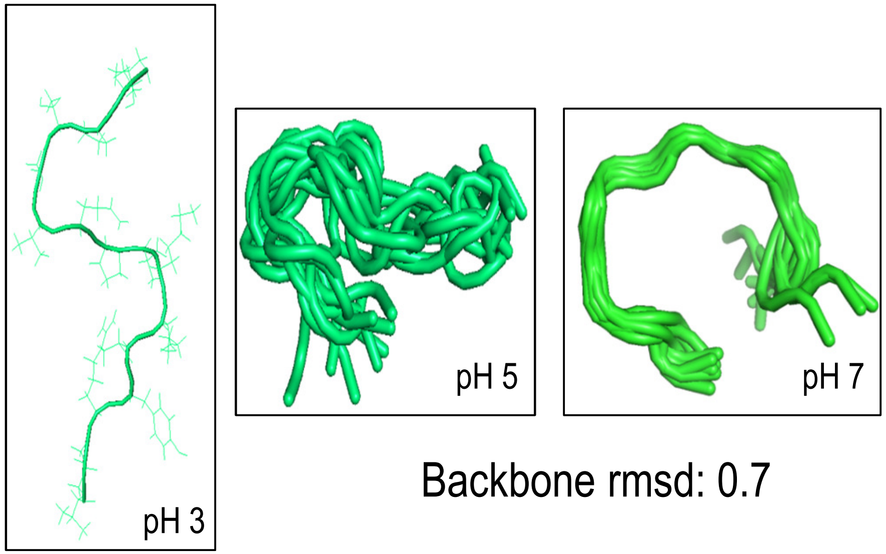
Through-bond couplings
- intraresidual signal assignment
- Dihedral angles

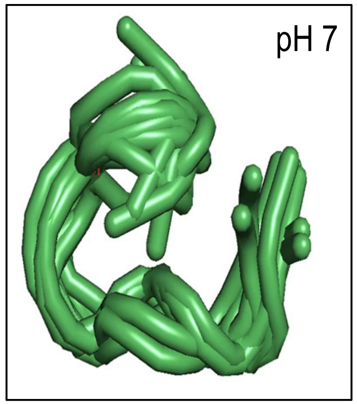
Through-space couplings
- Sequential signal assignment
- Intramolecular distances of up to 4/5 Å

EPI-X4, an HSA fragment and CXCR4 antagonist



Backbone rmsd: 0.7

JM21



Current work from Dr. Gönül Kizilsavas (SFB Project 1279)

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2D NMR distance calculation (time consuming, at least one week):

- T_1-relaxation time measurement
- Minimum 3 (mostly 5) 2D-NOESY with different mixing time,
- Finding of the linear increasing intensity range with increasing mixing time
- 2D volume integrations, using a well known reference distance (defined NOESY volume integration value),
- Calculation

Nuclear Magnetic Resonance (NMR)

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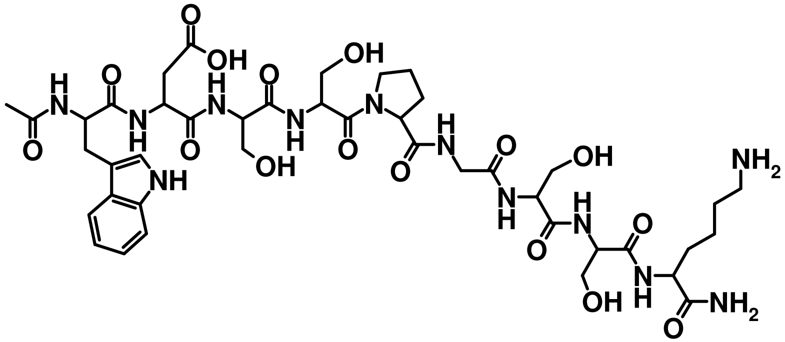
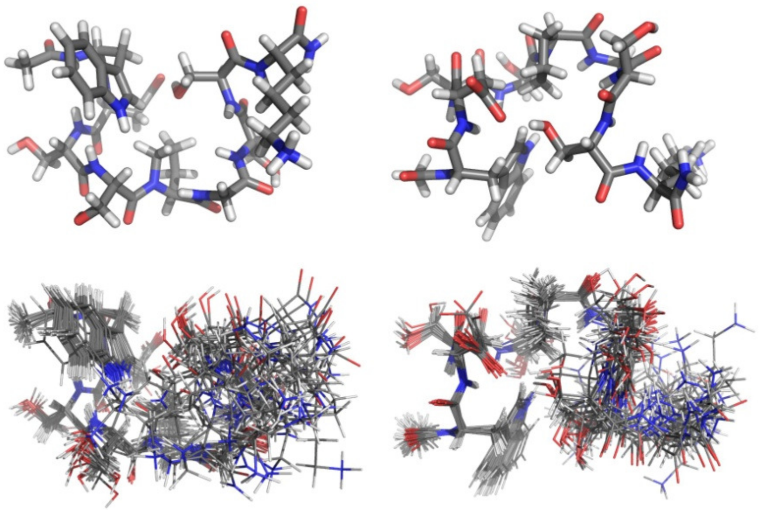
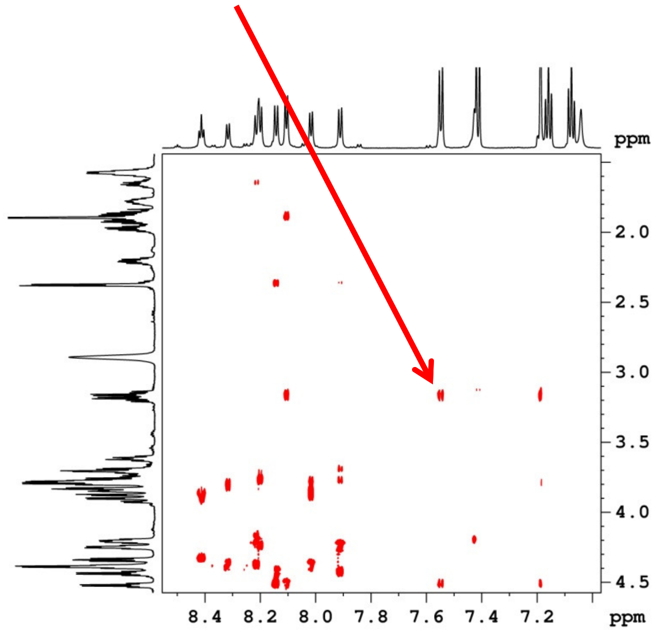
3D structure of peptide sequences with NOESY NMR

