Introduction to NMR

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Ljubljana, Slovenia

CONTACT Workshop
CERIC-CEI Training in advanced material characterisation in Large Scale Research Infrastructures
Basovizza, Italy, 26th June 2017


SLONMR


Austria
Hungary
Italy
Slovenia
Ljubljana
Croatia
Gulf of Venice
Adriatic Sea

Slovenian NMR Centre

Slovenian NMR Centre

- Institute facility
- National research infrastructure – academic & industrial users
- Centre of Excellence
- Partner facility; integration into C-ERIC services

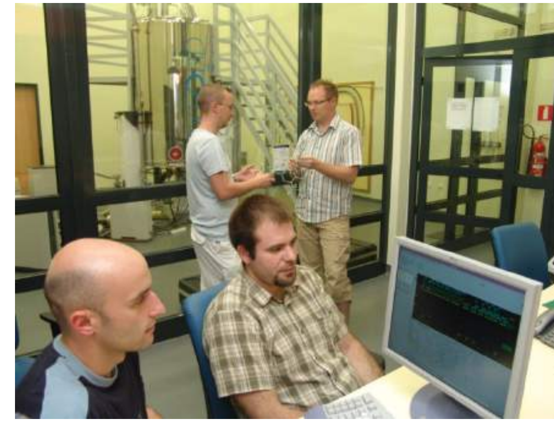
http://www.nmr.ki.si





NMR facility: up-to-date equipment and expertise

800 MHz Varian VNMRS spectrometer equipped with ^{1}H and ^{13}C enhanced triple resonance (HCN) cryogenic probe head

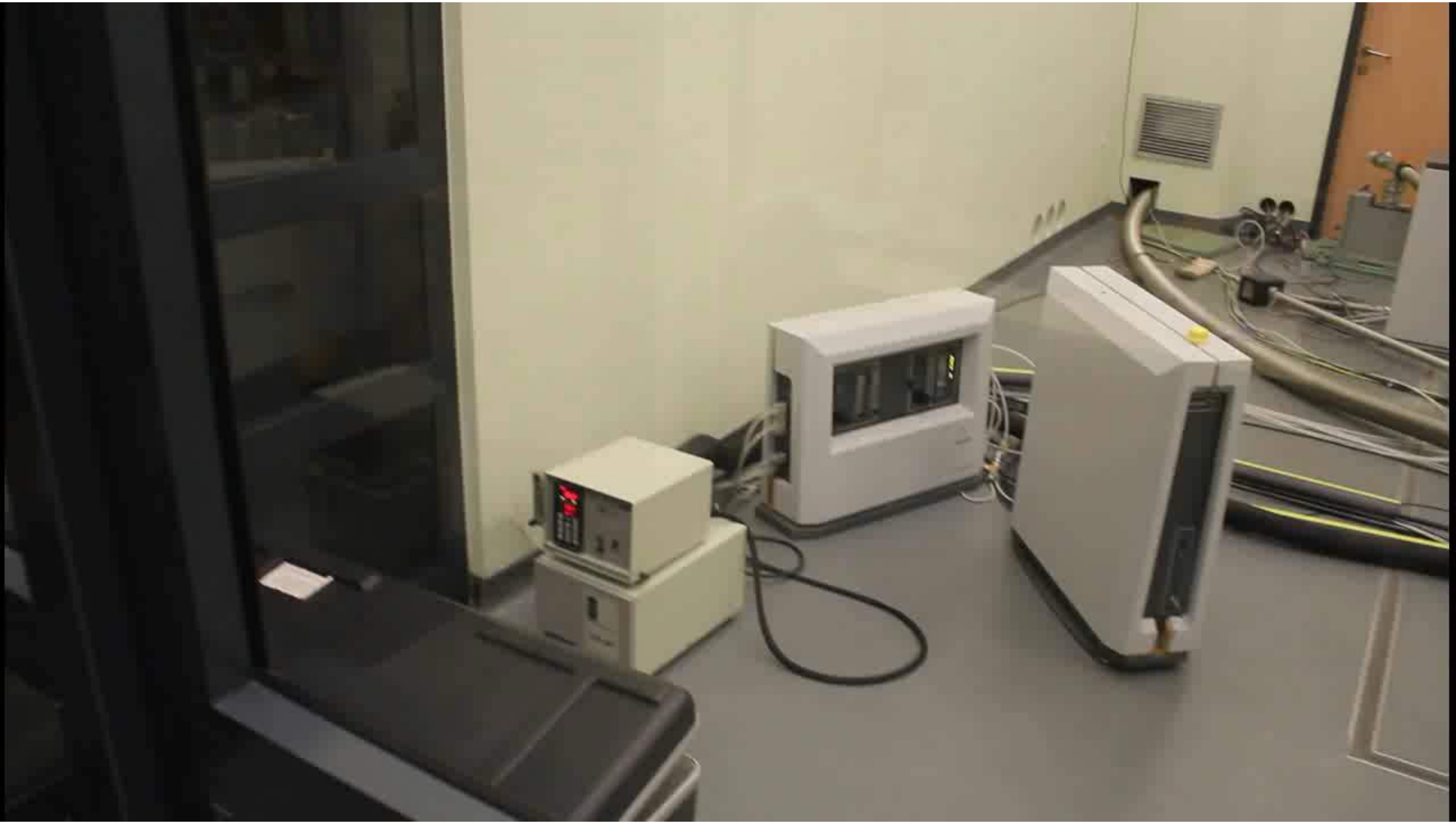


600 MHz Varian VNMRS spectrometer equipped with wide range of solid-state and liquid probe heads

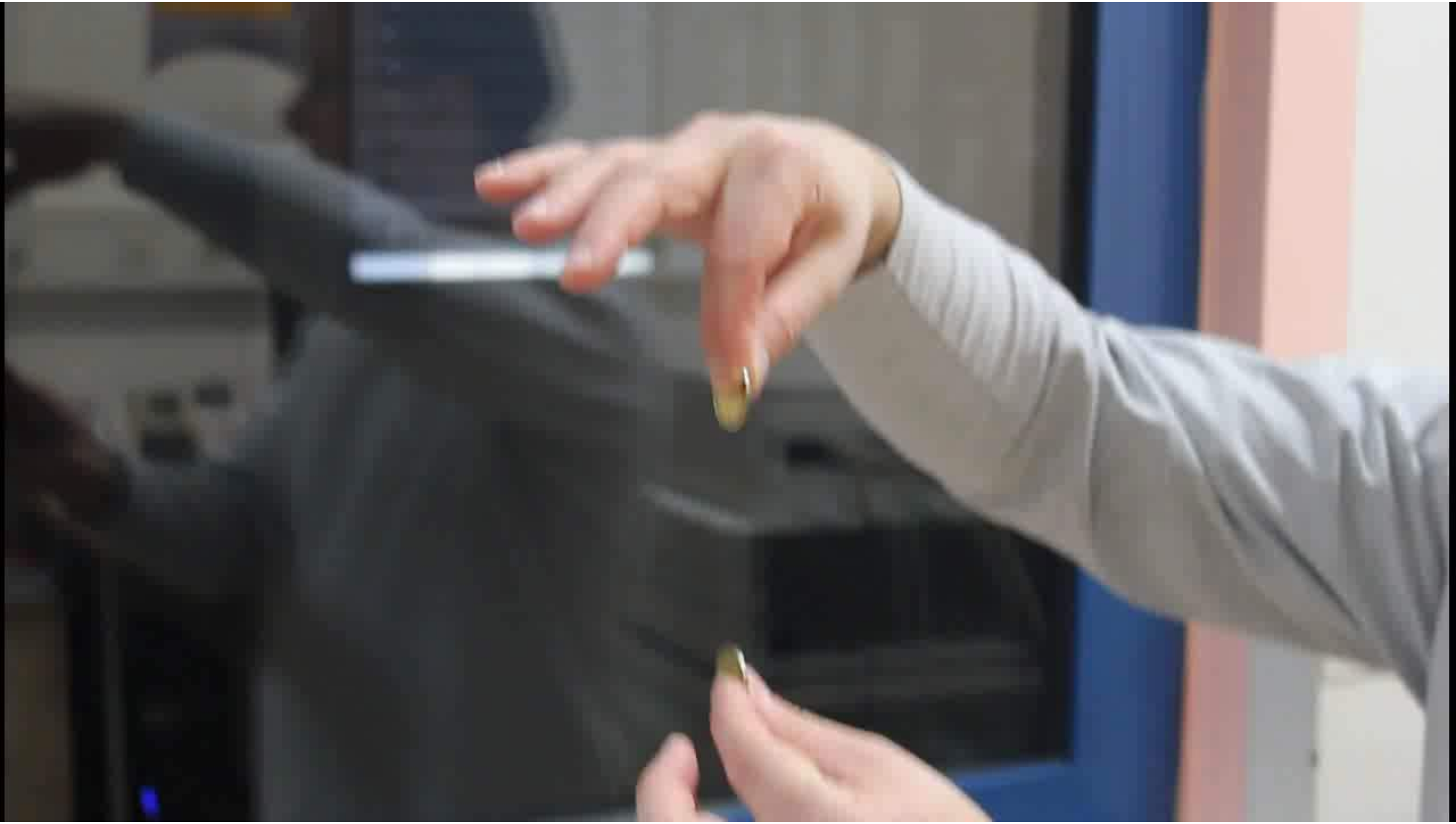
NEW 600 MHz Agilent DD2 spectrometer equipped with ^{1}H and ^{13}C enhanced triple resonance salt tolerant (HCN) cryogenic probe head

600 MHz NMR

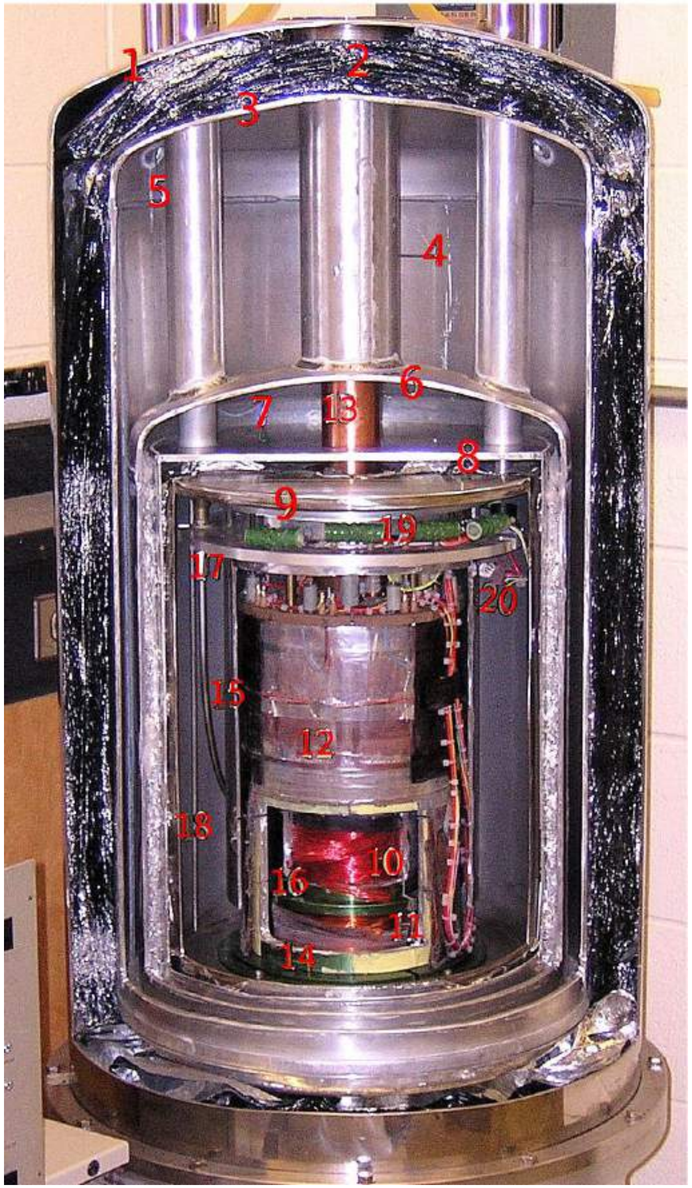
800 MHz NMR



Demonstration of the magnetic field



NMR He, N2



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MESSER GRIESHEIM
APOLLO

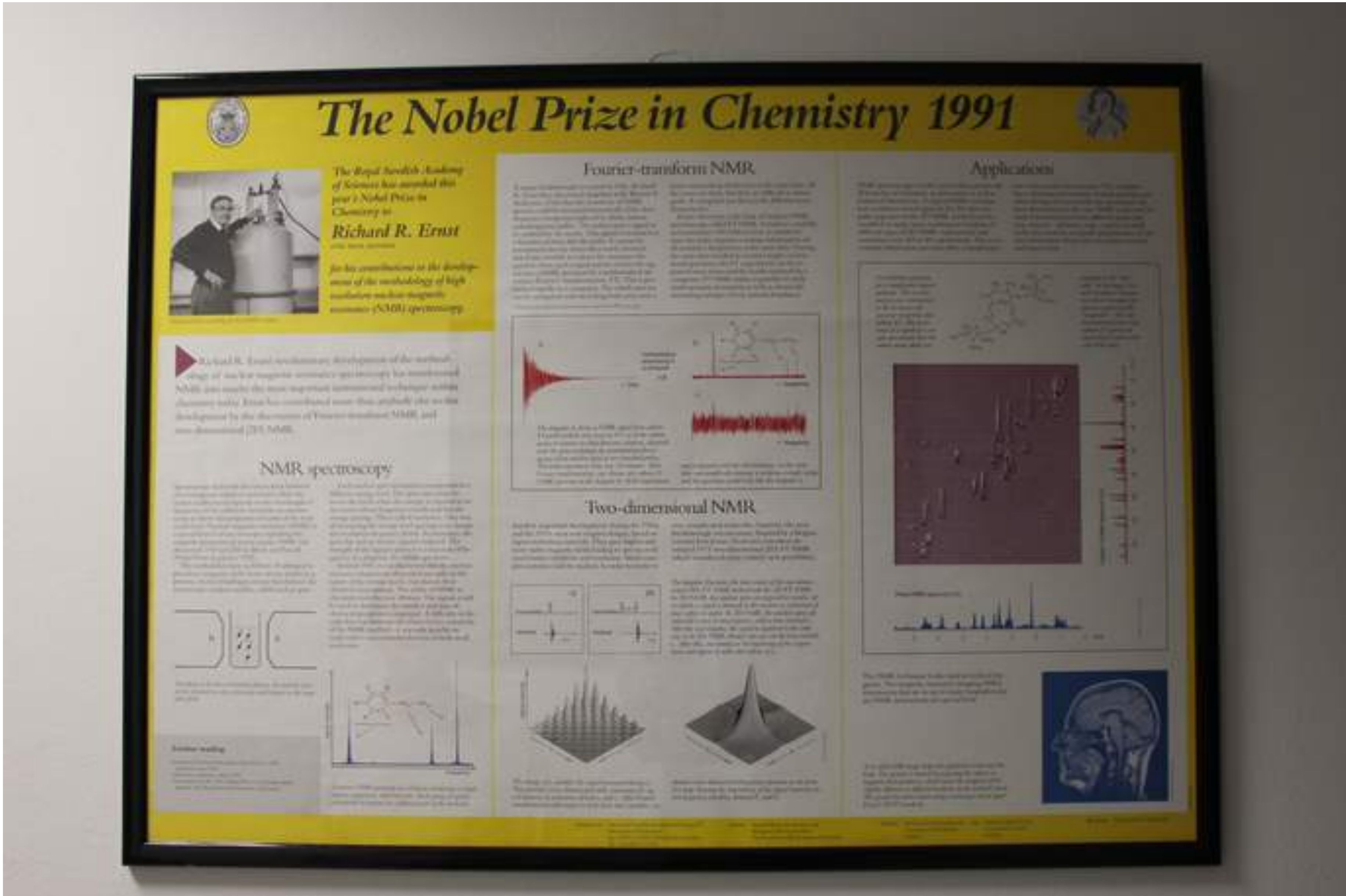
NMR History

1937 **Rabi** predicts and observes nuclear magnetic resonance
1946 **Bloch, Purcell** first nuclear magnetic resonance of bulk sample
1953 **Overhauser** NOE (nuclear Overhauser effect)
1966 **Ernst, Anderson** Fourier transform NMR
1975 **Jeener, Ernst** 2D NMR
1984 **Nicholson** NMR metabolomics
1985 **Wüthrich** first solution structure of a small protein (BPTI) from NOE derived distance restraints
1987 3D NMR + ^{13}C, ^{15}N isotope labeling of recombinant proteins (resolution)
1990 pulsed field gradients (artifact suppression)
1996/7 **residual dipolar couplings** (RDC) from partial alignment in liquid crystalline media
TROSY (molecular weight > 100 kDa)
2000s **Dynamic nuclear polarisation** (DNP) to enhance NMR sensitivity

Nobel prizes

1944 *Physics* Rabi (Columbia)
1952 *Physics* Bloch (Stanford), Purcell (Harvard)
1991 *Chemistry* Ernst (ETH)
2002 *Chemistry* Wüthrich (ETH)
2003 *Medicine* Lauterbur (University of Illinois in Urbana), Mansfield (University of Nottingham)

Nobel Prize


The Nobel Prize in Chemistry 1991
Richard R. Ernst
Fourier-transform NMR
Applications
NMR spectroscopy
Two-dimensional NMR

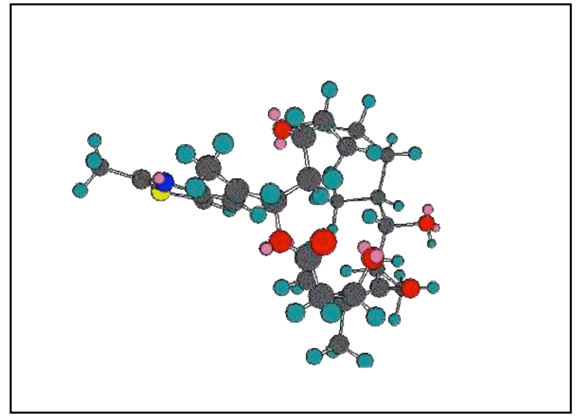
Nobel Prize

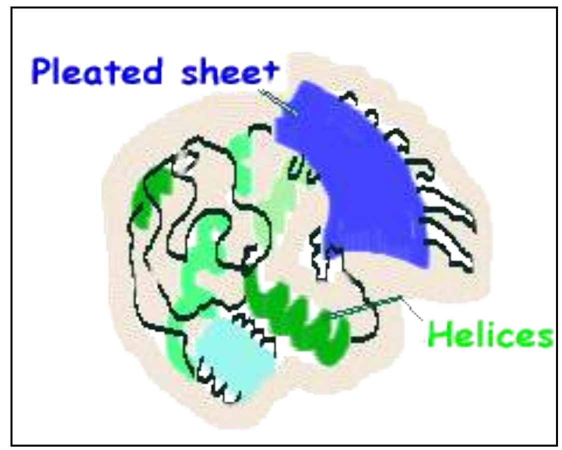
The problem the we want to solve by NMR

What we "really" see

What we want to "see"

NMR





NMR

- A physical phenomenon based upon the quantum mechanical magnetic properties of an atom's nucleus.
- Detects the absorption of radiofrequencies (electromagnetic radiation) by certain nuclei in a molecule.
- The nuclei of all atoms are characterized by:
  a nuclear spin quantum number (I)
- Only nuclei with ***spin number*** **(I) ≠ 0** can absorb/emit electromagnetic radiation. These should have an odd mass number.

