



Introduction to NMR

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



NATIONAL INSTITUTE OF CHEMISTRY



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

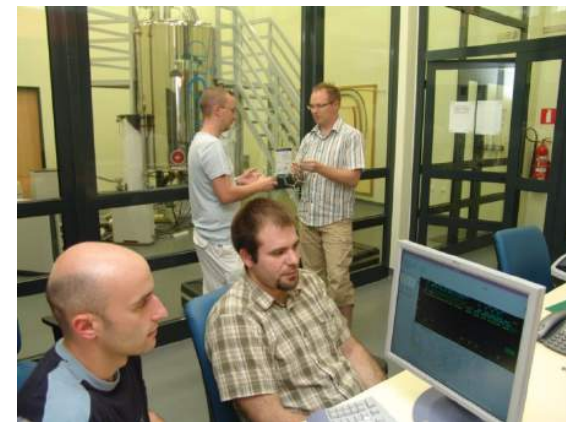
<http://www.nmr.ki.si>



NMR facility: up-to-date equipment and expertise



800 MHz Varian VNMRS spectrometer equipped with ^1H 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

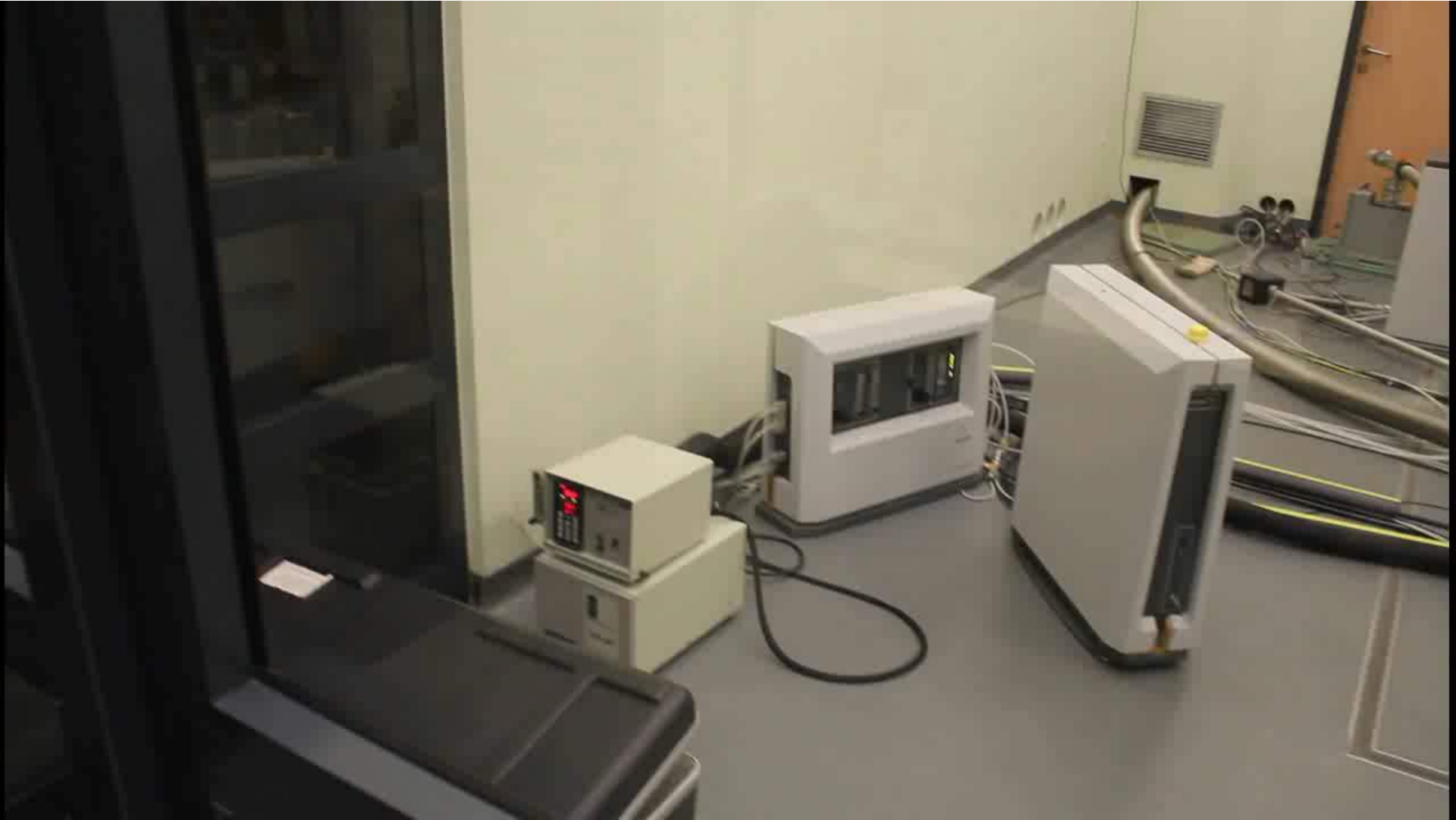
NEW 600 MHz Agilent DD2 spectrometer equipped with ^1H and ^{13}C enhanced triple resonance salt tolerant (HCN) cryogenic probe head