$^{1}H,^{1}H$ NOESY 2D Correlation together with 3D structure calculations generated from the NOESY cross peaks



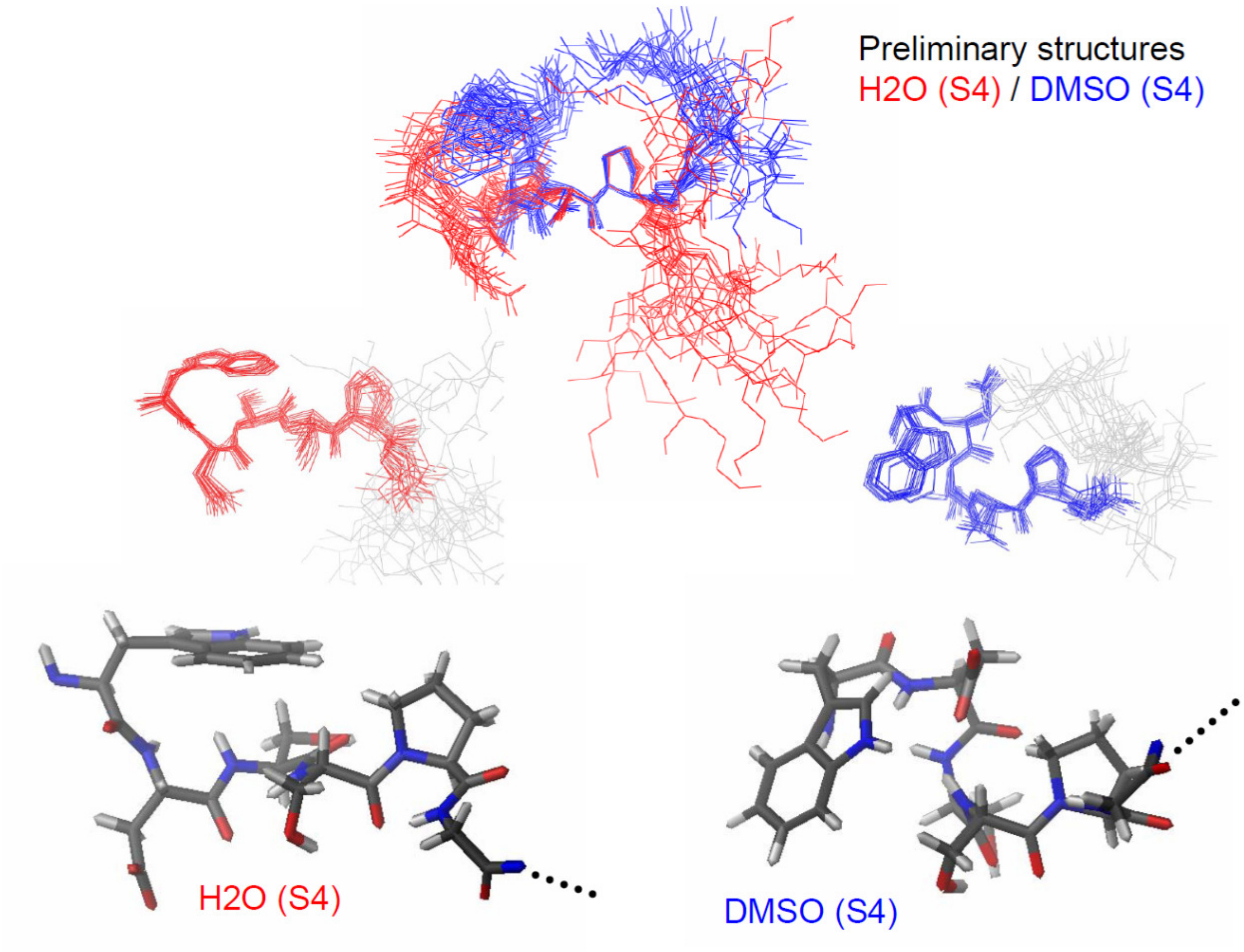




700 MHz Magnet BBI Proben ($^{1}H/X/^{2}H$) with z-Gradient

"Molecular Structure and Pronounced Conformational Flexibility of Doxorubicin in Free and Conjugated State within a Drug-Peptide