Mass number	Atomic #	I	Example
Odd	Even or odd	1/2, 3/2, 5/2,…	(^{1}H, ^{13}C, ^{15}N, ^{31}P)
Even	Even	0	(^{12}C, ^{16}O)
Even	Odd	1, 2, 3	(^{14}N, ^{2}H)

The two nuclei that are most useful to organic chemists are:

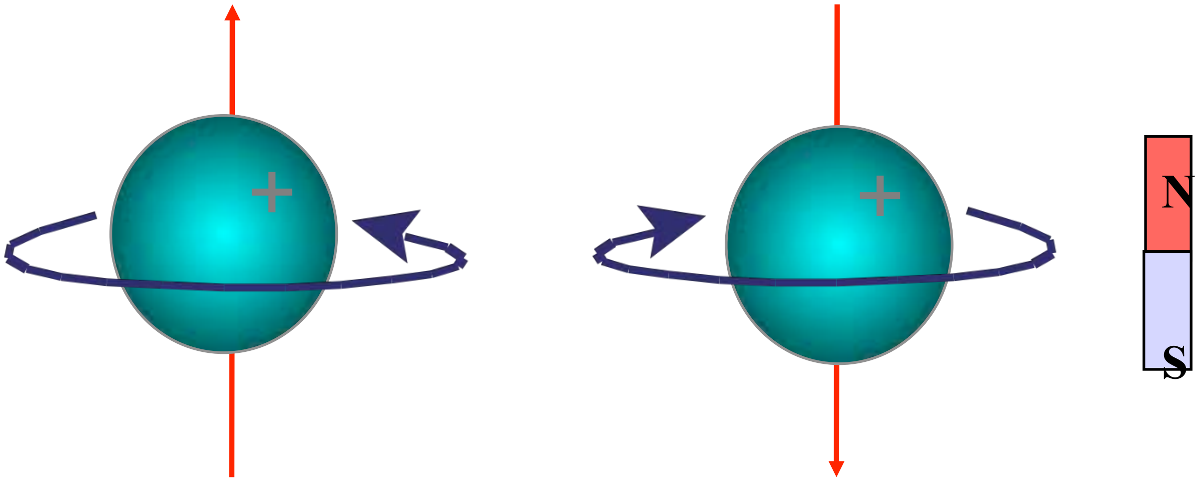
^{1}H and ^{13}C

both have spin = ±1/2

^{1}H is 99% at natural abundance

^{13}C is 1.1% at natural abundance

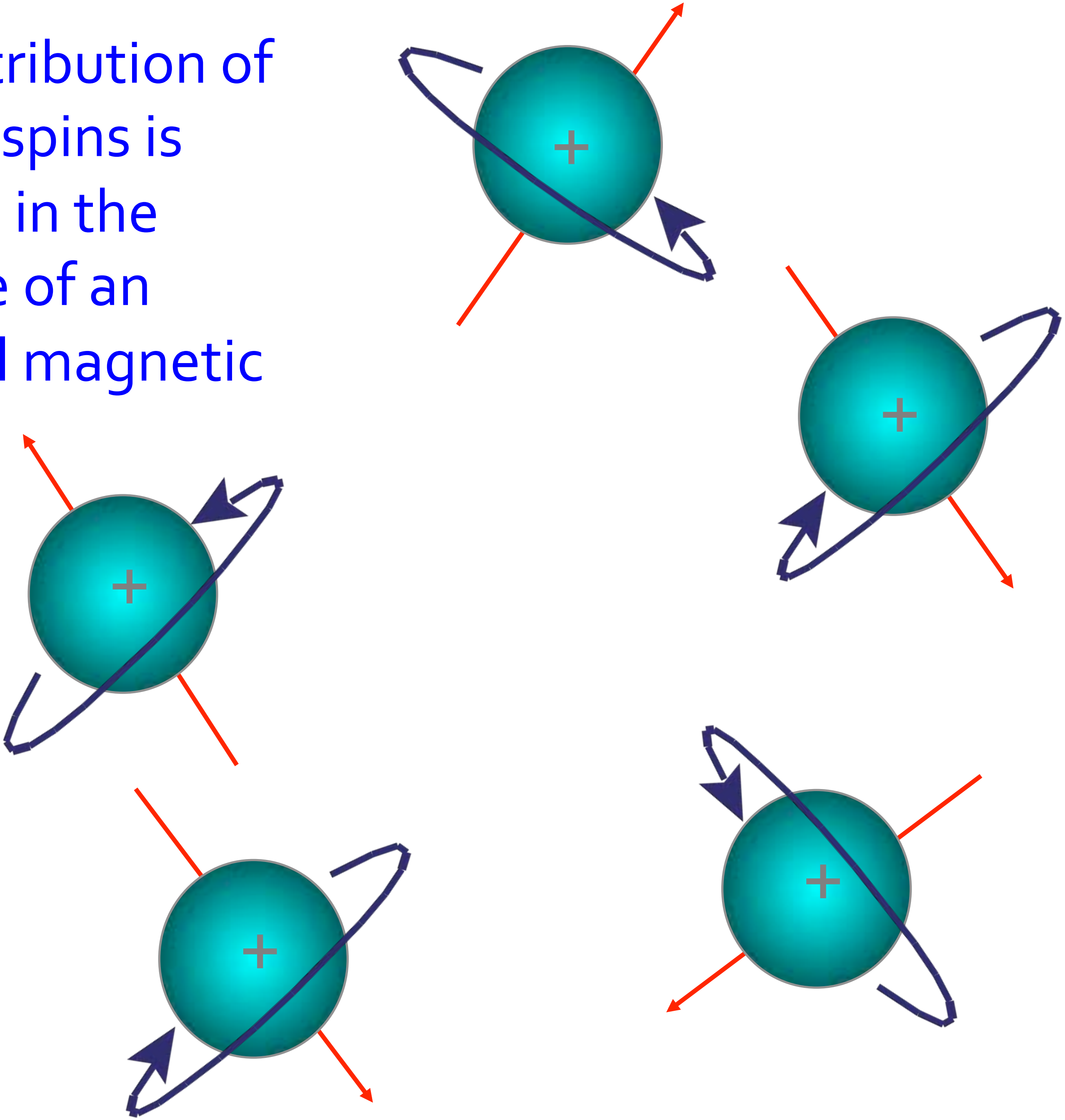
Nuclear Spin



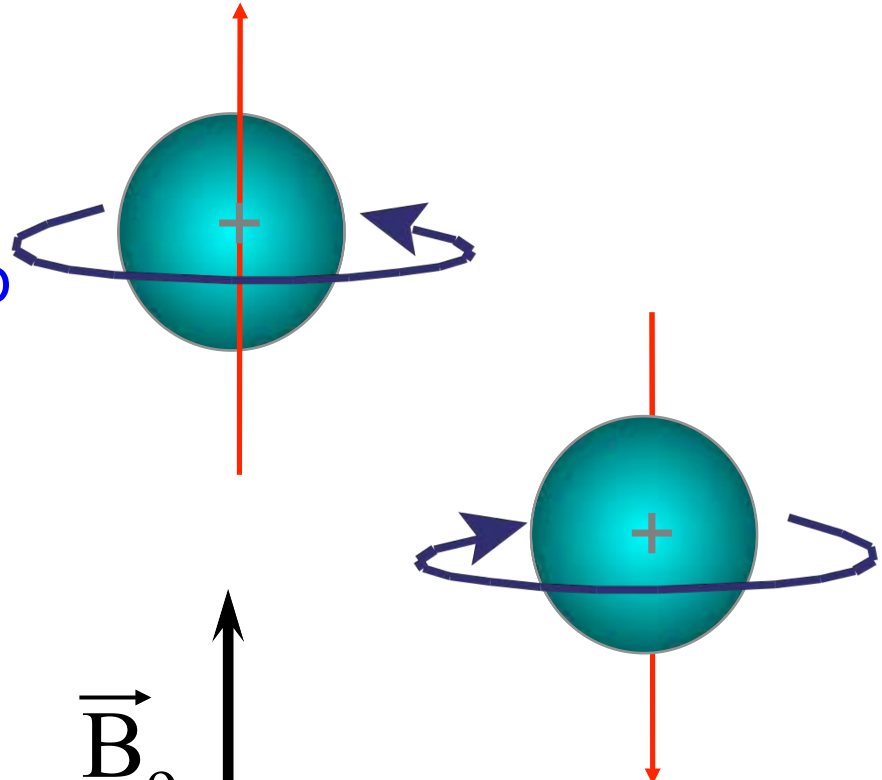
Each nucleus behaves like a bar magnet.

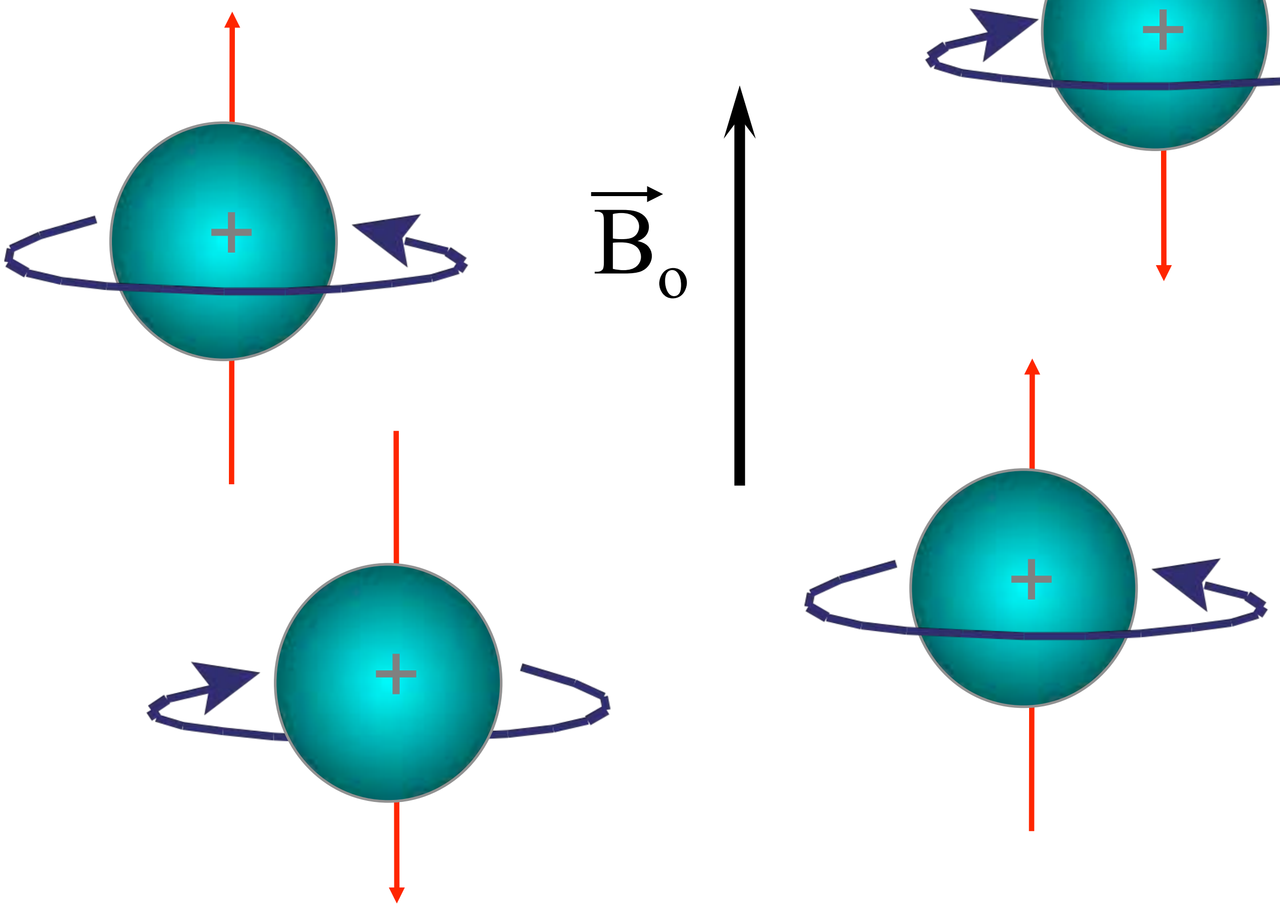
A spinning charge, such as the nucleus of ^{1}H or ^{13}C, generates a magnetic field. The magnetic field generated by a nucleus of spin +1/2 is opposite in direction from that generated by a nucleus of spin –1/2.

The distribution of nuclear spins is random in the absence of an external magnetic field.

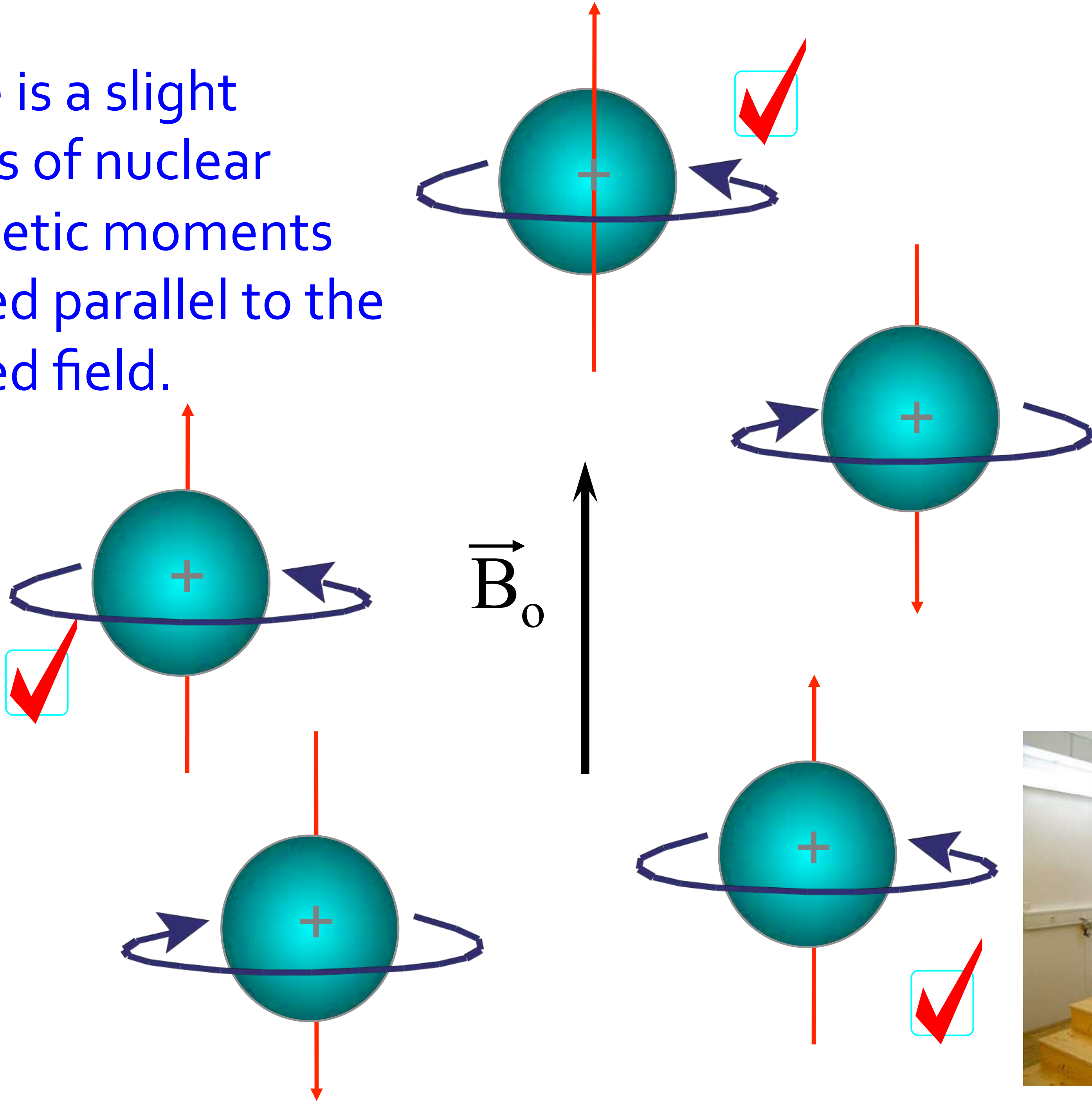


An external magnetic field causes nuclear magnetic moments to align parallel and antiparallel to applied field.

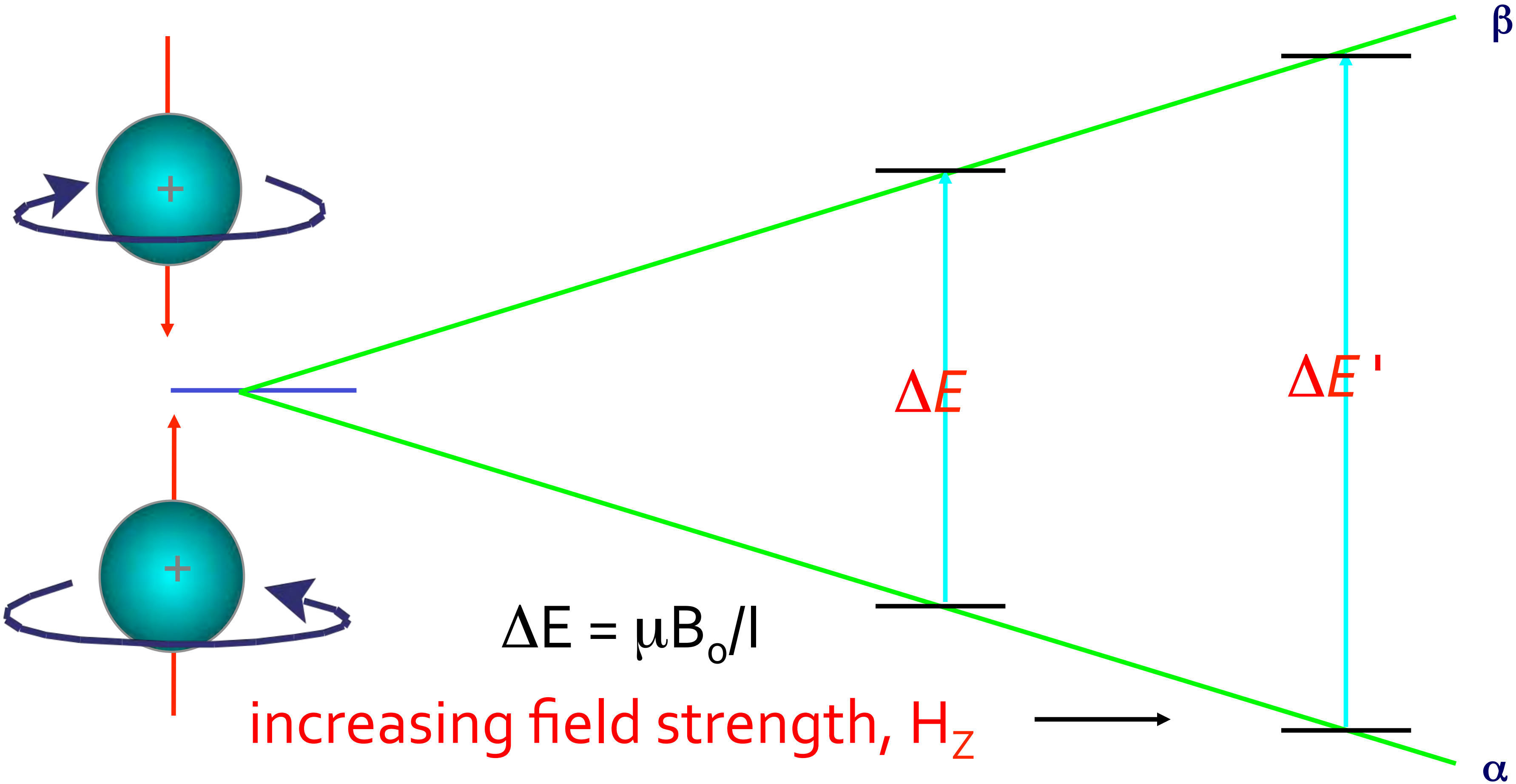




There is a slight excess of nuclear magnetic moments aligned parallel to the applied field.