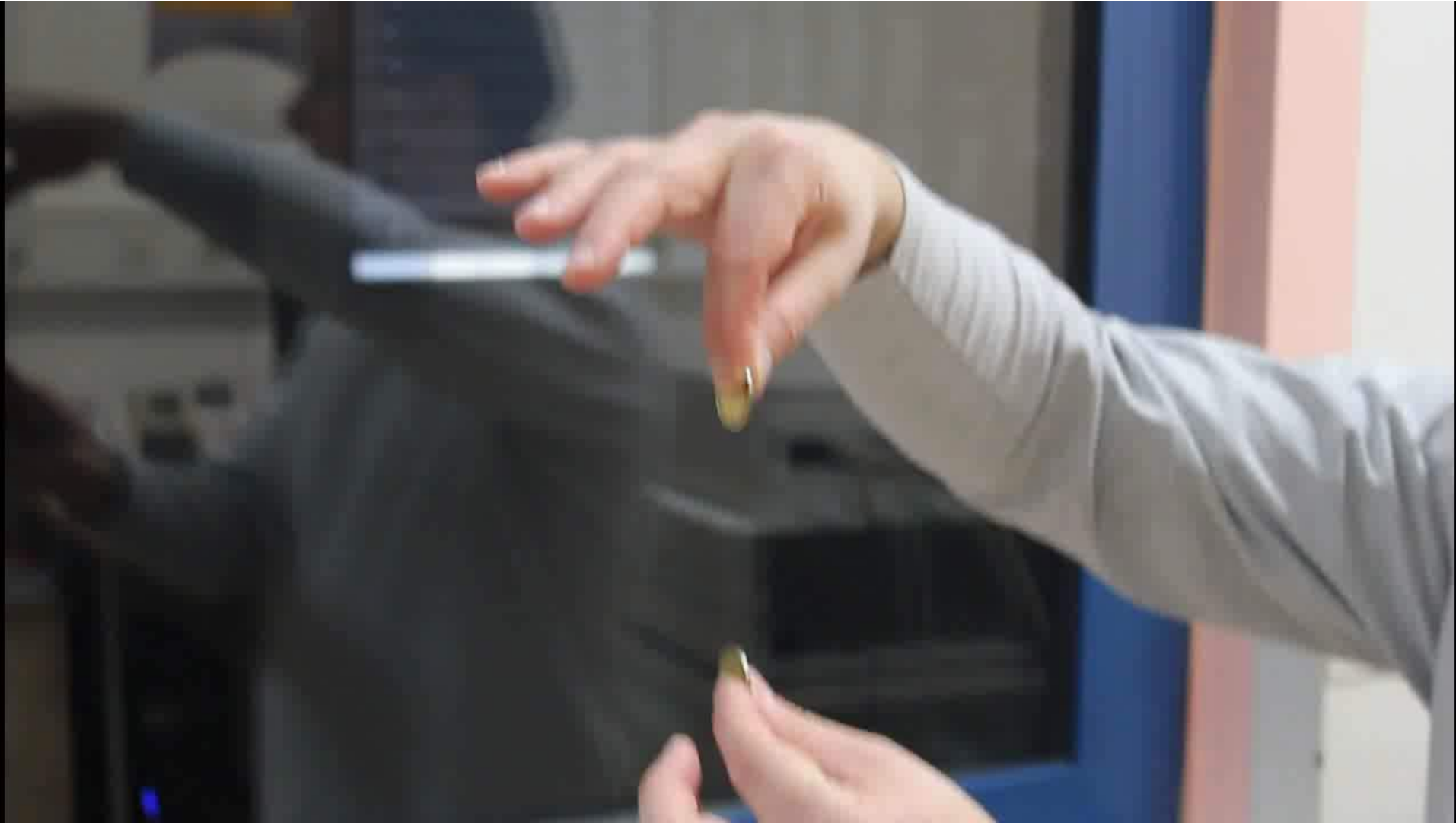
600 MHz NMR



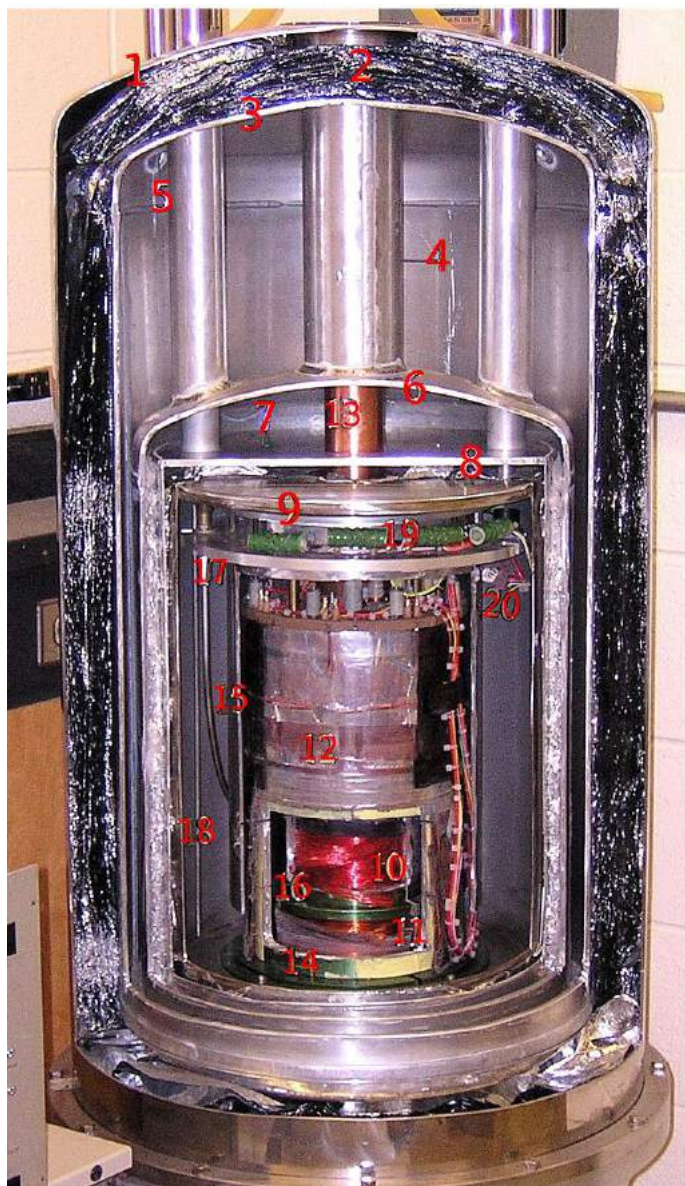
800 MHz NMR



Demonstration of the magnetic field



NMR He, N₂




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

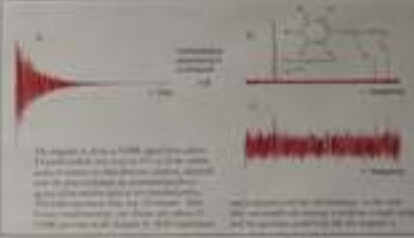




The Royal Swedish Academy of Sciences has awarded this year's Nobel Prize in Chemistry to
Richard R. Ernst
for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy

Fourier-transform NMR

Fourier-transform NMR (FTNMR) is a technique for measuring the NMR spectrum of a sample. It involves exciting the sample with a short pulse of radio waves, which causes the nuclei to precess and emit a free induction decay (FID) signal. This signal is then digitized and transformed into a spectrum using a Fourier transform. FTNMR offers several advantages over traditional NMR, including a higher signal-to-noise ratio and the ability to measure the entire spectrum in a single scan.