Compound", Tsoneva, Y., Jonker, H. R. A., Wagner, M., Tadjer, A., Lelle, M., Peneva, K., Ivanova, A.; JOURNAL OF PHYSICAL CHEMISTRY B, Vol.: 119, Issue: 7, 3001-3013, DOI: 10.1021/jp509320q, FEB 19 2015

Nuclear Magnetic Resonance (NMR)



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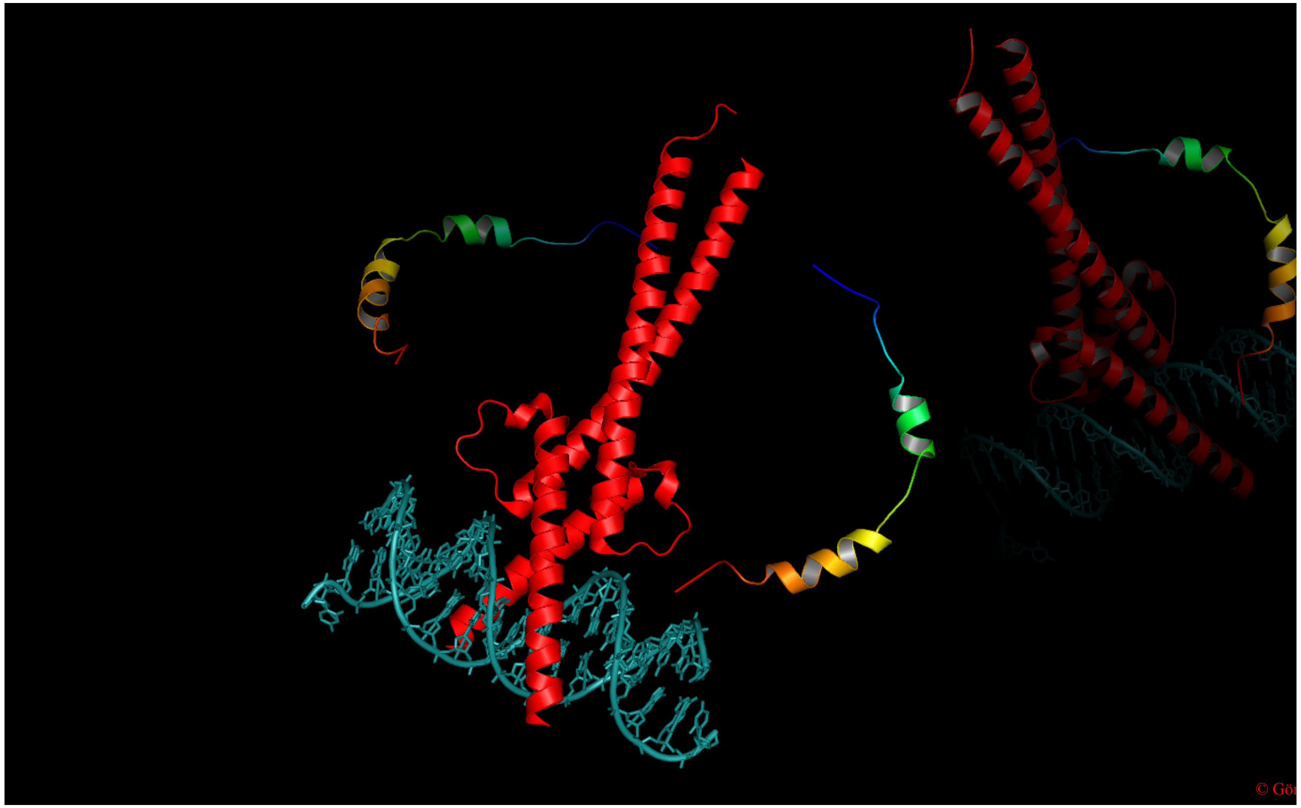
Protein NMR