Energy Differences Between Nuclear Spin States



μ – magnetic moment

no energy difference in absence of magnetic field

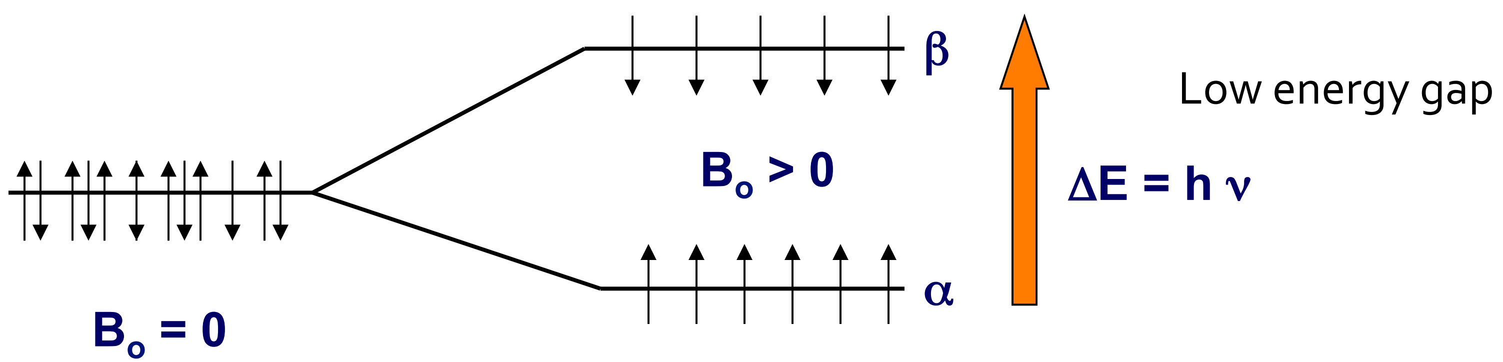
proportional to strength of external magnetic field

NMR Signal

- The applied magnetic field causes an energy difference between the aligned (α) and unaligned (β) nuclei

- NMR signal results from the transition of spins from the α to β state

- Strength of the signal depends on the population difference between the α and β spin states

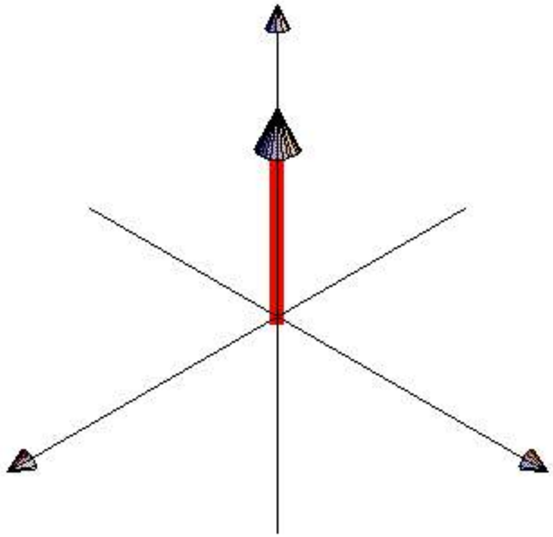


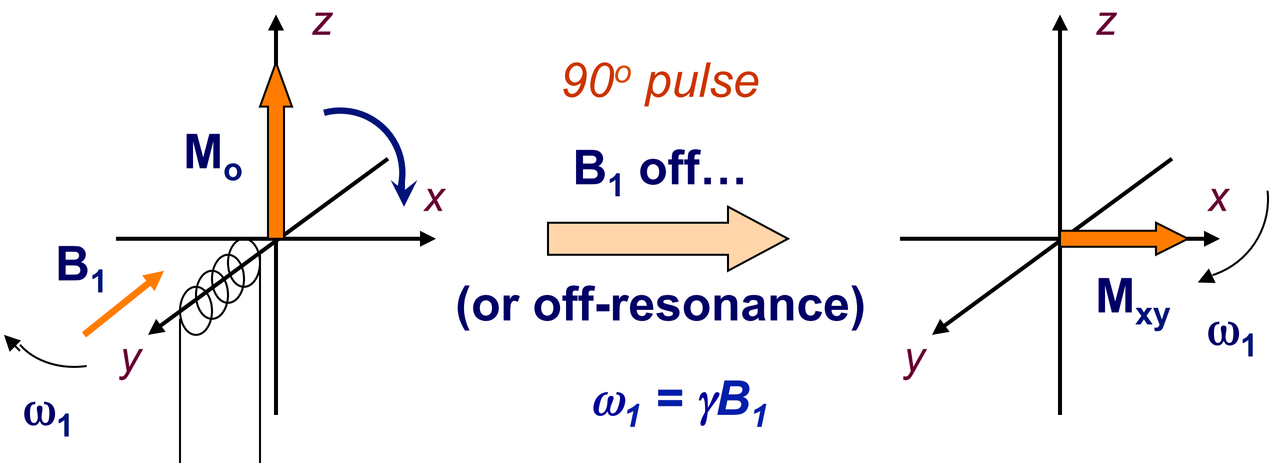
- The population (**N**) difference can be determined from the Boltzmann distribution and the energy separation between the a and b spin states:

$$N_\alpha / N_\beta = e^{\Delta E / kT}$$

Classical Description

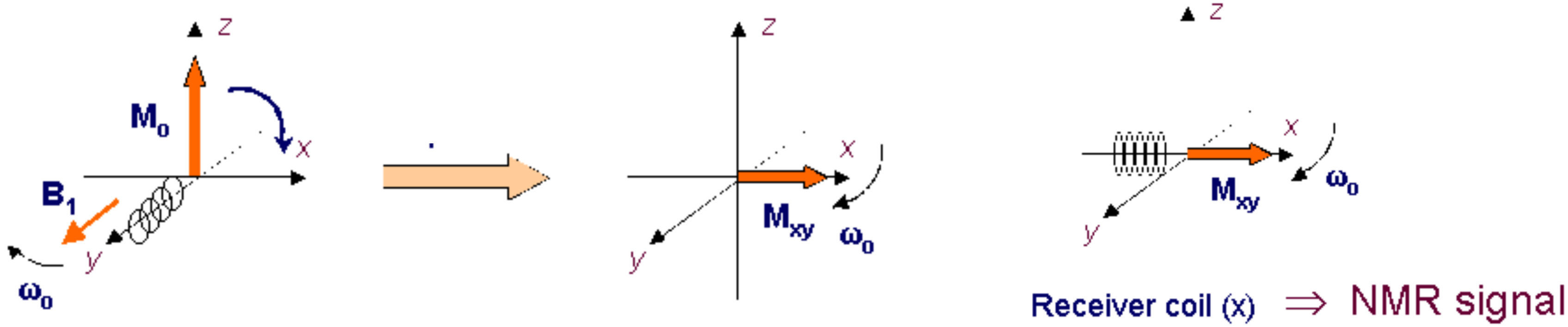
- *NMR Pulse*
  - Applying the B_1 field for a specified duration (Pulse length or width)
  - Net Magnetization precesses about B_1 a defined angle (90°, 180°, etc)

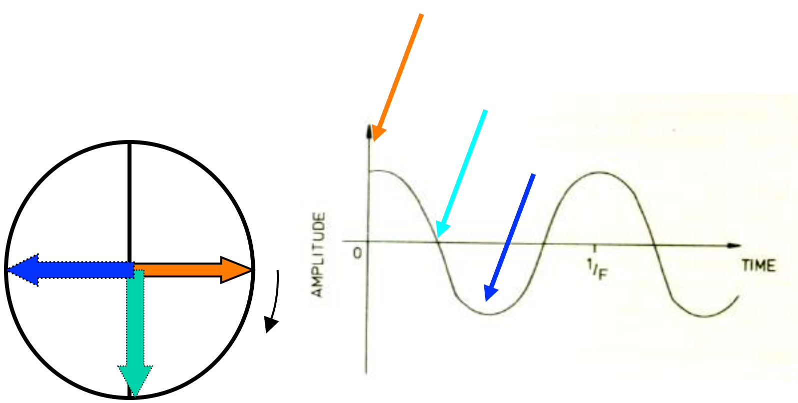




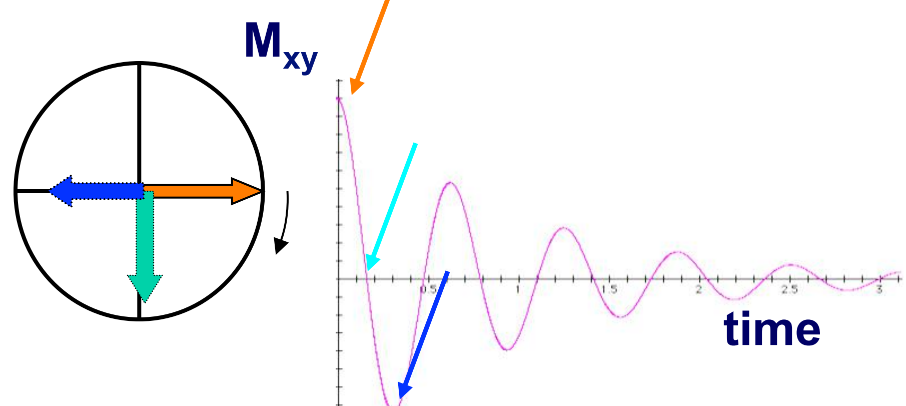
Collecting NMR signals

- The detection of NMR signal is on the xy plane. The oscillation of Mxy generate a current in a coil , which is the NMR signal.
- Due to the "relaxation process", the time dependent spectrum of nuclei can be obtained. This time dependent spectrum is called "free induction decay" (FID)



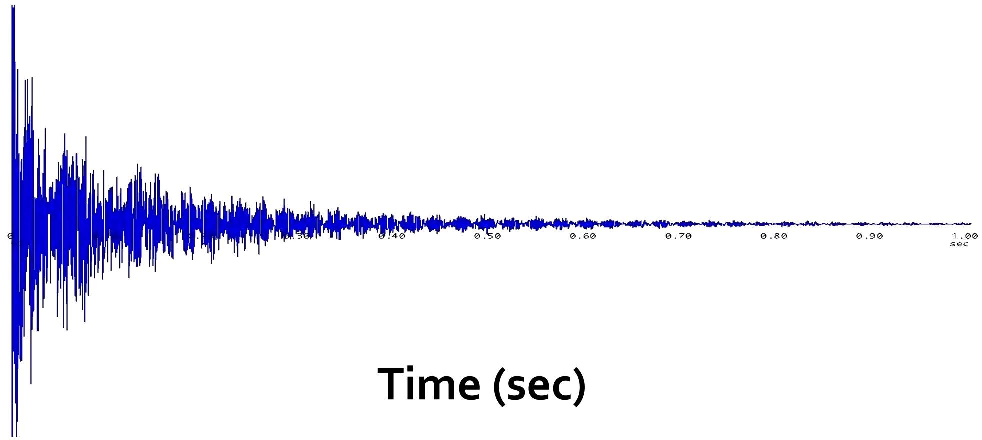


(if there's no relaxation)



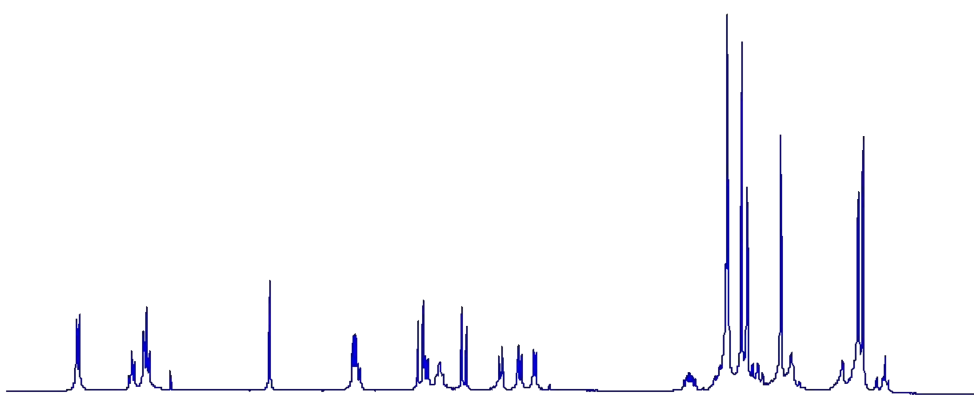
(the real case with T1 &T2)

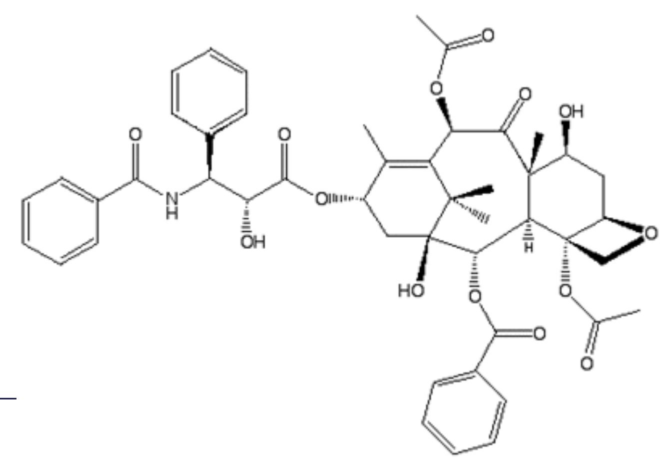
•In addition, most molecules examined by NMR have several sets of nuclei, each with a different precession frequency.



Time (sec)

•The FID (free induction decay) is then **Fourier transform** **to frequency domain to obtain each $v_{pression}$ (chemical shift) for different nuclei.**





frequency (Hz)

NMR Periodic Table

NMR “active” Nuclear Spin (I) = ½:

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

biological and chemical relevance

Odd atomic mass

I = +½ & -½

NMR “inactive” Nuclear Spin (I) = 0:

^{12}C, ^{16}O

Even atomic mass & number

Quadrupole Nuclei Nuclear Spin (I) > ½:

^{14}N, ^{2}H, ^{10}B

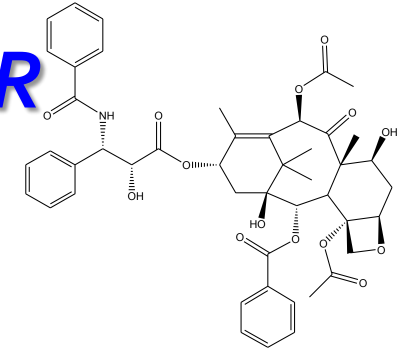
Even atomic mass & odd number

I = +1, 0 & -1

Table 1.1 Nuclear properties of some of the elements

Element	*Atomic mass*	*Spin I*	*Natural abundance (%)*	*Receptivity (^{13}C = 1.00)*	*Quadrupole moment (10^{30} m^2)*	*Resonant frequency (MHz) at 2.348 T*
Hydrogen	1	1/2	99.985	5670	None	100.00
Deuterium	2	1	0.015	0.0082	0.287	15.35
Tritium	3	1/2	Radioactive	–	None	106.66
Helium	3	1/2	0.00014	0.0035	None	76.18
Lithium	6	1	7.42	3.58	–0.064	14.72
Lithium	7	3/2	92.58	1540	–3.7	38.87
Beryllium	9	3/2	100	78.8	5.3	15.06
Boron	10	3	19.58	22.1	7.4	10.75
Boron	11	3/2	80.42	754	4.1	32.08
Carbon	13	1/2	1.108	1.00	None	25.15
Nitrogen	14	1	99.63	5.70	1.67	7.23
Nitrogen	15	1/2	0.37	0.022	None	10.14
Oxygen	17	5/2	0.037	0.061	–2.6	13.56
Fluorine	19	1/2	100	4730	None	94.09
Neon	21	3/2	0.257	0.0036	9	7.90
Sodium	23	3/2	100	524	10	26.43
Magnesium	25	5/2	10.13	1.54	22	6.13
Aluminium	27	5/2	100	1170	14	26.08
Silicon	29	1/2	4.7	2.1	None	19.87
Phosphorus	31	1/2	100	377	None	40.48
Sulfur	33	3/2	0.76	0.098	–6.4	7.67
Chlorine	35(37)	3/2	75.53	20.2	–8.2	9.81
Potassium	39	3/2	93.1	2.69	5.5	4.67
Calcium	43	7/2	0.145	0.053	–5	6.74
Scandium	45	7/2	100	1720	–22	24.33
Titanium	49(47)	7/2	5.51	1.18	24	5.64
Vanadium	51(50)	7/2	99.76	2170	–5.2	26.35
Chromium	53	3/2	9.55	0.49	–15	5.64
Manganese	55	5/2	100	1014	40	24.84
Iron	57	1/2	2.19	0.00425	None	3.24
Cobalt	59	7/2	100	1560	42	23.73
Nickel	61	3/2	1.19	0.24	16	8.93
Copper	63(65)	3/2	69.09	368	–22	26.51
Zinc	67	5/2	4.11	0.67	15	6.25
Gallium	71(69)	3/2	39.6	322	11	30.58
Germanium	73	9/2	7.76	0.62	–17	3.48
Arsenic	75	3/2	100	144	29	17.18
Selenium	77	1/2	7.58	3.02	None	19.07
Bromine	81(79)	3/2	49.46	279	27	27.10
Krypton	83	9/2	11.55	1.24	27	3.86
Rubidium	87(85)	3/2	27.85	280	13	32.84
Strontium	87	9/2	7.02	1.08	16	4.35
Yttrium	89	1/2	100	0.676	None	4.92
Zirconium	91	5/2	11.23	6.05	–21	9.34
Niobium	93	9/2	100	2770	–32	24.55
Molybdenum	95(97)	5/2	15.72	2.92	–1.5	6.55
Technetium	99	9/2	Radioactive	–	–0.13	22.51
Ruthenium	99(101)	5/2	12.72	0.815	7.6	4.61
Rhodium	103	1/2	100	0.18	None	3.16
Palladium	105	5/2	22.23	1.43	65	4.58
Silver	109(107)	1/2	48.18	0.28	None	4.65
Cadmium	113(111)	1/2	12.26	7.69	None	22.18

Typical Applications of NMR



Taxol (natural product)

1.) Structural (chemical) elucidation

, Natural product chemistry

, Synthetic organic chemistry

- analytical tool of choice of synthetic chemists
- used in conjunction with MS and IR

2.) Study of dynamic processes

, reaction kinetics

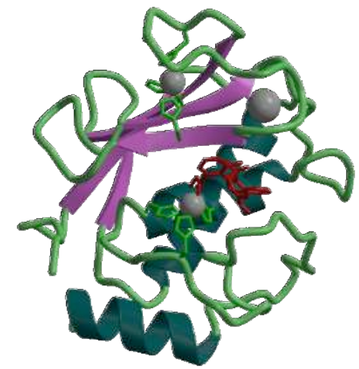
, study of equilibrium (chemical or structural)

3.) Structural (three-dimensional) studies

, Proteins, Protein-ligand complexes

, DNA, RNA, Protein/DNA complexes

, Polysaccharides



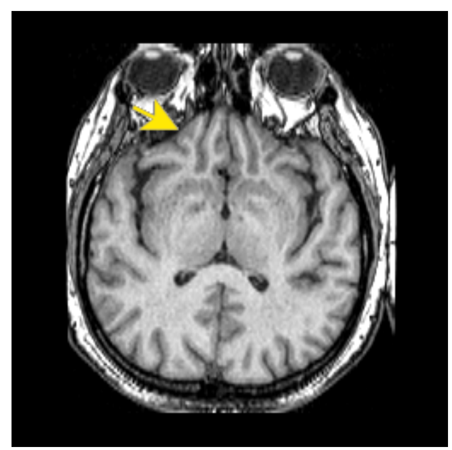
NMR Structure of MMP-13 complexed to a ligand