Applications

FTNMR has a wide range of applications in chemistry and physics. It is used to study the structure and dynamics of molecules, to identify unknown compounds, and to investigate the properties of materials. In particular, FTNMR is used in the study of polymers, proteins, and other complex systems. It is also used in the development of new materials and in the study of catalytic reactions.

Richard R. Ernst's revolutionary development of the methodology of two-dimensional magnetic resonance spectroscopy has revolutionized NMR and made this most important spectroscopic technique widely accessible. Ernst has contributed more than anybody else to the development of the techniques of Fourier transform NMR and two-dimensional (2D) NMR.

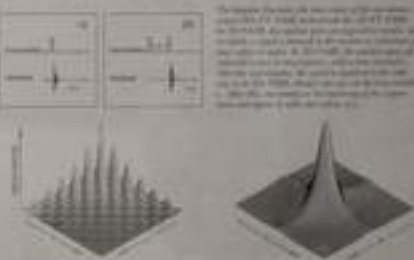
NMR spectroscopy

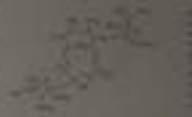


Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique used to determine the structure of molecules. It is based on the interaction of the magnetic moments of certain nuclei with an external magnetic field. The resulting NMR spectrum provides information about the chemical environment of the nuclei, allowing chemists to identify unknown compounds and study the properties of materials.




Two-dimensional NMR

Two-dimensional (2D) NMR spectroscopy is a technique for measuring the NMR spectrum of a sample. It involves exciting the sample with a series of pulses, which causes the nuclei to precess and emit a 2D signal. This signal is then digitized and transformed into a 2D spectrum using a Fourier transform. 2D NMR offers several advantages over traditional NMR, including the ability to measure the correlation between different nuclei and to study the dynamics of molecules.



The NMR technique is also used in the study of the brain. It is used to study the structure and function of the brain, and to investigate the effects of drugs and other substances. In particular, NMR is used in the study of neurodegenerative diseases and in the development of new treatments.



Nobel Prize

THE NOBEL PRIZE IN CHEMISTRY 2002



Proteins in close-up

Using tools created at Uppsala, researchers have taken the first steps towards understanding the structure of proteins, which include the proteins involved with our health and with our environment, which is happening inside the cell. This important step is being taken by "seeing" with the eye of a Nobel Prize laureate.

HOW: To understand, we need to see. The first step is to determine the structure of a protein. This is done by using X-ray crystallography. The protein is crystallized and then X-rays are shined through it. The resulting diffraction pattern is then analyzed to determine the structure of the protein.

FROM EARLY DIAGNOSIS TO NEW MEDICINES

Understanding the structure of proteins is crucial for developing new medicines. It allows scientists to design drugs that can specifically target a protein, leading to more effective treatments for various diseases.

Along peaks in the spectrum - the protein

The structure of a protein is determined by the sequence of amino acids. This sequence is encoded in the DNA. The protein is then synthesized and folds into a specific shape. This shape is determined by the interactions between the amino acids.