Investigating Structure, Dynamics and Interactions with NMR



PhD work from Dr. Gönül Kizilsavas

Nuclear Magnetic Resonance (NMR)

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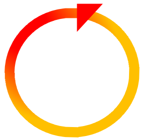
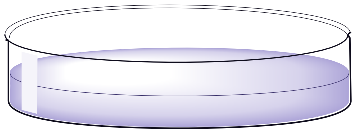
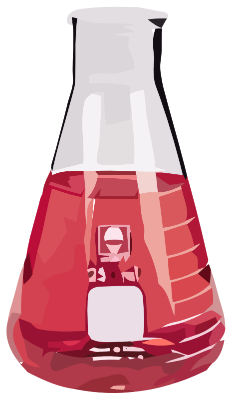
Mostly done in E.coli strains, but also possible in insect cells, especially recommended for PTM

Sample Preparation



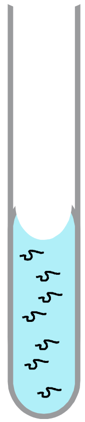
Vector with the DNA information of the protein





Grow in a minimal medium with $^{15}NH_4Cl$ and ^{13}C-Glucose

Specifically for the protein optimized purification protocol



i. Stable,
ii. Isotopically labelled,
iii. soluble protein
iv. 600 µl, ~500 µM

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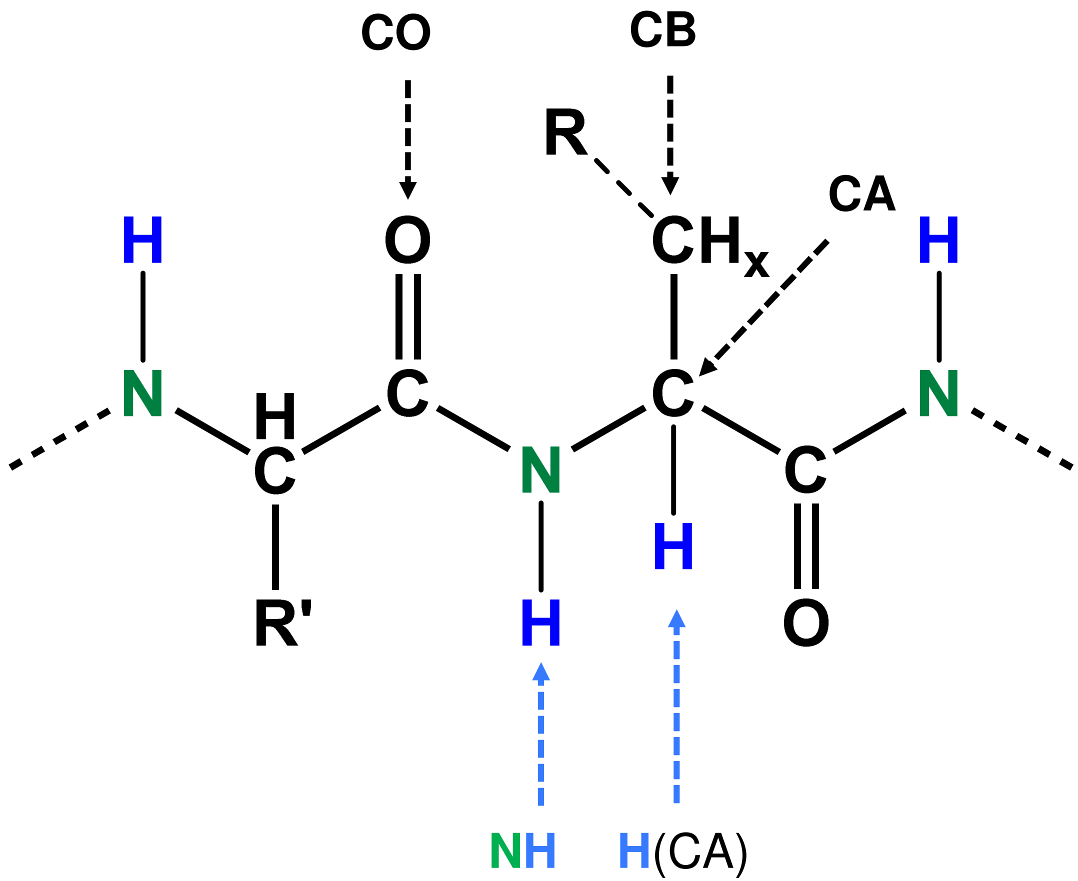
^{1}H, ^{13}C and ^{15}N Backbone and Side Chain Signal Assignment

Several 2D and 3D NMR measurements:

HNCO,
HNCA,
HN(CO)**CA, HN**(CA)**CO**
H(CA)**NH,**
CBCA(CO)**NH**
CBCANH
HNCACB
....