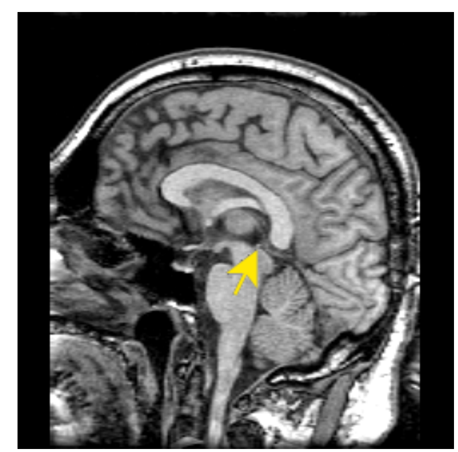
4.) Metabolomics

5.) Drug Design

, Structure Activity Relationships by NMR

6.) Medicine -MRI

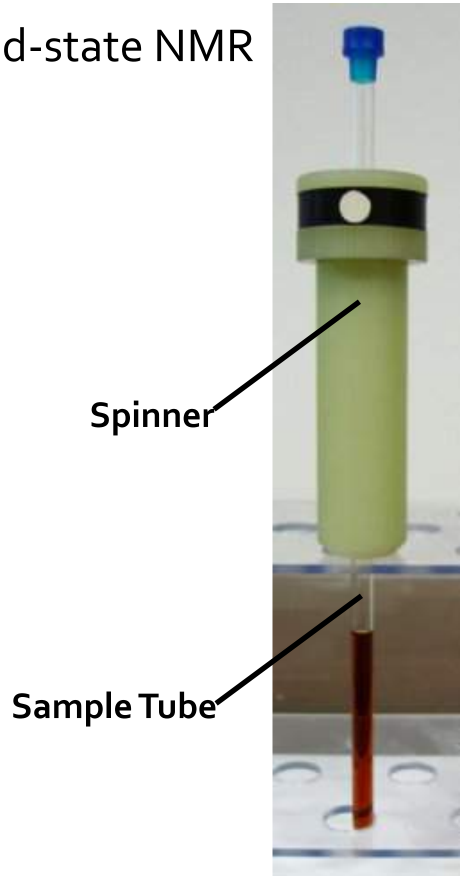


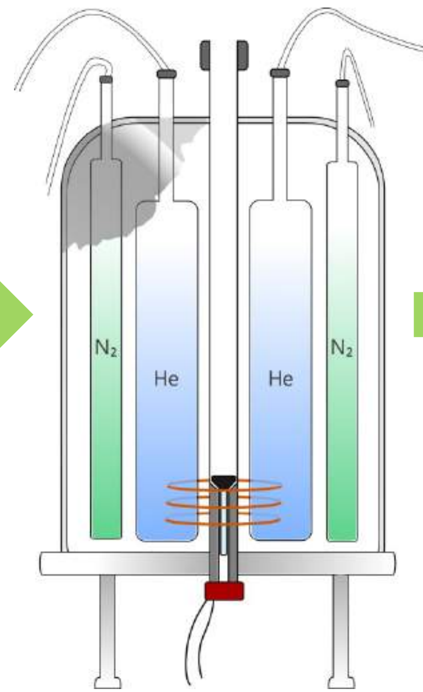


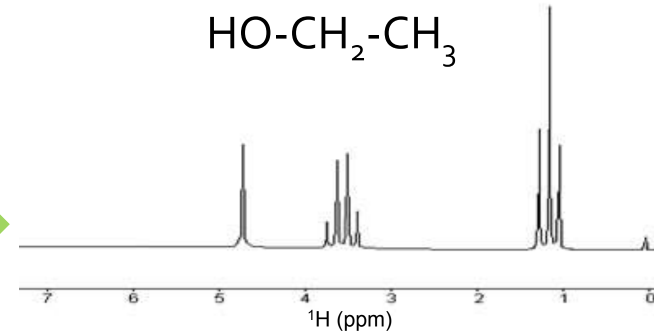
MRI images of the Human Brain

NMR is a widely used structural tool

Liquid-state NMR

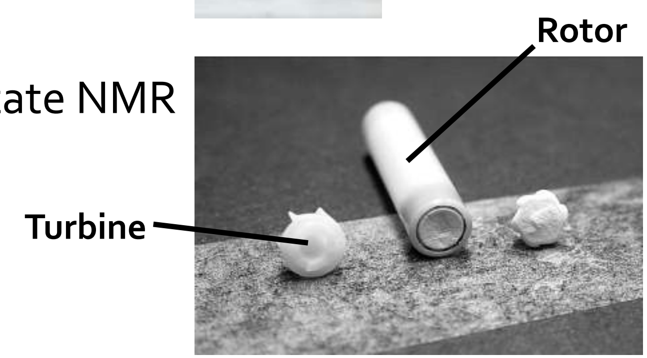


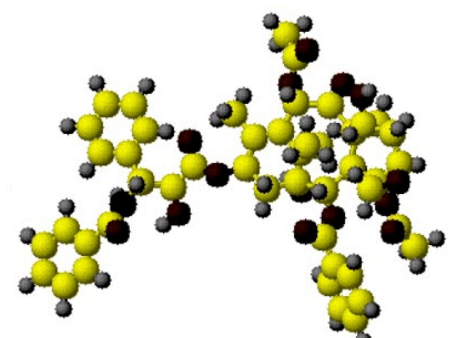




- Chemical shift
- Signal intensity
- Scalar couplings

Solid-state NMR



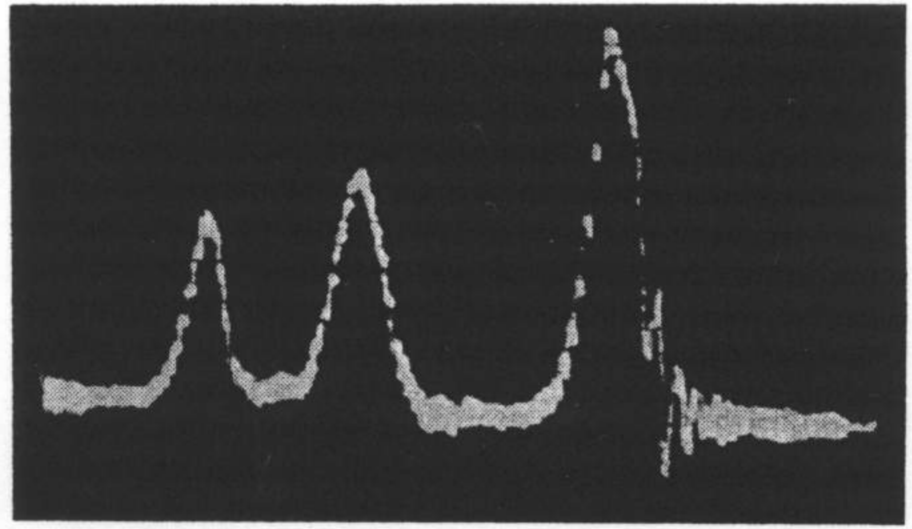


NMR History

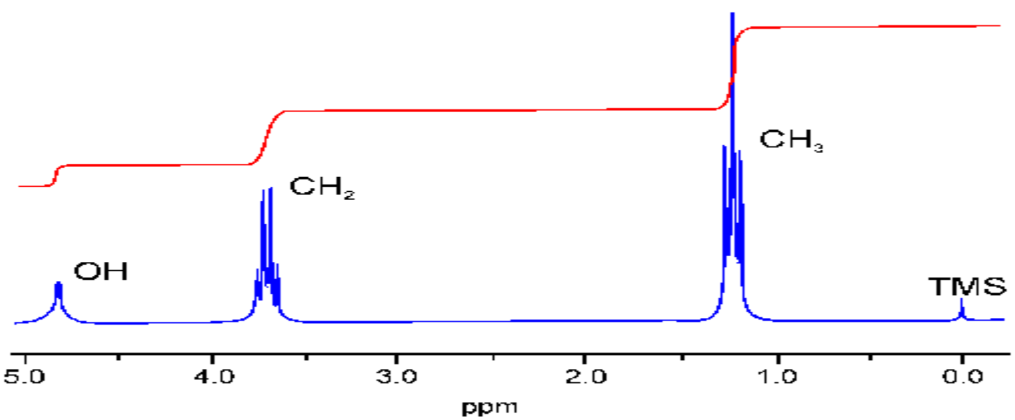
First Observation of the Chemical Shift

¹H NMR spectra ethanol

CH_3CH_2OH

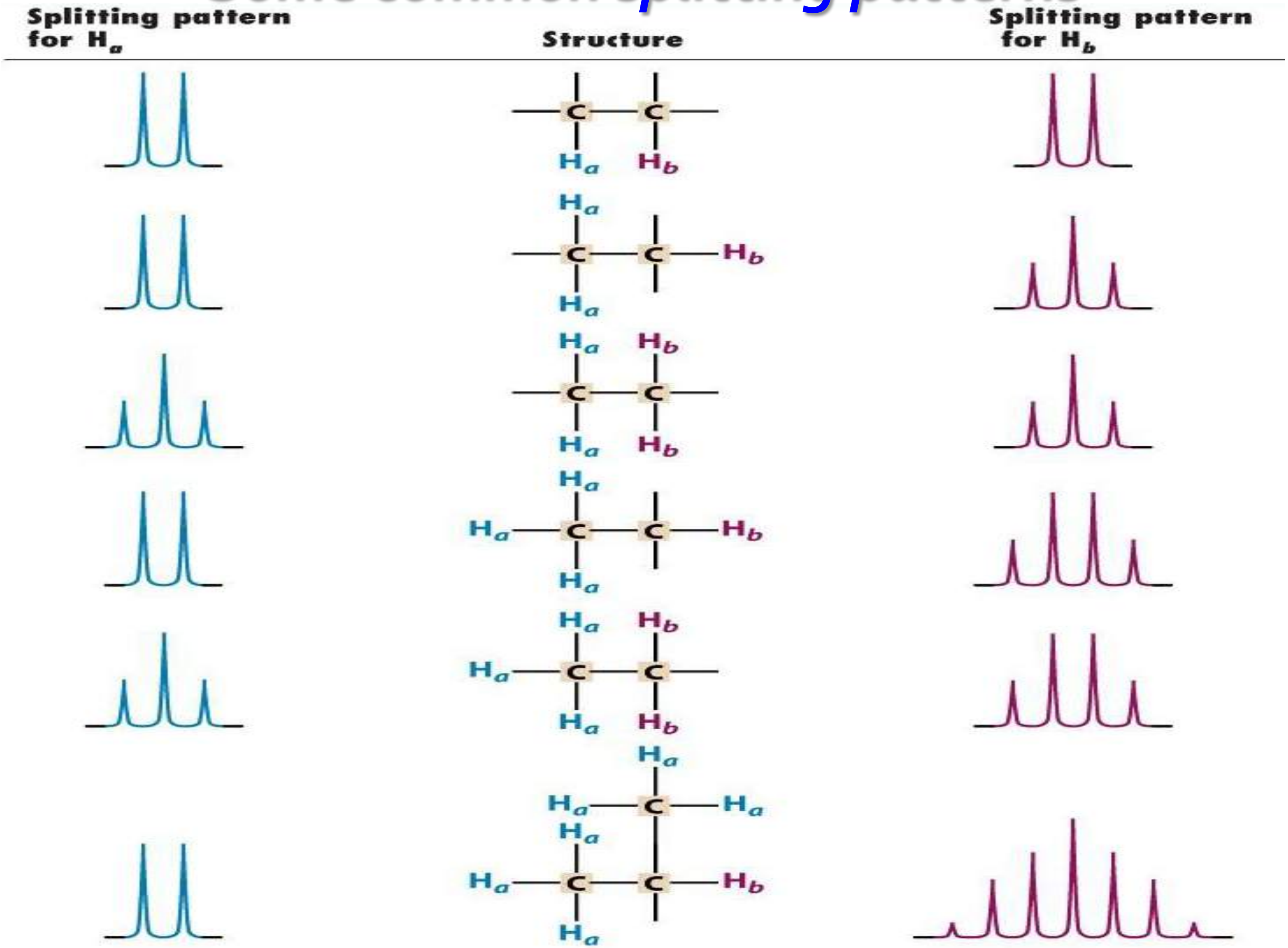


Modern ethanol spectra



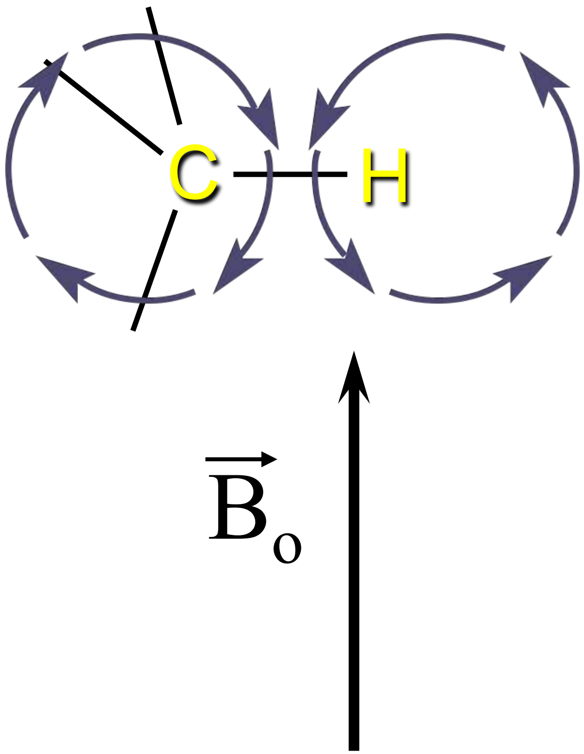
Arnold, J.T., S.S. Dharmatti, and M.E. Packard, J. Chem. Phys., 1951. **19**: p. 507.

Some common splitting patterns



Chemical shift

An external magnetic field affects the motion of the electrons in a molecule, inducing a magnetic field within the molecule.

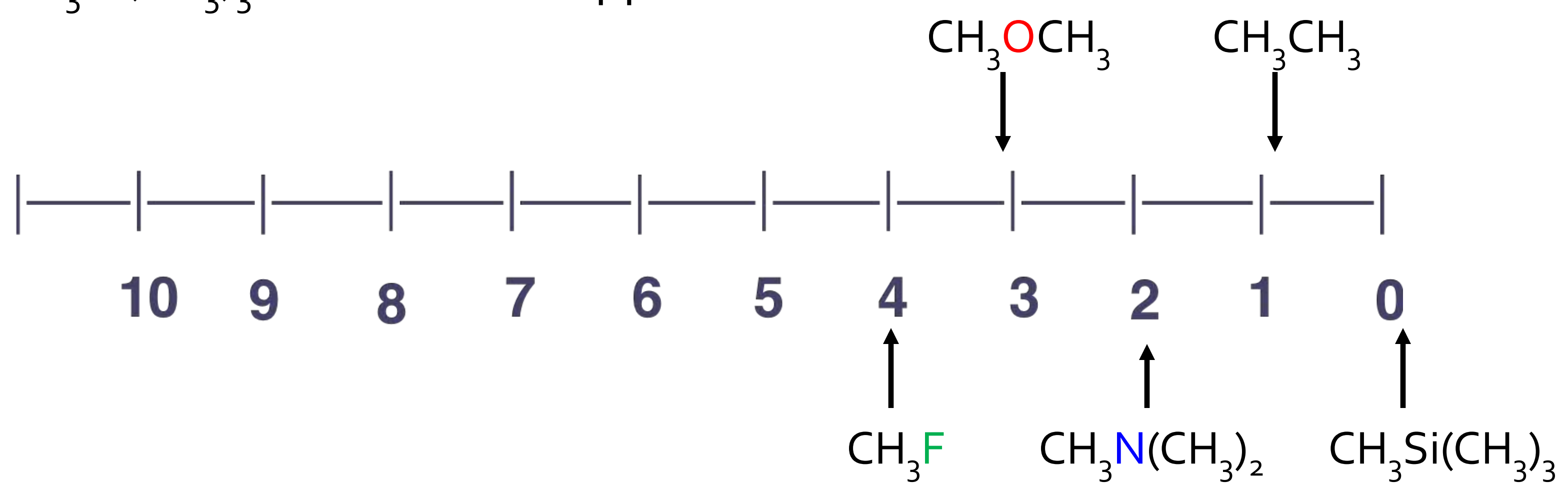


Chemical shift is a measure of the degree to which a nucleus in a molecule is shielded.

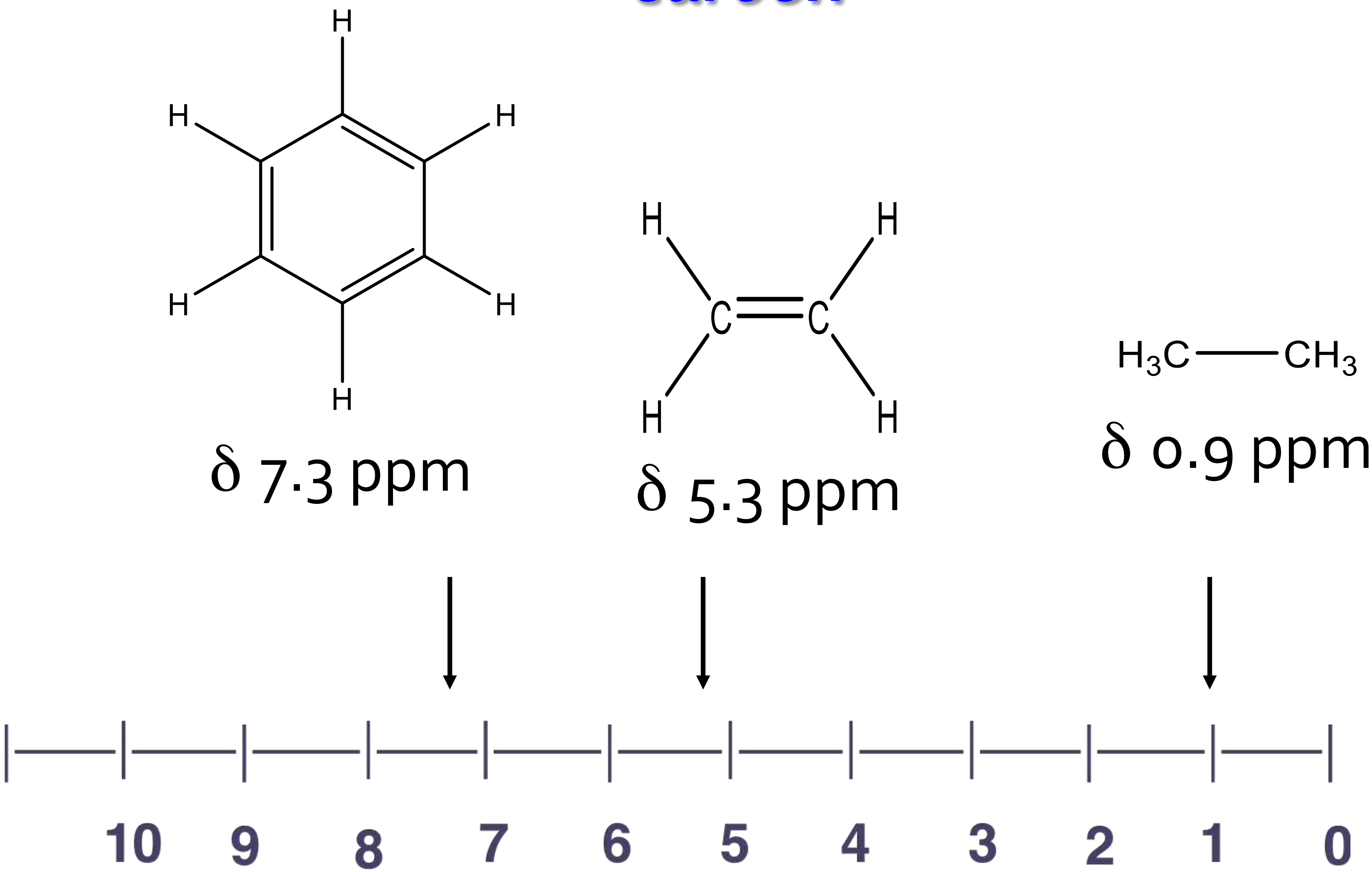
Protons in different environments are shielded to greater or lesser degrees; they have different chemical shifts.