Nuclear Magnetic Resonance (NMR)

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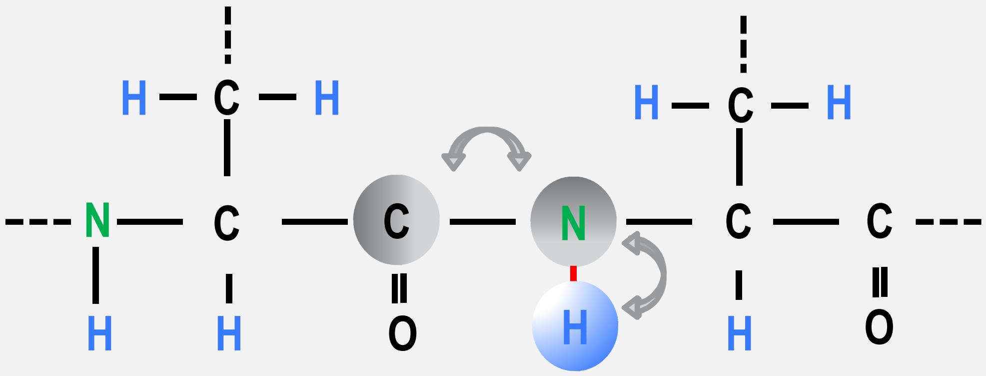
HNCO

$^{1}H_{i}^{N} - {}^{15}N_{i} - {}^{13}CO_{i-1}$

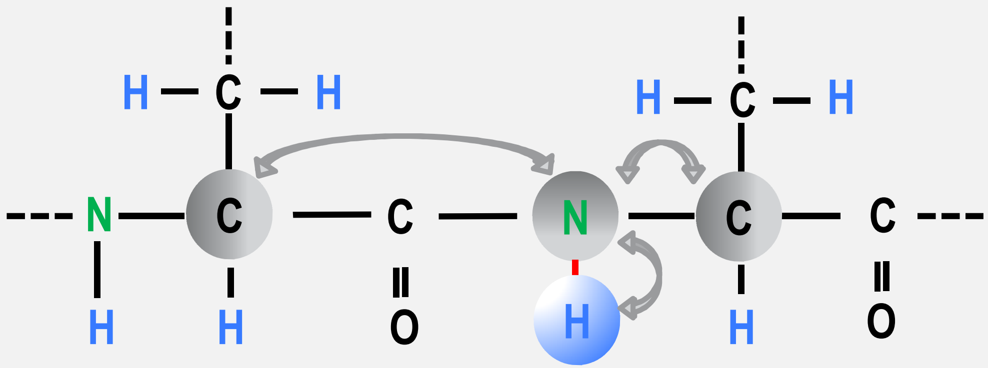


^{1}JNH: ~ 91 Hz ^{1}JNCO: ~ 15 Hz

HNCA

$^{1}H_{i}^{N} - {}^{15}N_{i} - {}^{13}C^{\alpha}_{i}$

$^{1}H_{i}^{N} - {}^{15}N_{i} - {}^{13}C^{\alpha}_{i-1}$



^{1}JNH: ~ 91 Hz ^{1}JNC$^{\alpha}$: ~ 7 - 11 Hz ^{2}JNC$^{\alpha}$: ~ 4 – 9 Hz

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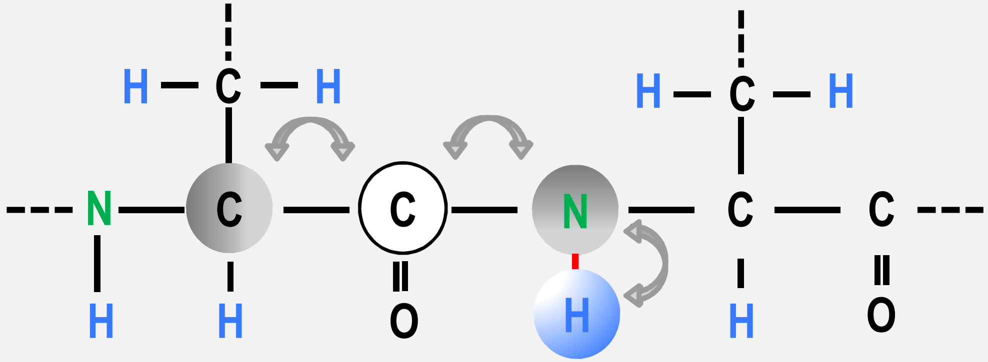
HN(CO)CA

$^{1}H_{i}^{N} - {}^{15}N_{i} - {}^{13}C^{\alpha}_{i-1}$

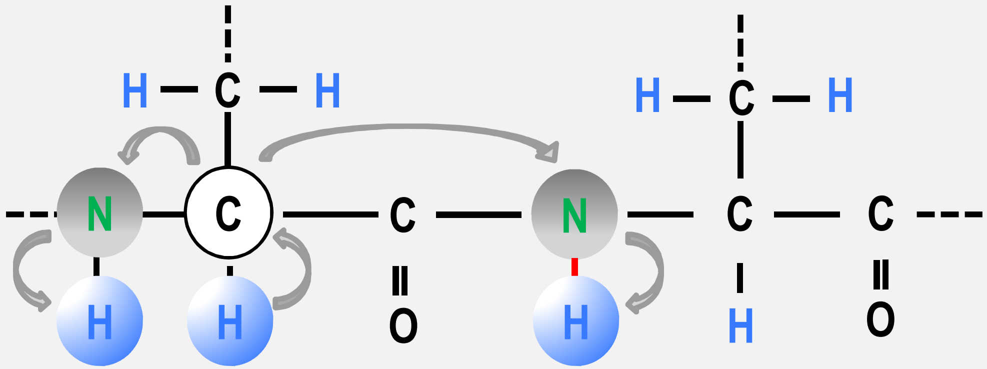


^{1}JNH: ~ 91 Hz ^{1}JNCO: ~ 15 Hz ^{1}JC$^{\alpha}$CO: ~ 55 Hz

H(CA)NH

$^{1}H_{i}^{N} - {}^{15}N_{i} - {}^{1}H_{i}^{N}$

$^{1}H_{i}^{N} - {}^{15}N_{i+1} - {}^{1}H_{i+1}^{N}$



^{1}JNH: ~ 91 Hz ^{1}JNC$^{\alpha}$: ~ 91 Hz ^{1}JC$^{\alpha}$H$^{\alpha}$: ~ 140 Hz ^{2}JNC$^{\alpha}$: ~ 4 – 9 Hz

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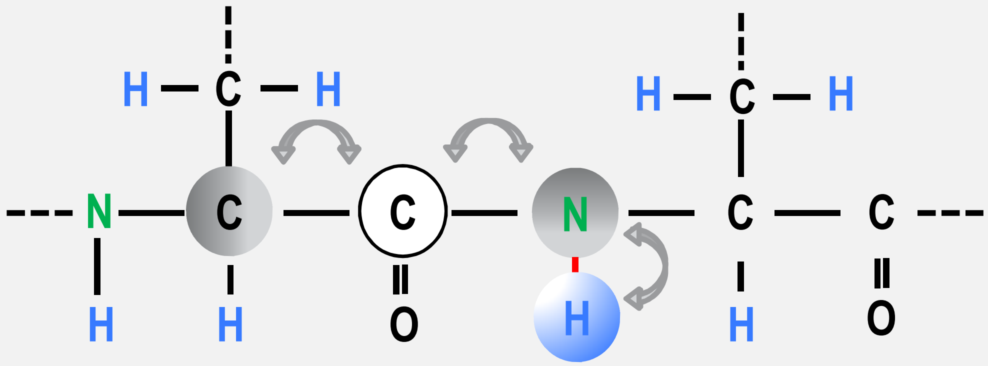
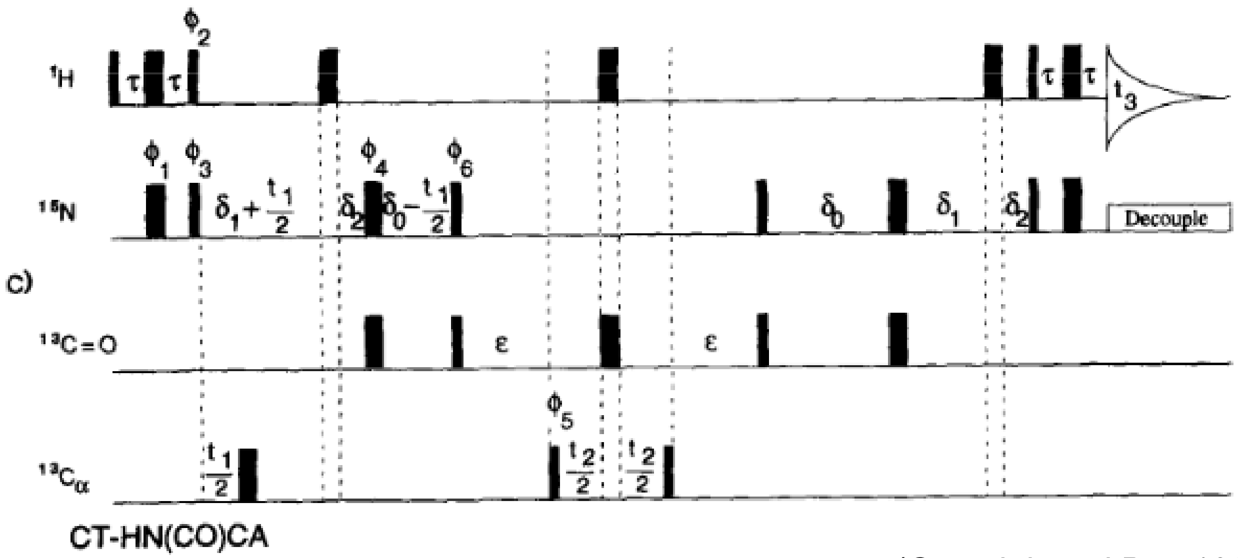
HN(CO)CA