Electronegative substituents decrease the shielding of methyl groups

CH_3F	δ 4.3 ppm
CH_3OCH_3	δ 3.2 ppm
$CH_3N(CH_3)_2$	δ 2.2 ppm
CH_3CH_3	δ 0.9 ppm
$CH_3Si(CH_3)_3$	δ 0.0 ppm

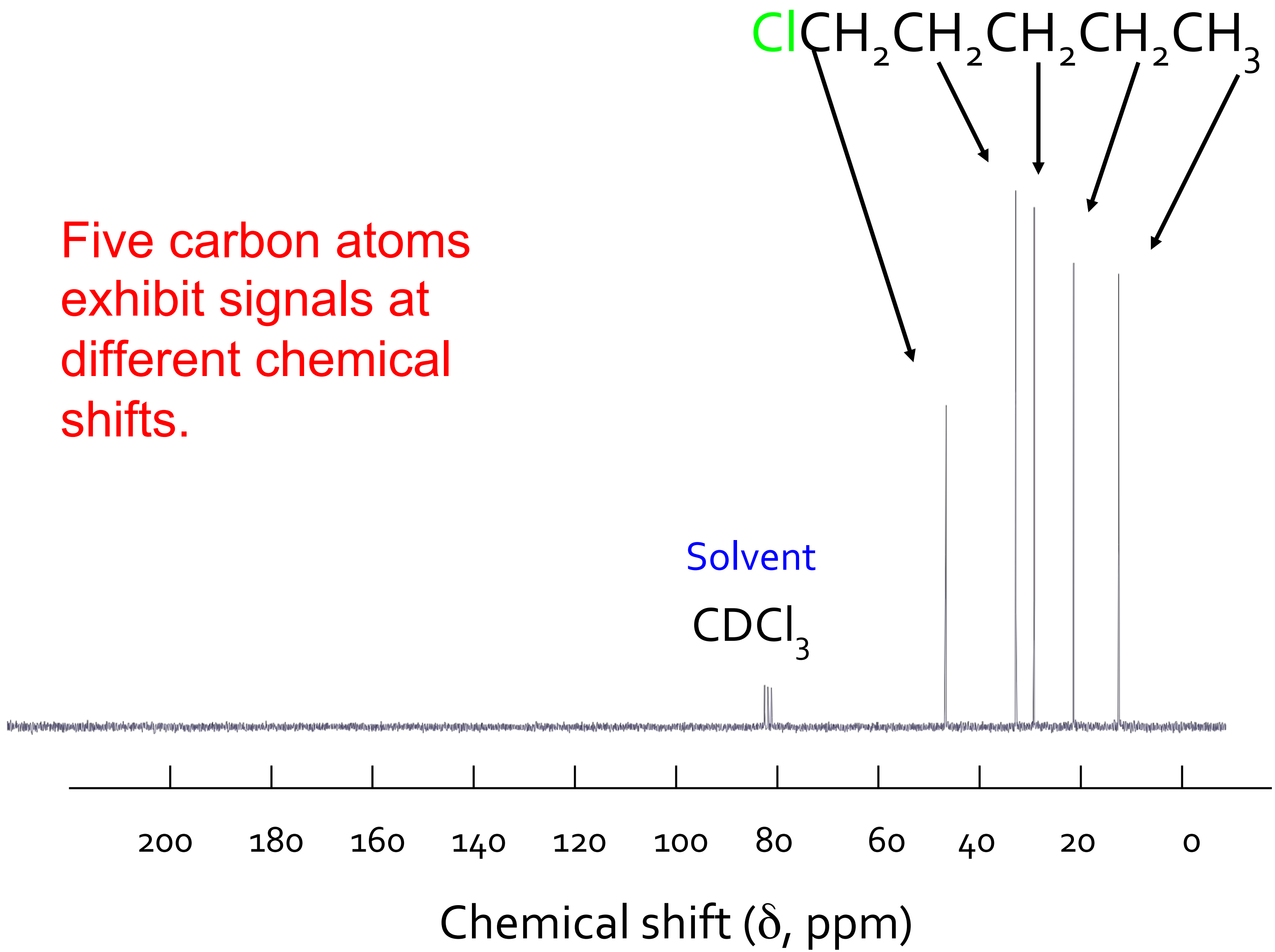


Protons attached to sp^2 hybridized carbon are less shielded than those attached to sp^3 hybridized carbon



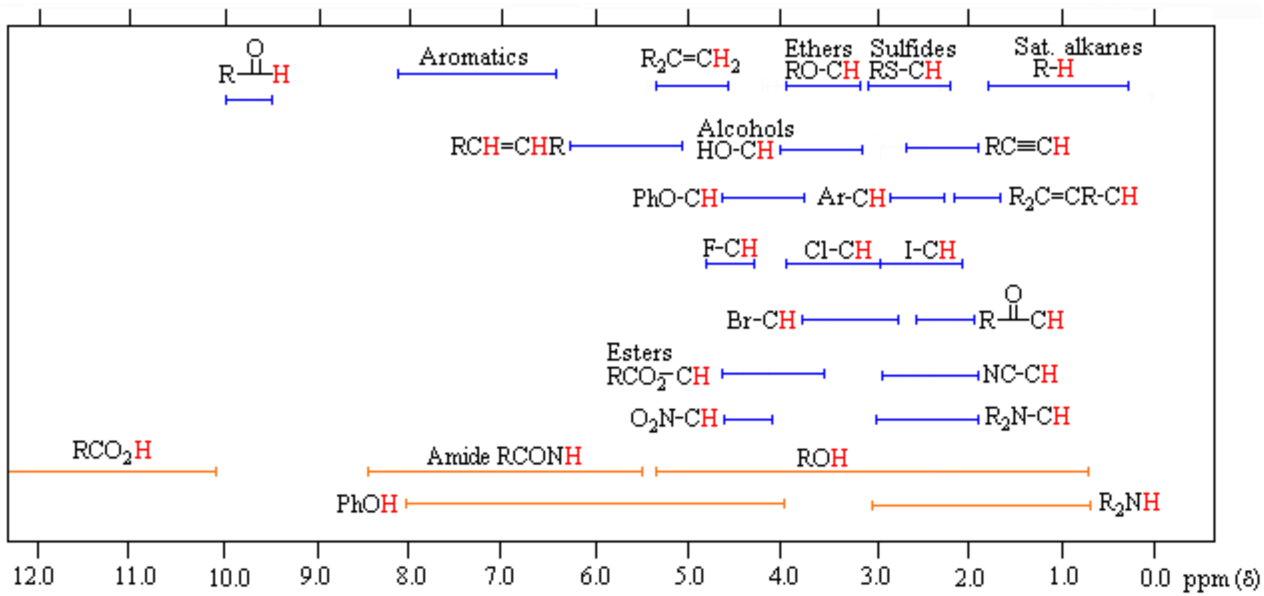
^{13}C NMR spectrum

Five carbon atoms exhibit signals at different chemical shifts.



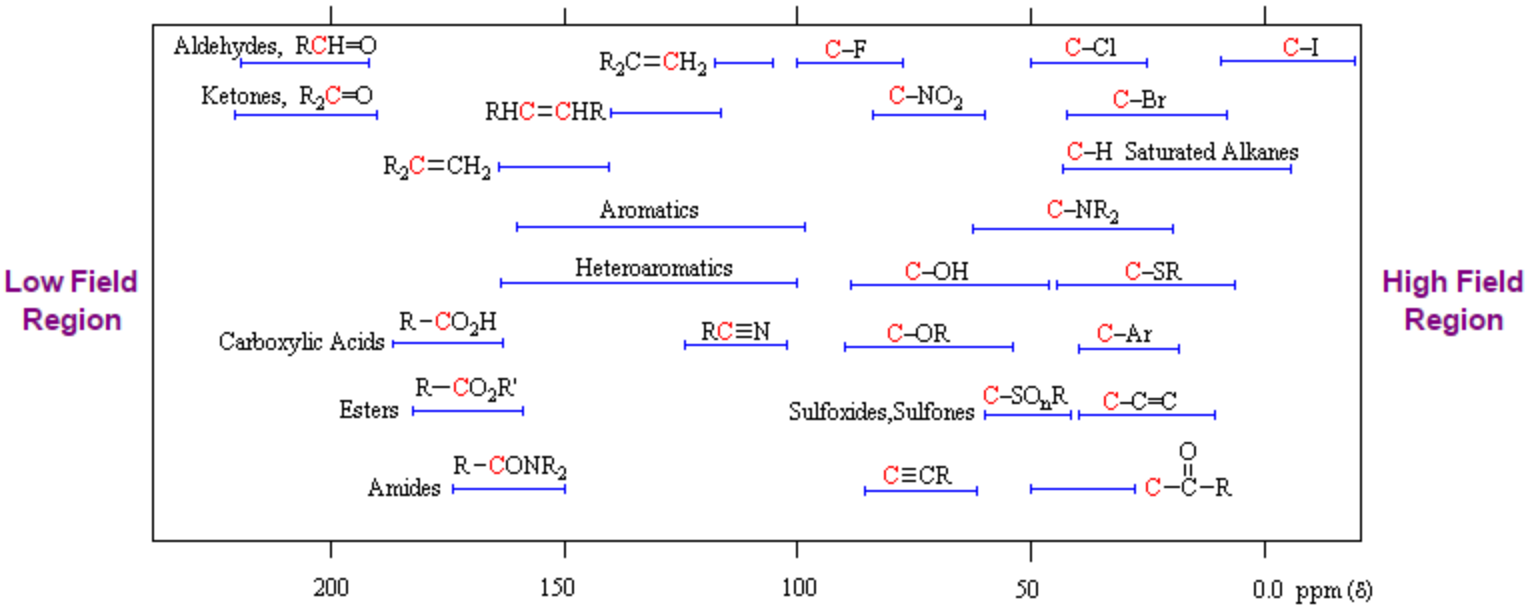
Proton chemical shift ranges

Low Field Region



High Field Region

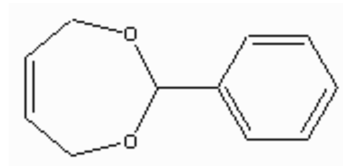
Carbon chemical shift ranges



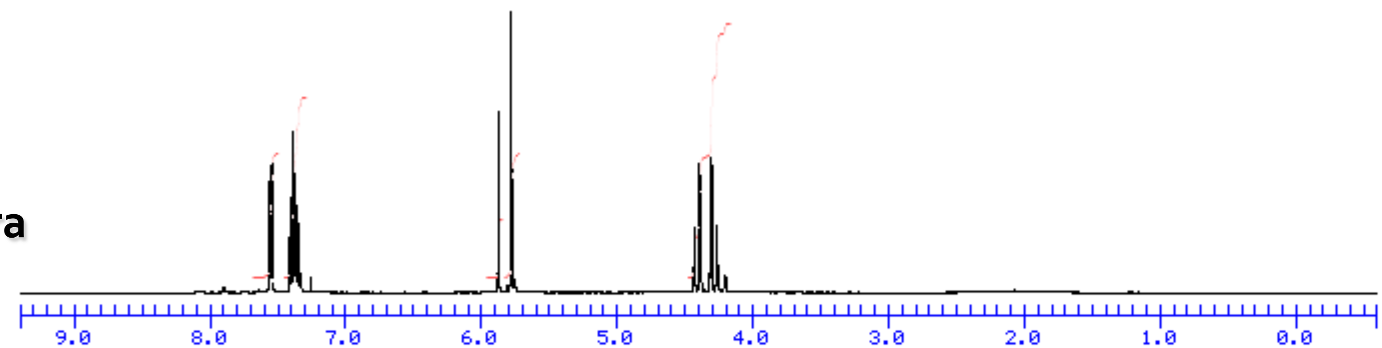
Each NMR observable nuclei yields a peak in the spectra