$^{1}H_i^{N} - {}^{15}N_i - {}^{13}C^{\alpha}_{i-1}$





(Grzesiek and Bax 1992)

Nuclear Magnetic Resonance (NMR)

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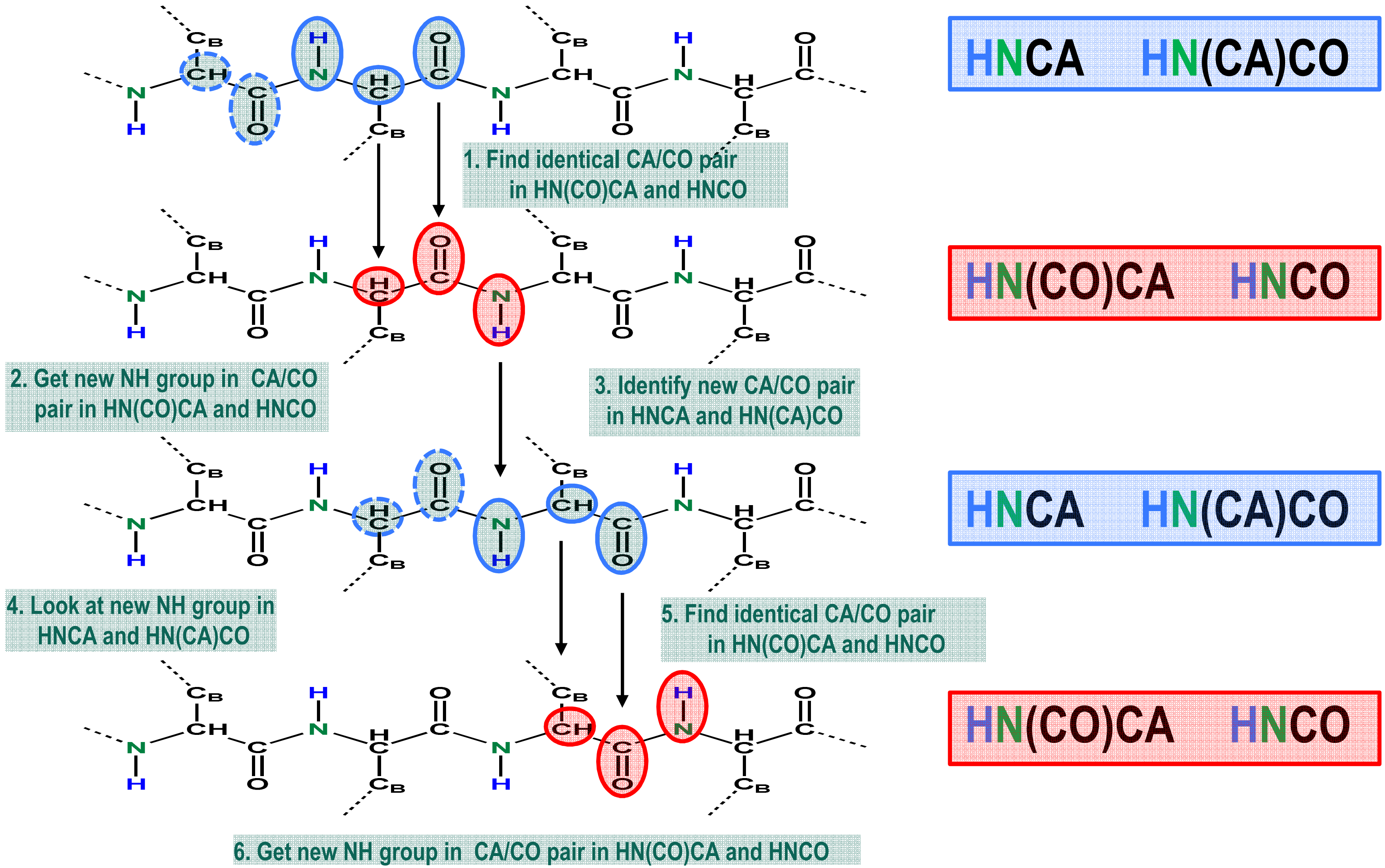
^{1}H, ^{13}C and ^{15}N Backbone and Side Chain Signal Assignment



Nuclear Magnetic Resonance (NMR)

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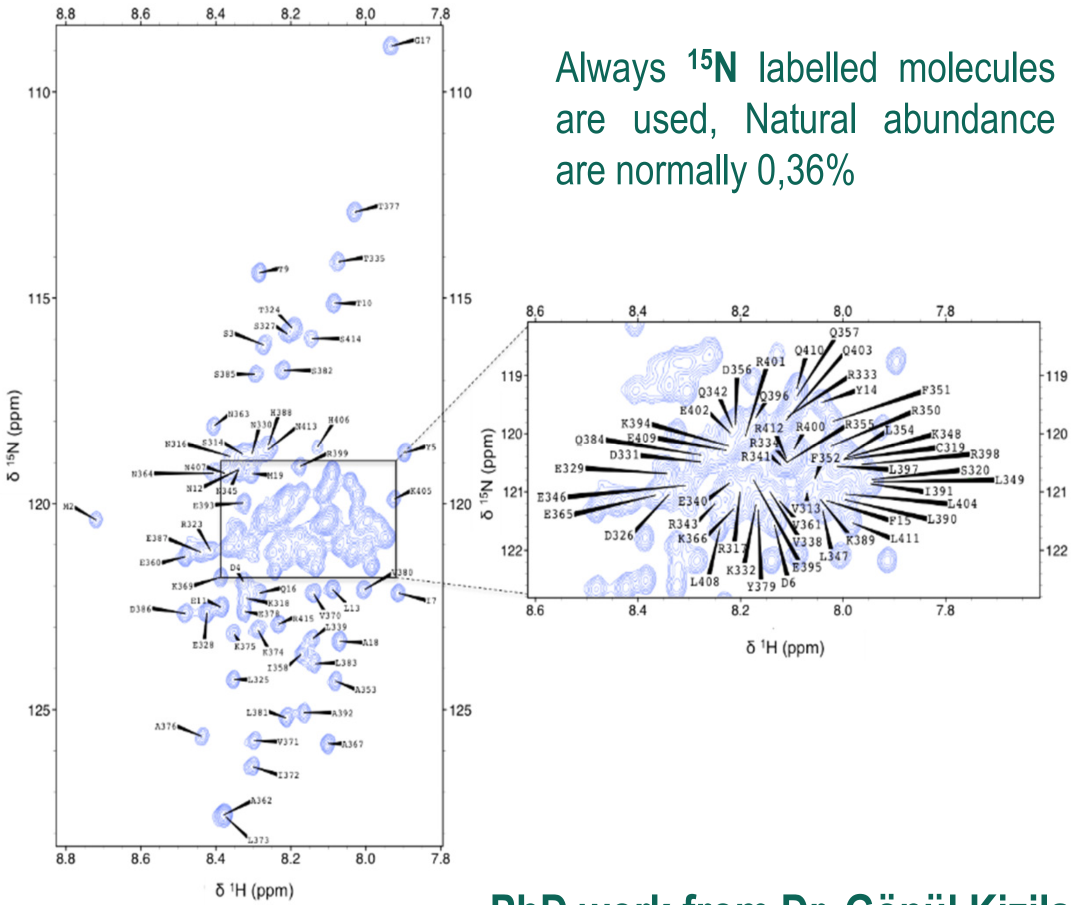
^{1}H, ^{13}C and ^{15}N Backbone and Side Chain Signal Assignment

Example: ^{1}H, ^{15}N signal assignment of v-Myc (102 amino acids) depicted on a ^{1}H,^{15}N-HSQC



Always **^{15}N** labelled molecules are used, Natural abundance are normally 0,36%

Calculation of the **3D** structure needs long time (months) and the full assignment of a new protein sometimes longer depending on the size.

PhD work from Dr. Gönül Kizilsavas

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When searching for analytical methods, don't be limited by your imagination!!!

Asking others that are not experts in your field is really helpful!!

Sometimes the “negative” result has a reason!!

Knowing “why it doesn’t work” is sometimes the beginning of a new idea!!

Be curious!! Always.

Thanks for your attention