"fingerprint" of the structure

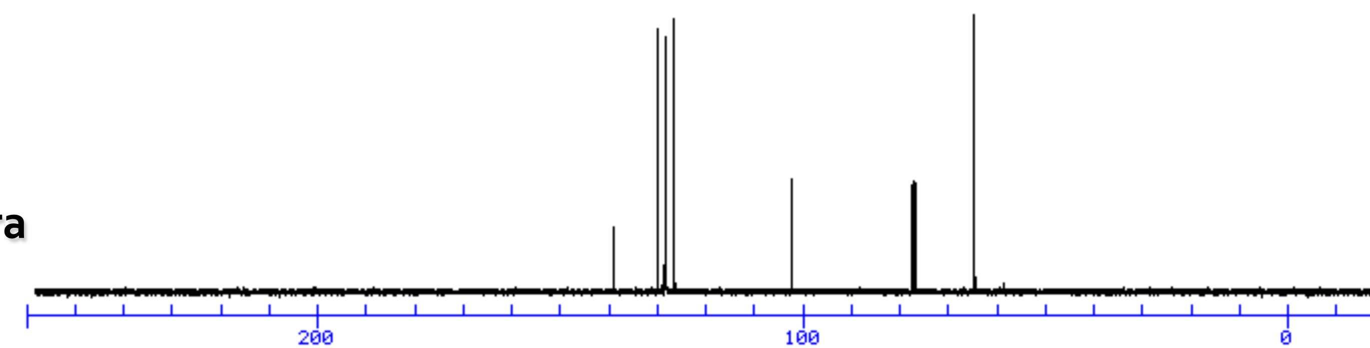
2-phenyl-1,3-dioxep-5-ene



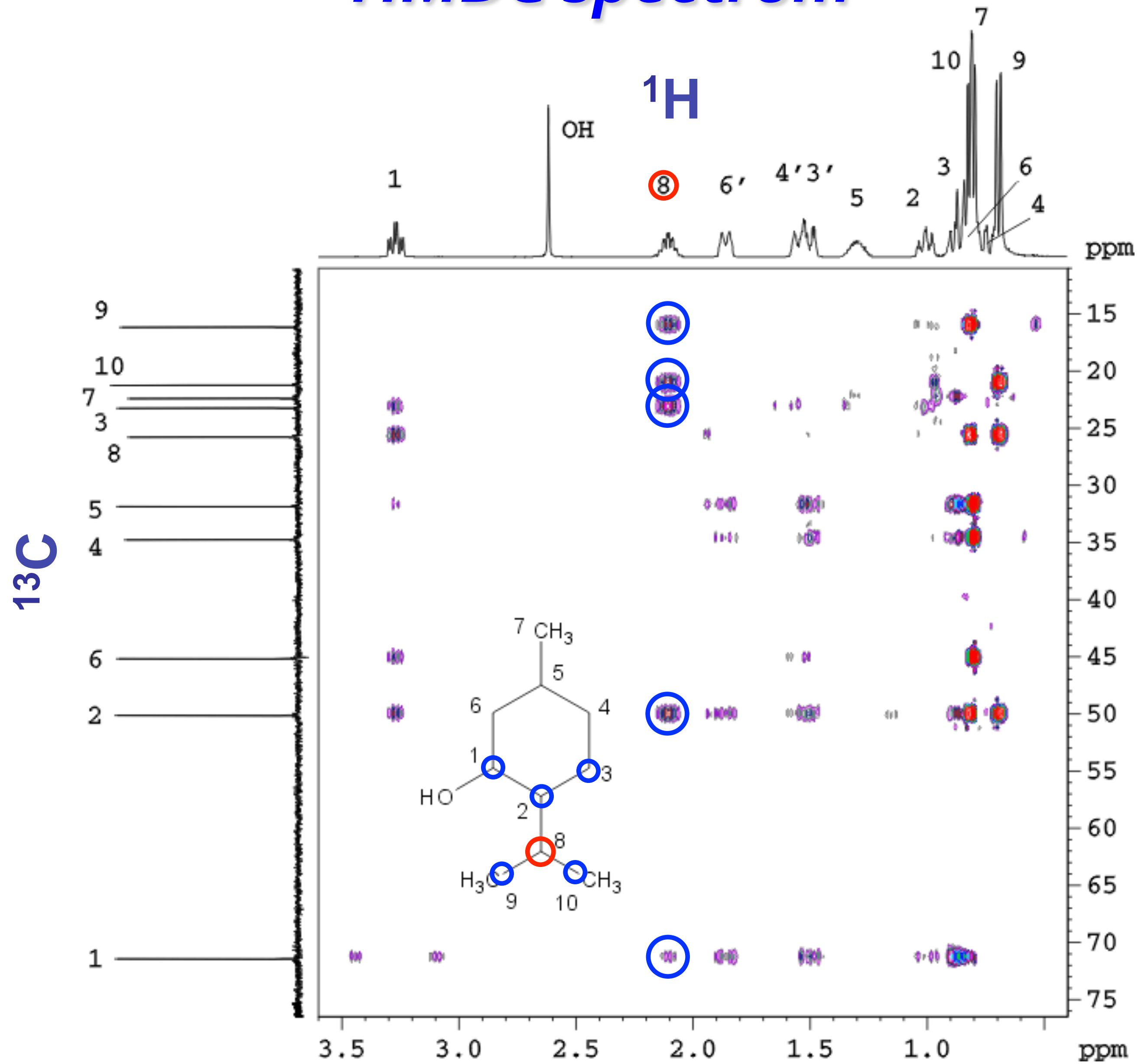
^{1}H NMR spectra



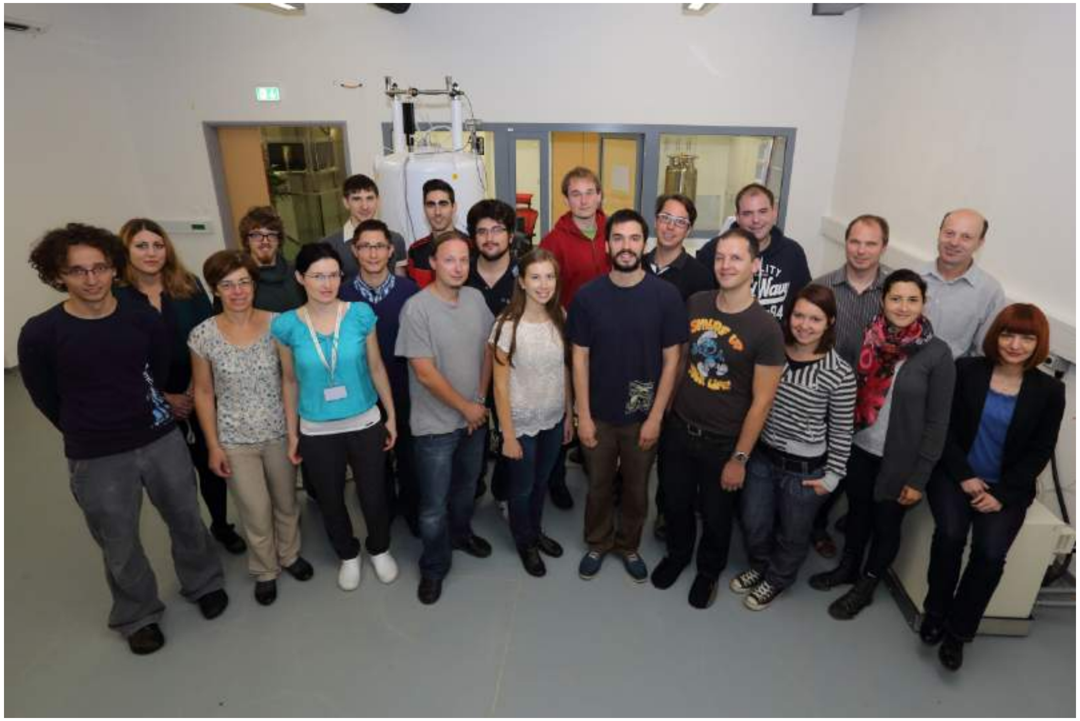
^{13}C NMR spectra



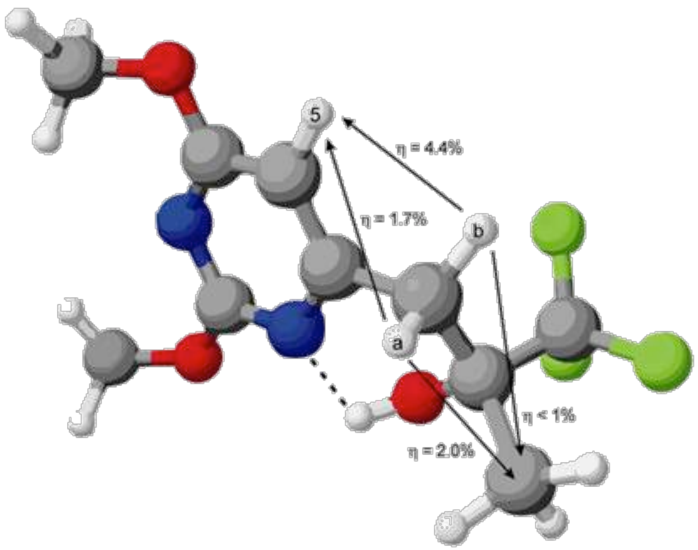
HMBC spectrum



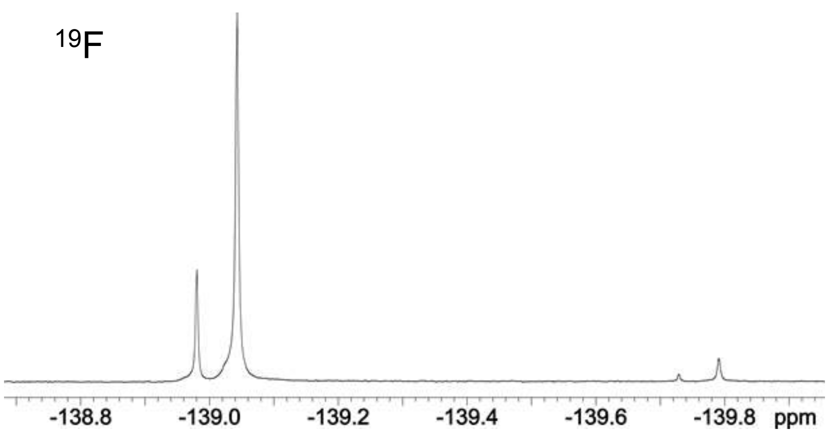
Liquid state NMR

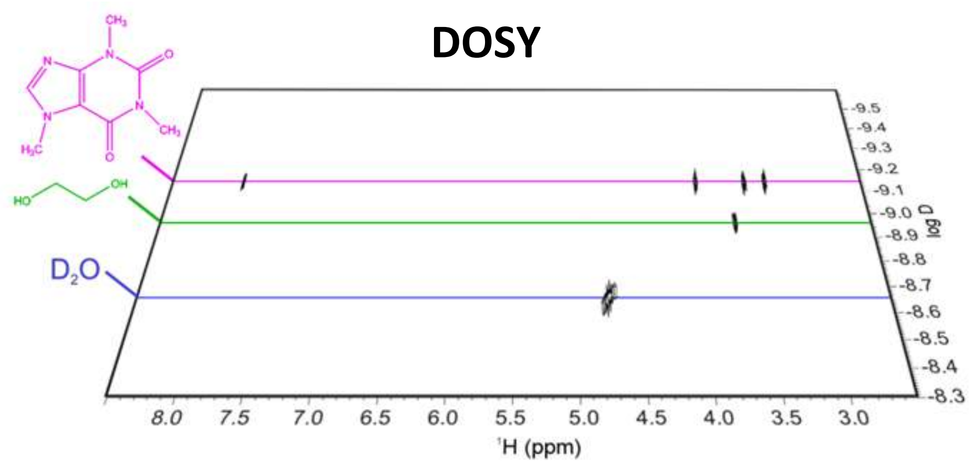


Analysis of organic compounds in solution

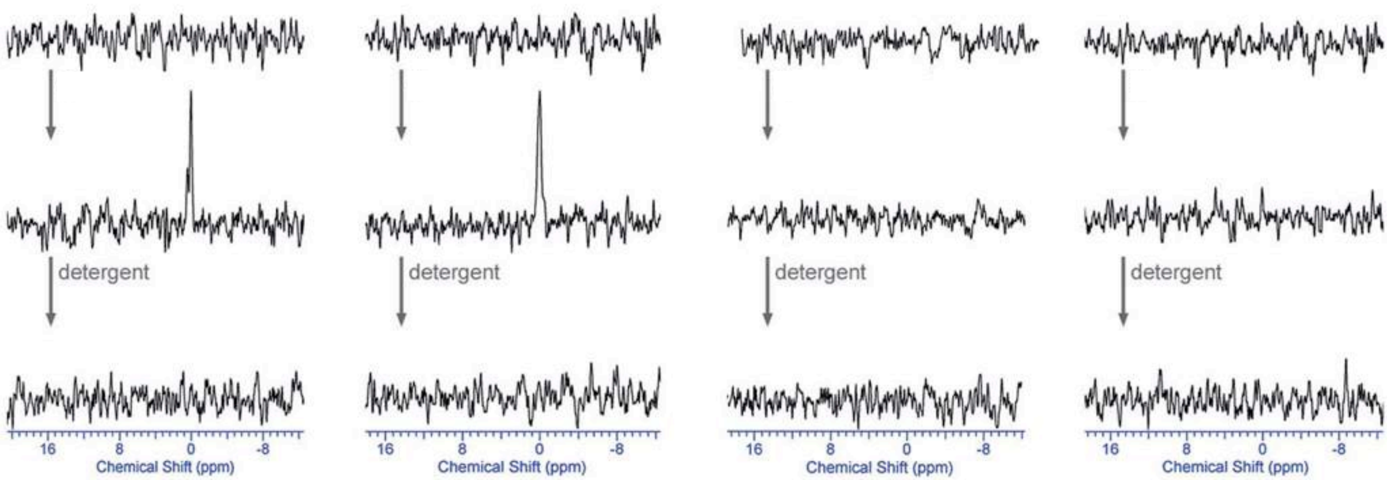


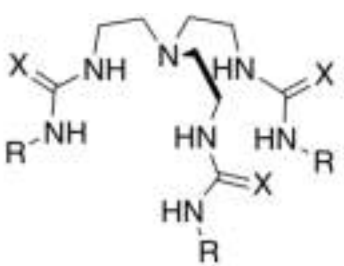
- Complete structure determination
- Study of dynamics of molecule (flexibility)
- Determination of kinetics
- Determination of structures inside mixtures



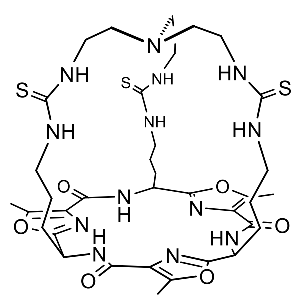


Chloride/sulfate transport
^{33}S NMR experiments

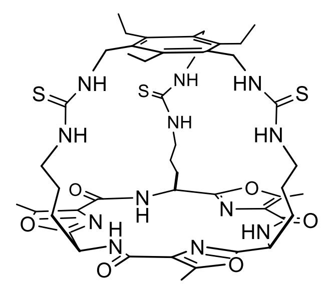




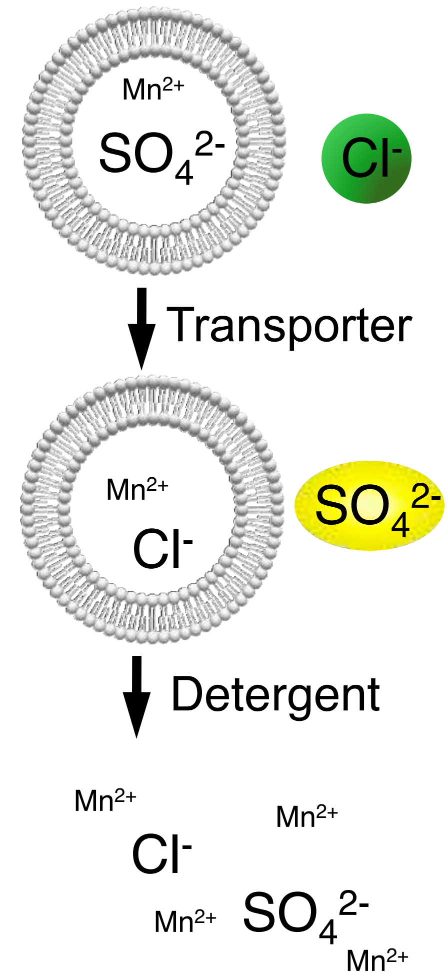
X=O, R=C_6F_5 X=S, R=C_6F_5



PY34



PY45

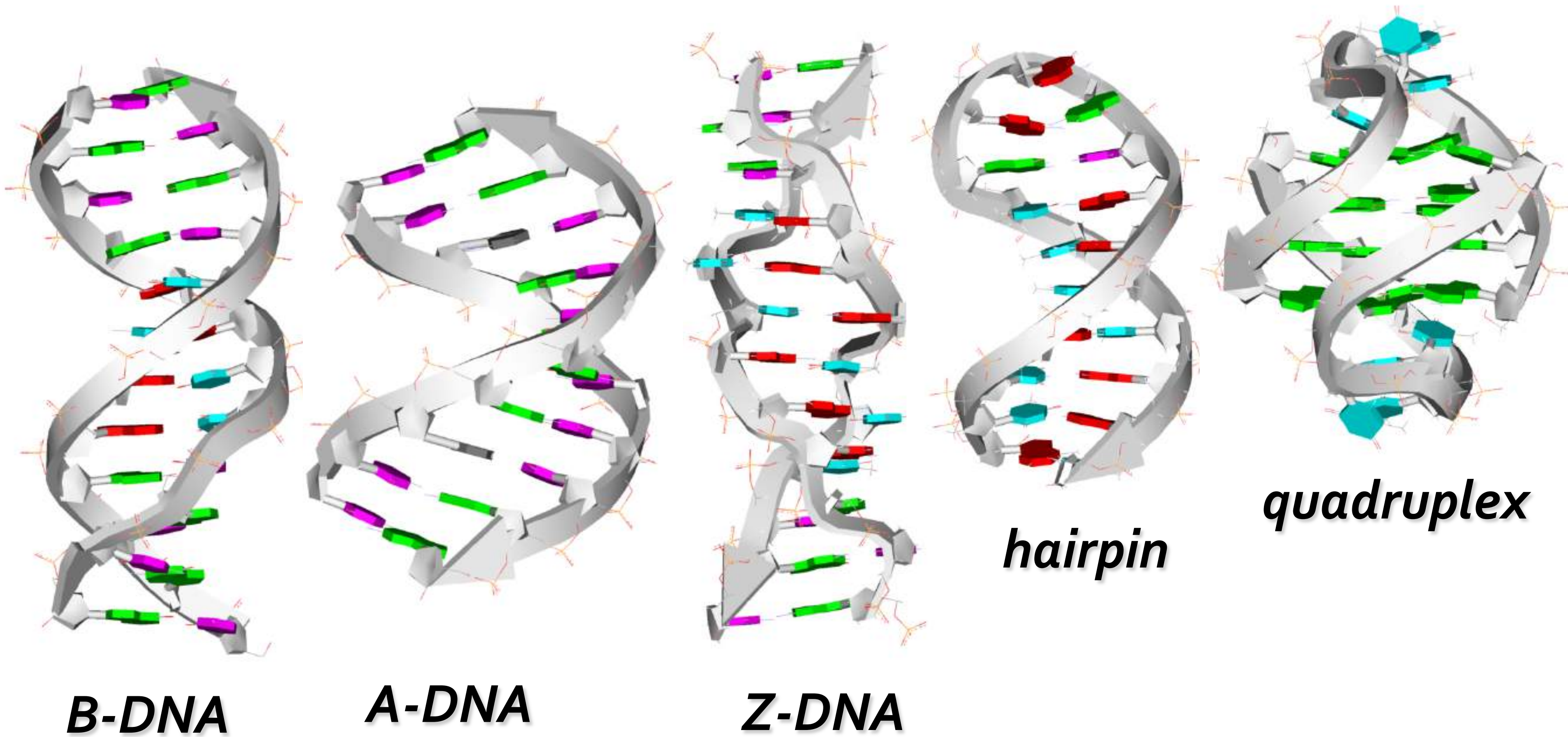


Busschaert et al., *Chem. Sci.* **2013**,
DOI: 10.1039/C3SC52006D.

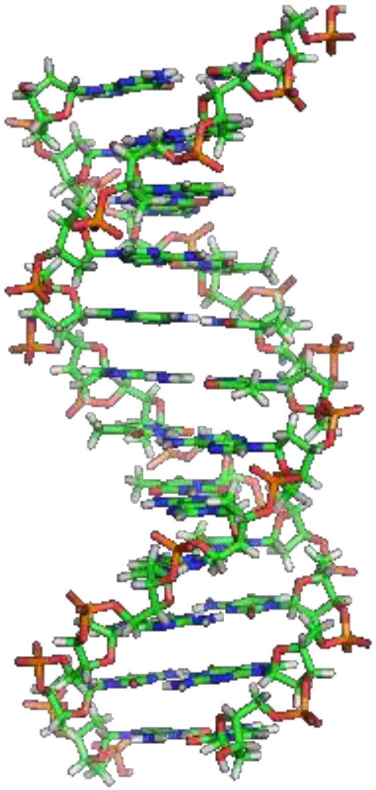
Nucleic acids

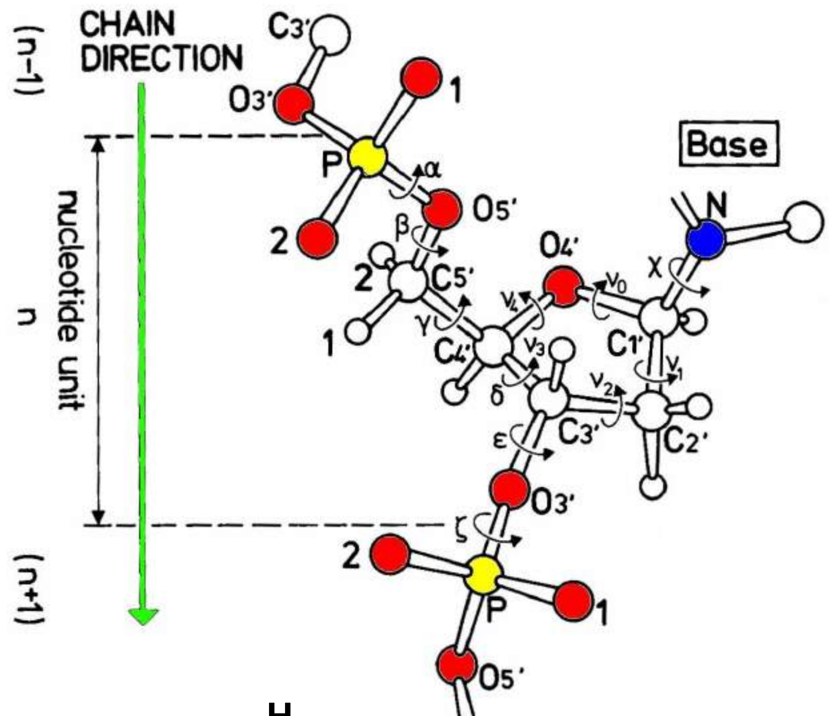
Nucleic acids are biological molecules necessary for life, including DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).

Together with proteins, nucleic acids form the most essential macromolecules.

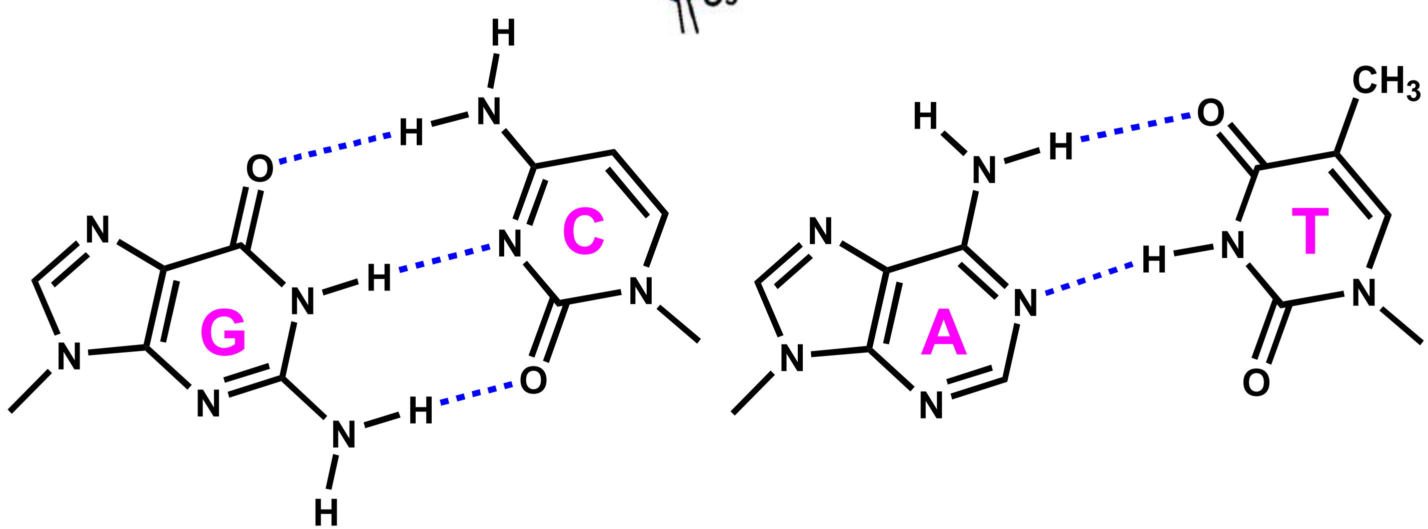


DNA - double-helix, Watson-Crick base pairs, …

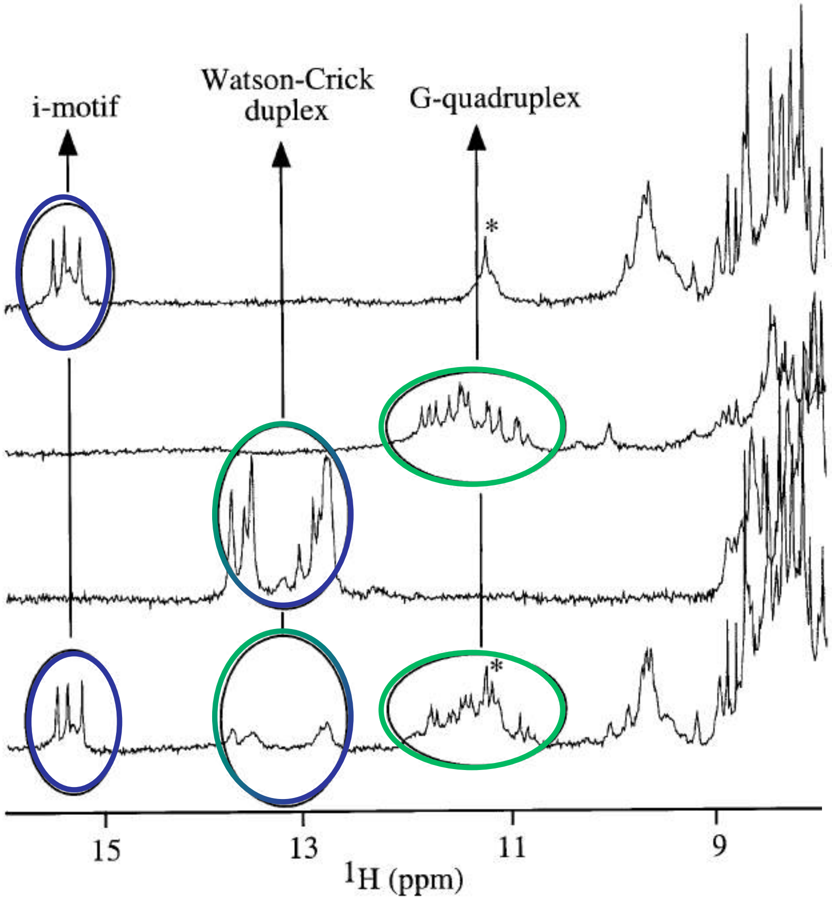




B-DNA

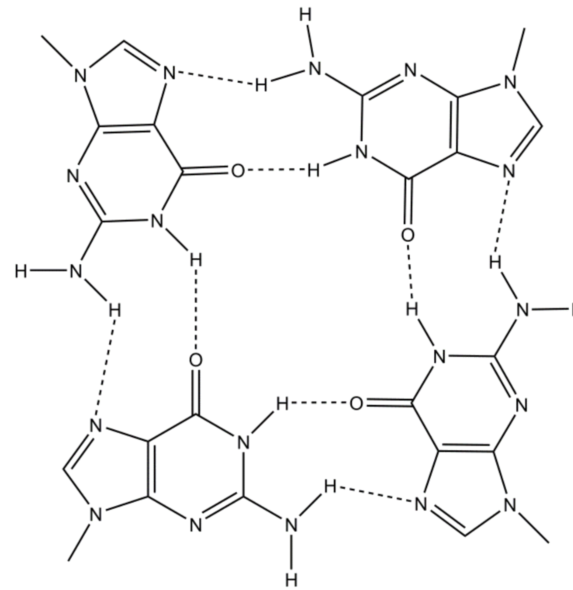


NMR easily distinguishes different structures

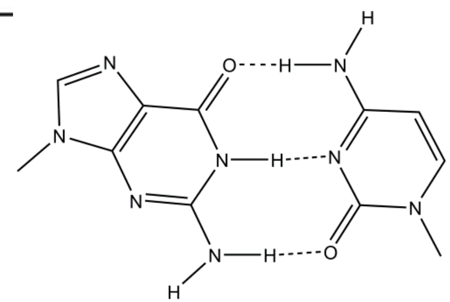


A.T. Phan and JL. Mergny, NAR, 2002, 30, 21)

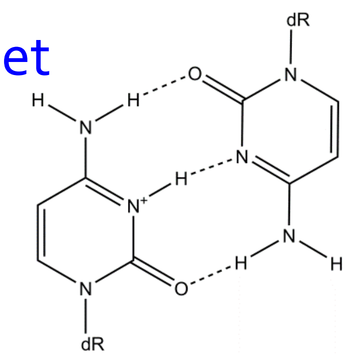
5'-AGGGTTAGGGTTAGGGTTAGGG-3'
3'-TCCCAATCCCAATCCCAATCCC-5'



G-quartet



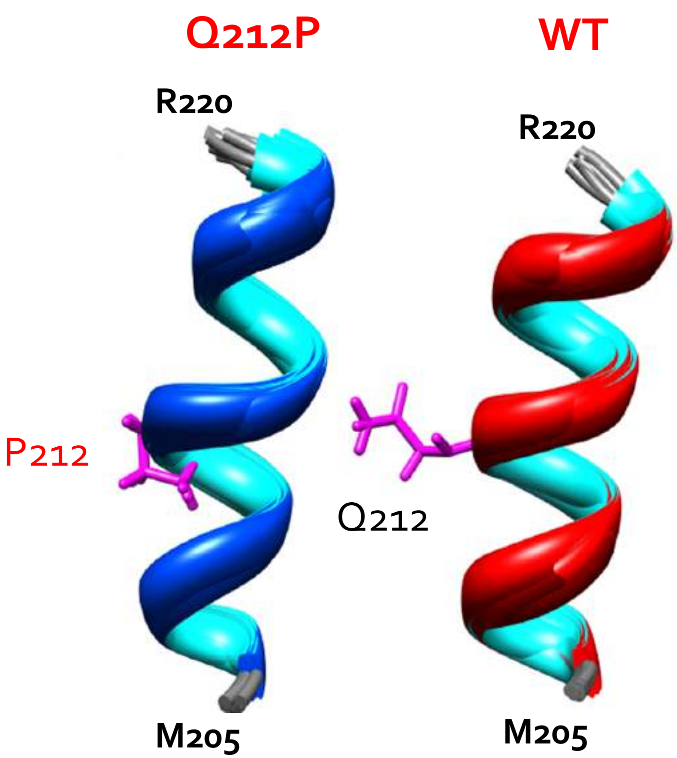
GC base pair



C^{+}-C base pair

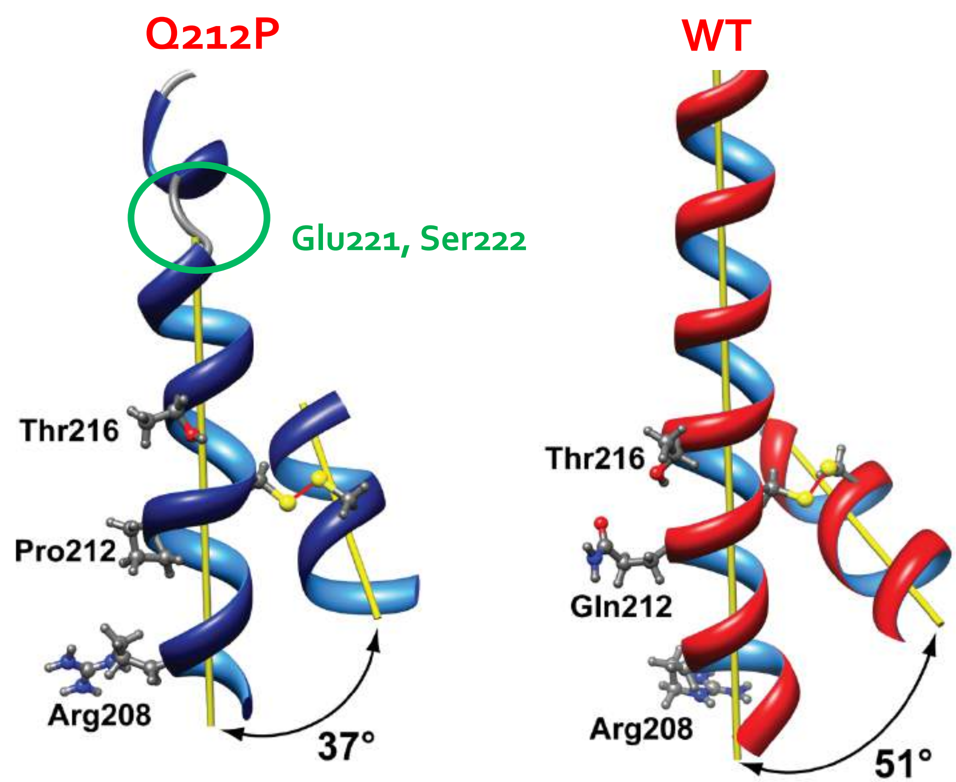
Structural features of HuPrP(Q212P)

The Q212P mutation is associated with Gerstmann-Sträussler-Scheinker (GSS) syndrome, a slowly progressive hereditary autosomal dominant disease.



r.m.s.d. = 0.7 Å

Broken α_3 helix



α_2-α_3 interhelical angle

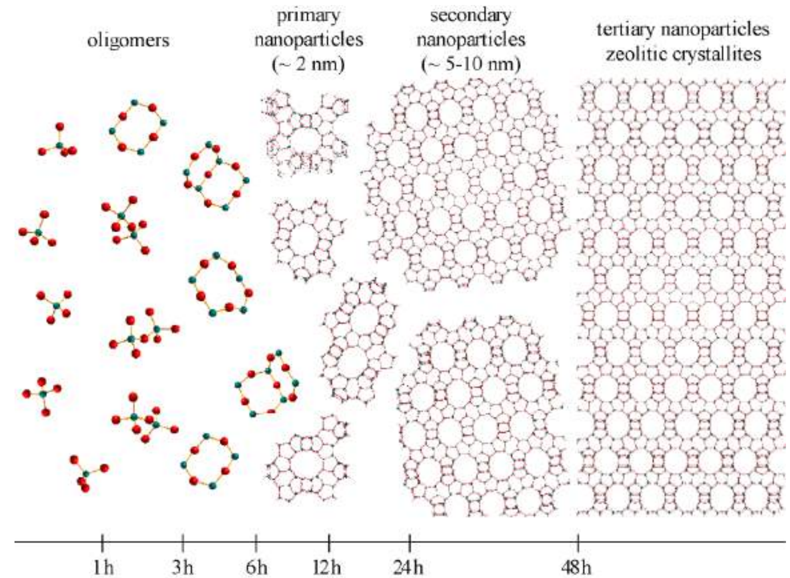
Solid state NMR

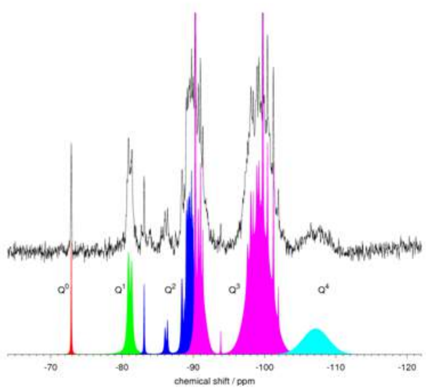
NMR spectroscopy of porous materials

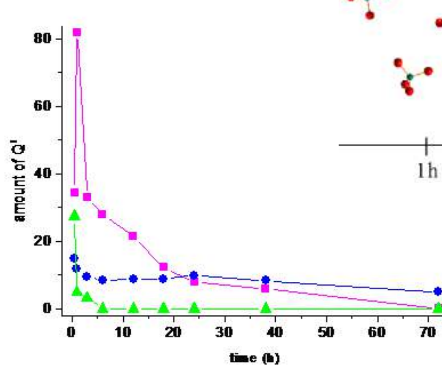
- **formation of porous materials**

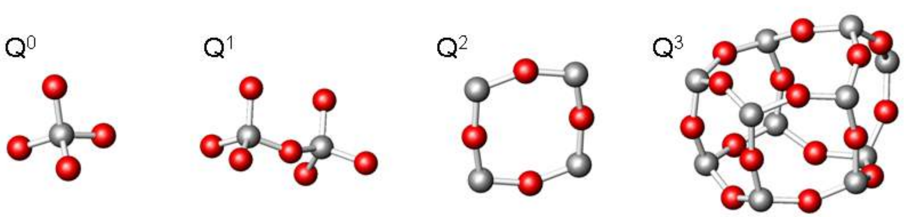
micro- and mesoporous silicates, alumino-phosphates, metal-organic frameworks

NMR can follow formation from the initial solution to the final solid product





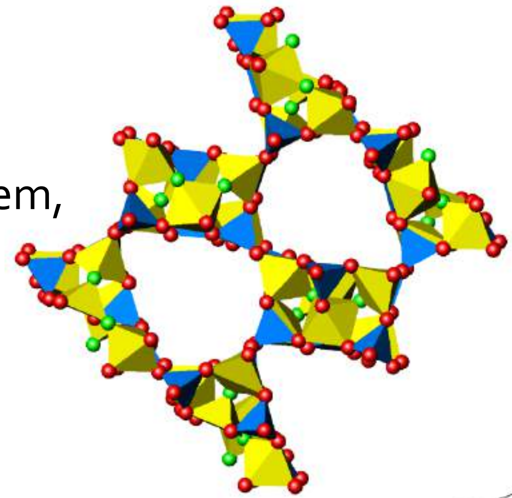


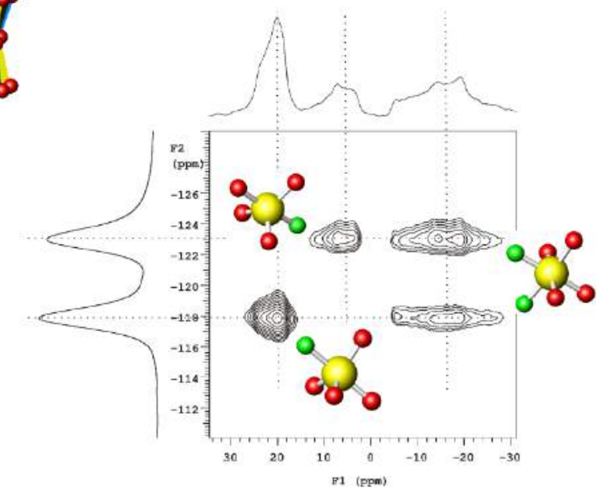


• structure of porous materials

inequivalent sites, connectivities among them,

motifs that do not exhibit long-range order

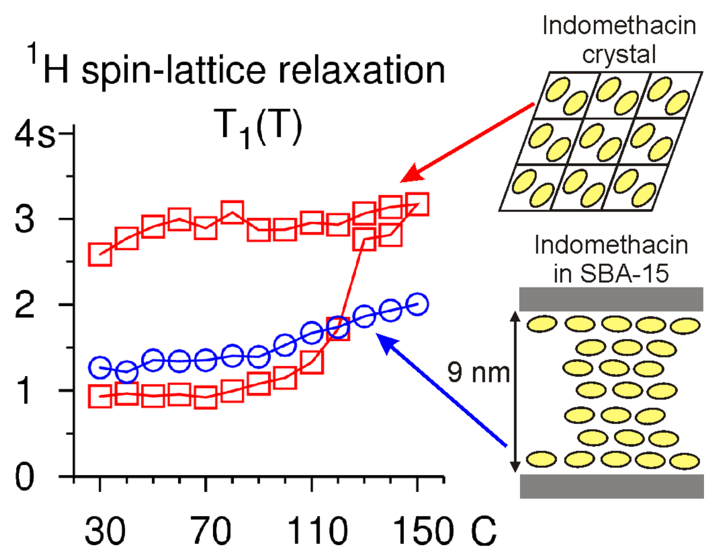




• molecules embedded within the pores

drug-delivery, gas-storage,
heat-storage systems

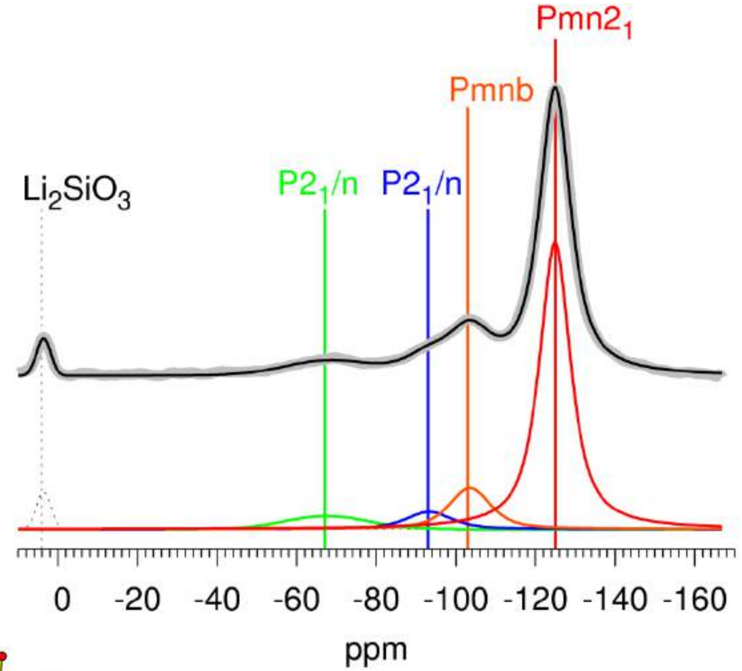
structural information, dynamics,
interactions

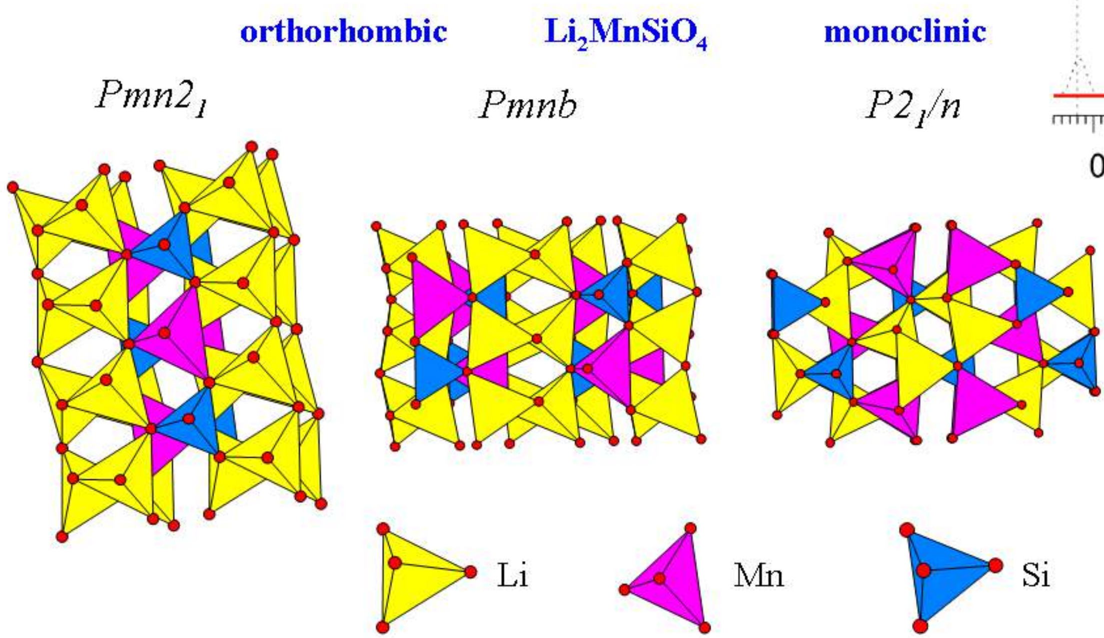


New materials for Li-ion batteries

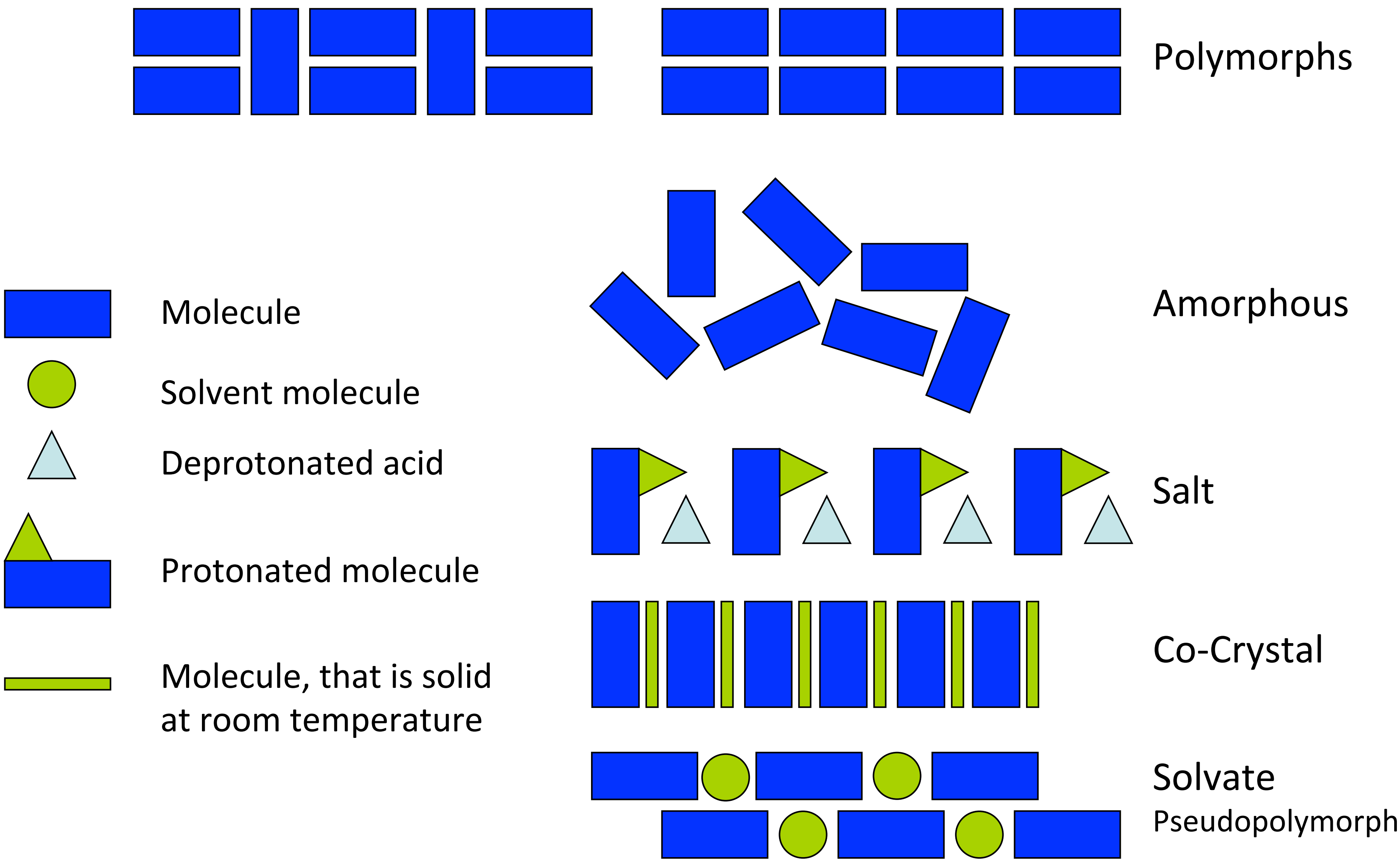
• **composition of materials**

impurities, mixtures of polymorphs





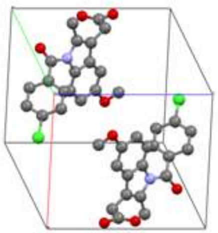
Polymorphism

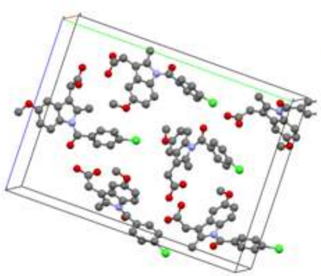


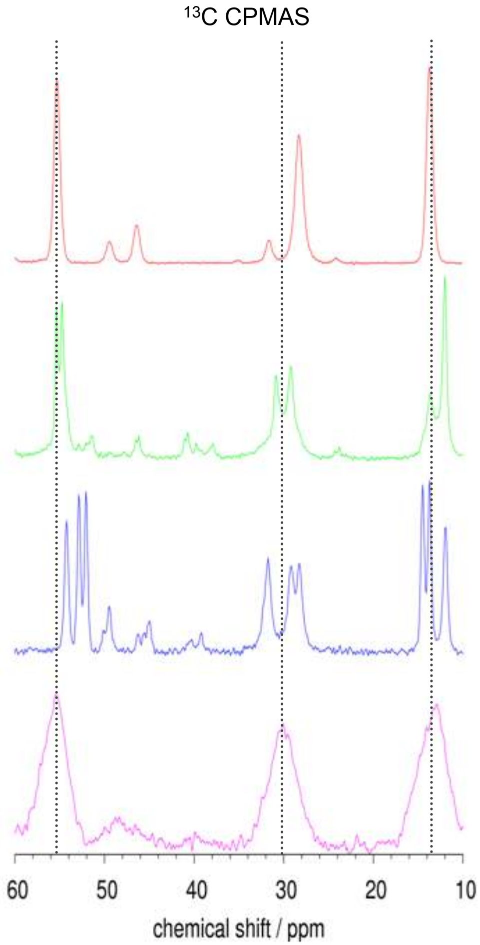
Structure of polymorphs

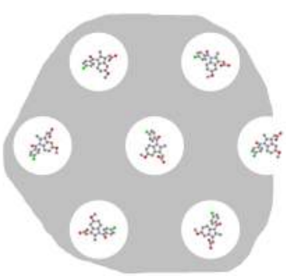
- Solid-state NMR distinguishes between polymorphs
- Determines the number of molecules within the asymmetric crystallographic unit
- Provides information about local environment

Indomethacin

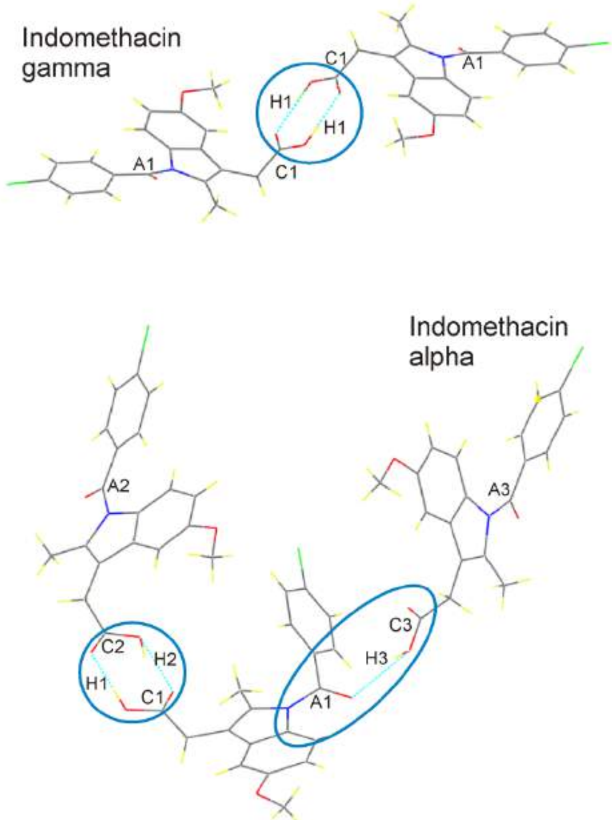


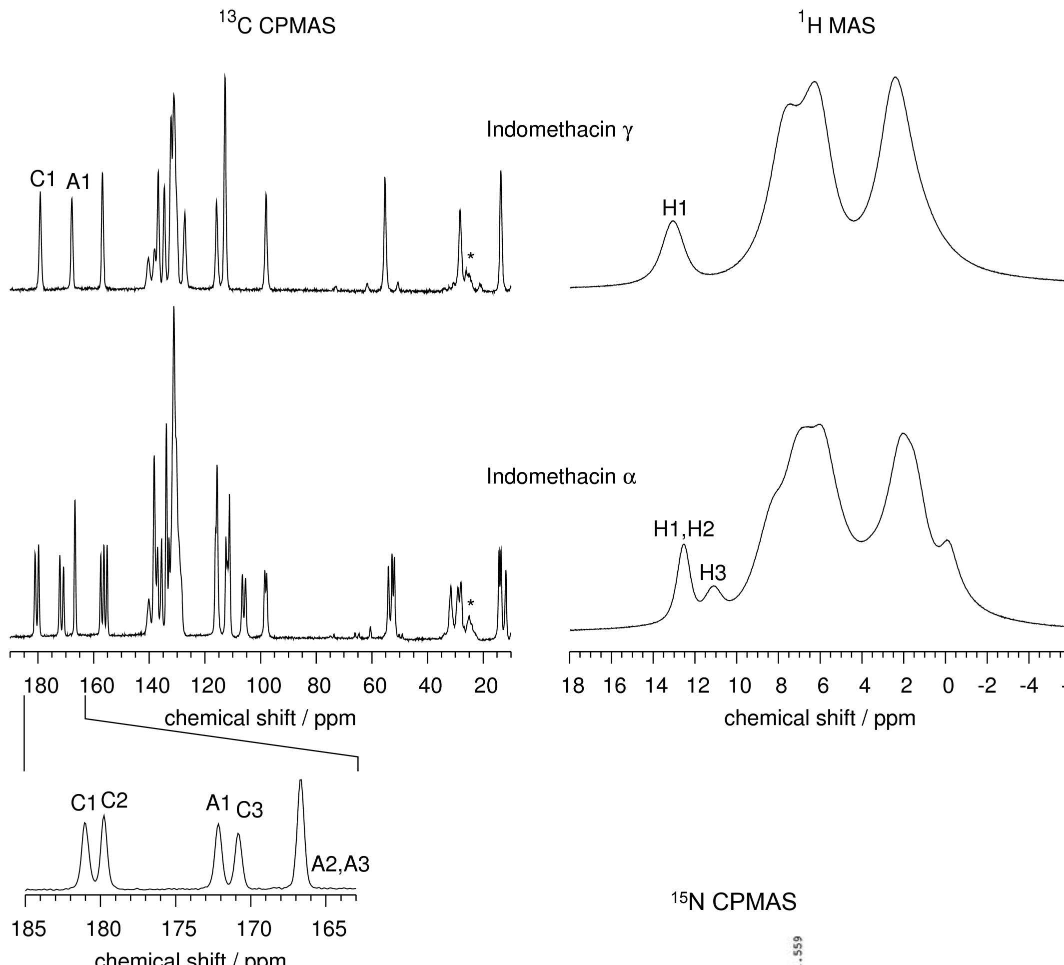


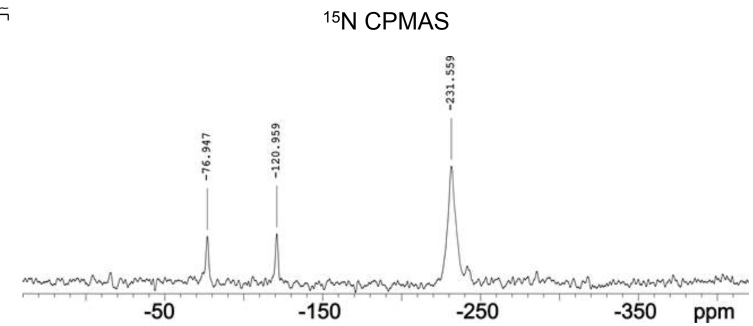




Interactions between molecules



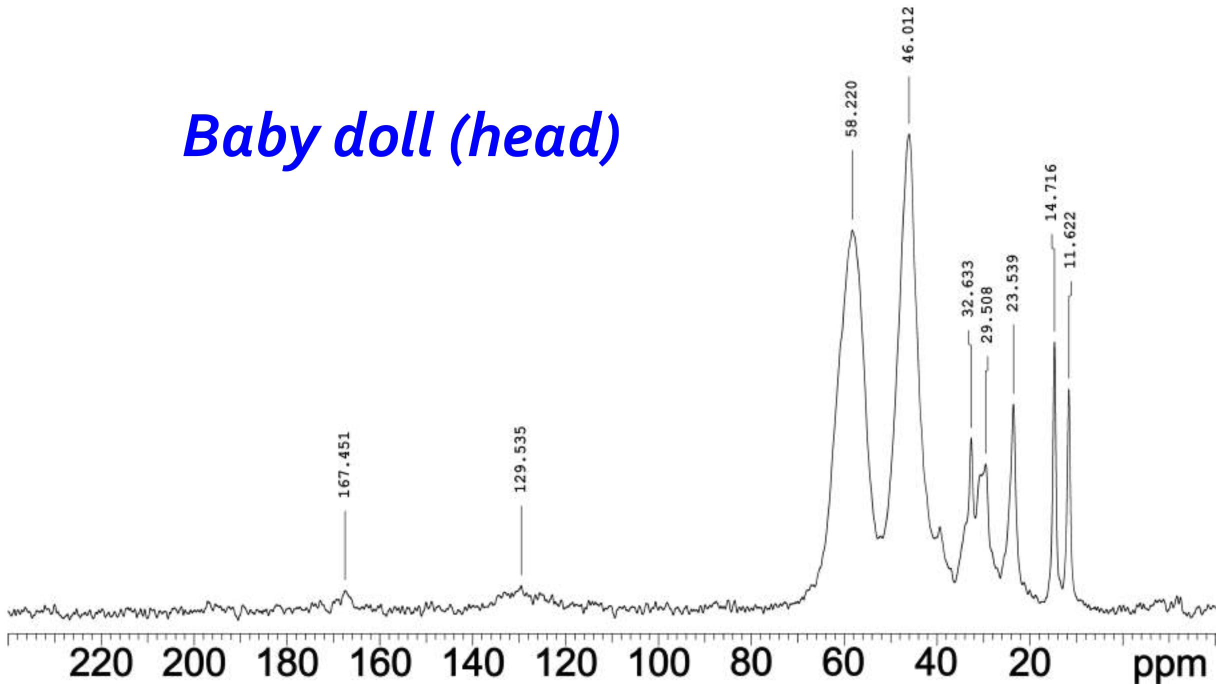




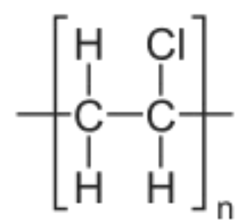
- Useful information about hydrogen bonding

HC-CPMAS NMR spectra of different plastics

Baby doll (head)

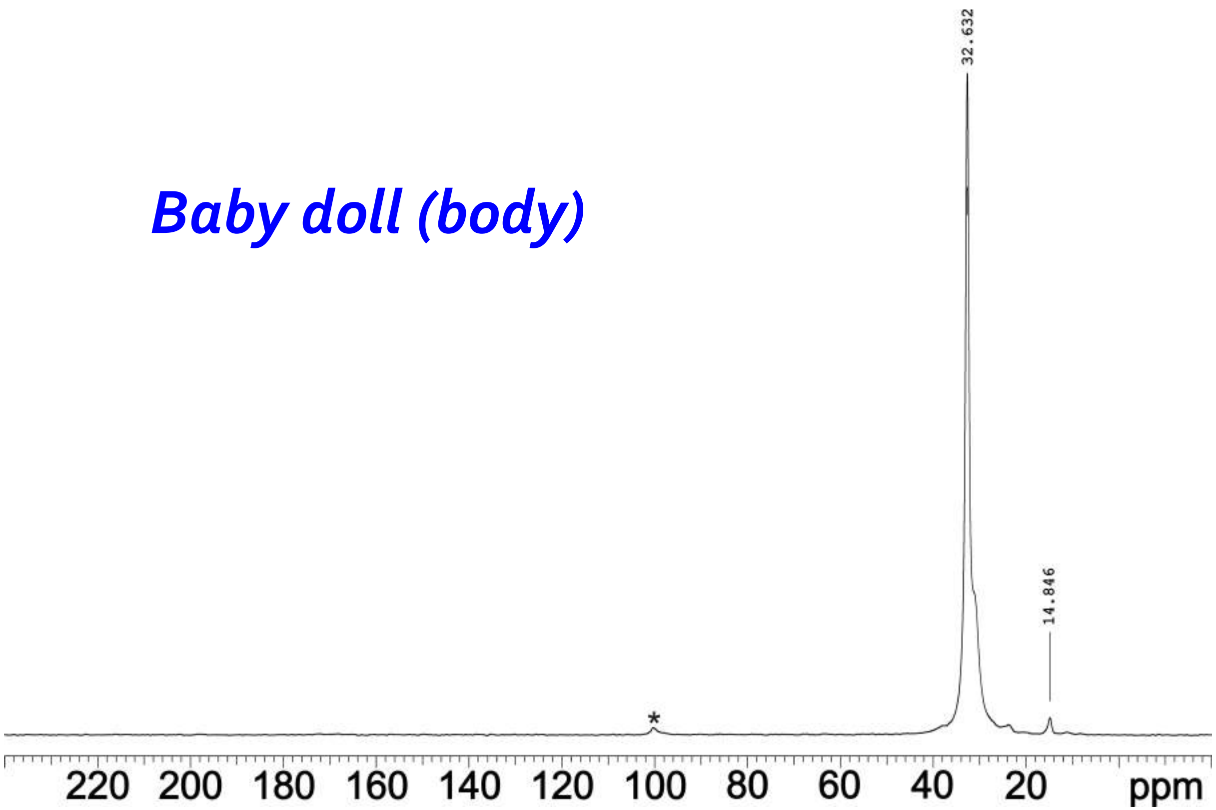


polyvinylchloride (PVC)

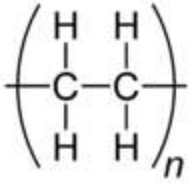


HC-CPMAS NMR spectra of different plastics

Baby doll (body)

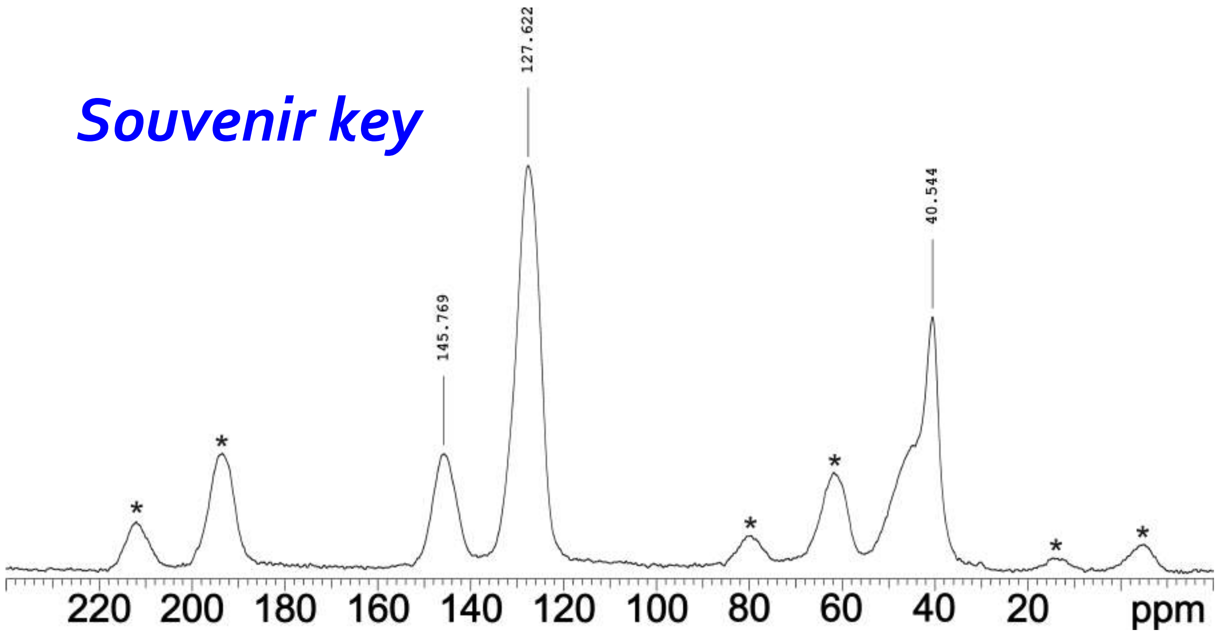


polyethylene (PE)

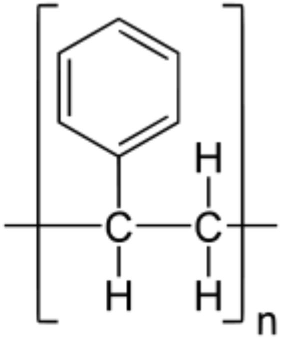


HC-CPMAS NMR spectra of different plastics

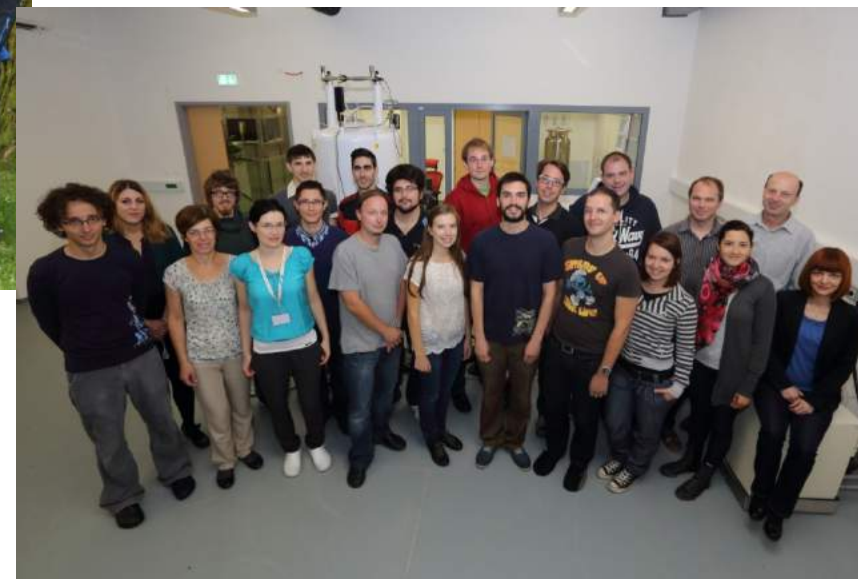
Souvenir key



polystyrene (PS)



Acknowledgments



SLOVENIAN RESEARCH AGENCY

EAST-NMR

Bio-NMR

Thank you for your attention