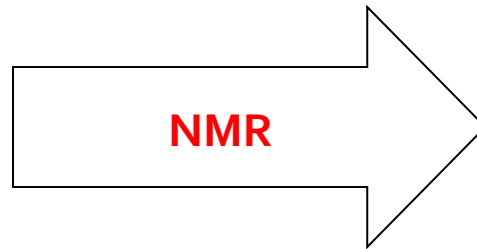


Uppsala University

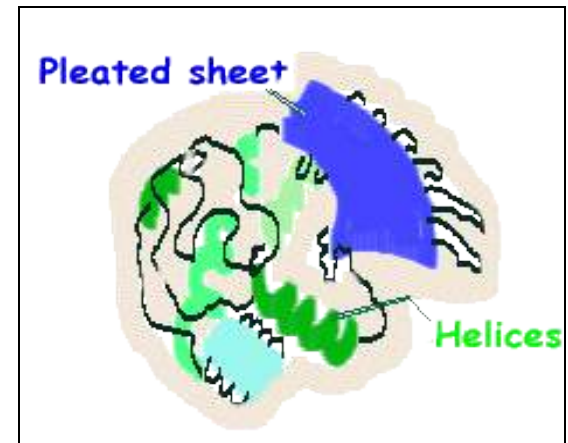
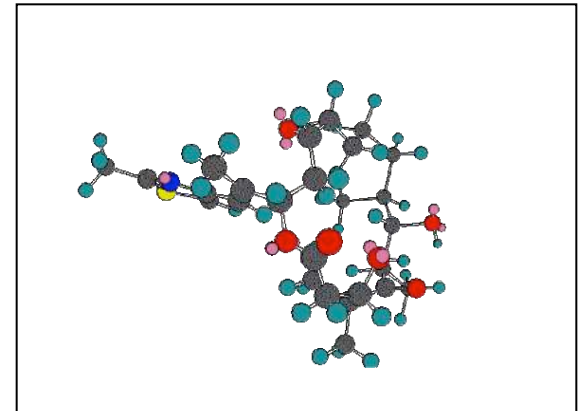
SCIENCE VETERINÄRMEDICINEN

The problem the we want to solve by NMR

What we “really” see



What we want to “see”



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) $\neq 0$ can absorb/emit electromagnetic radiation. These should have an odd mass number.

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

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

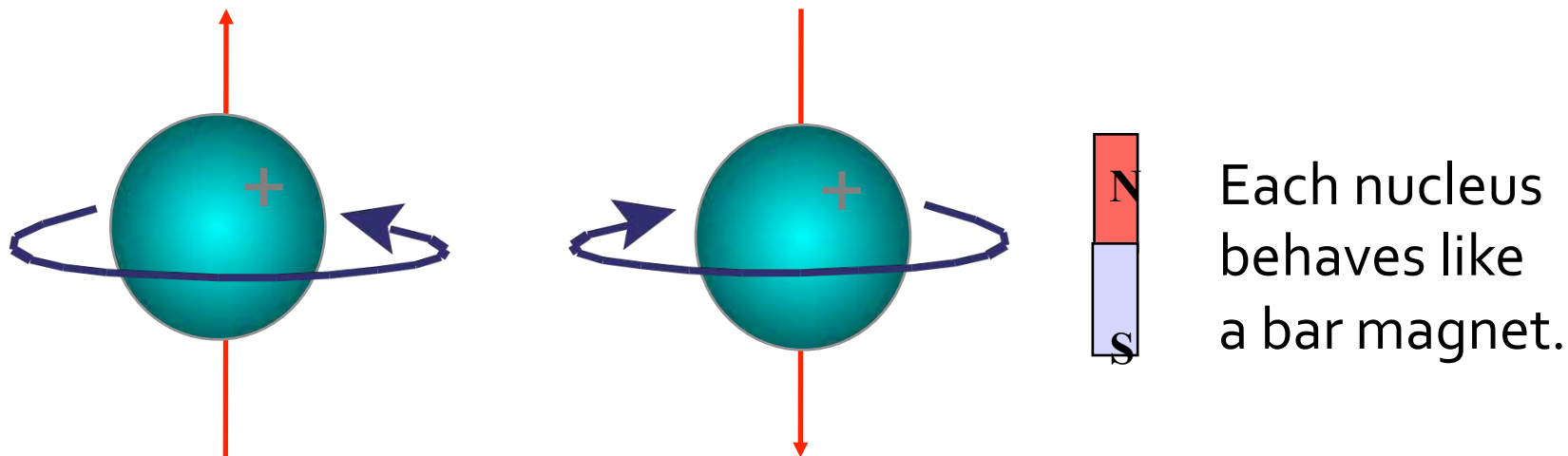
^1H and ^{13}C

both have spin = $\pm 1/2$

^1H is 99% at natural abundance

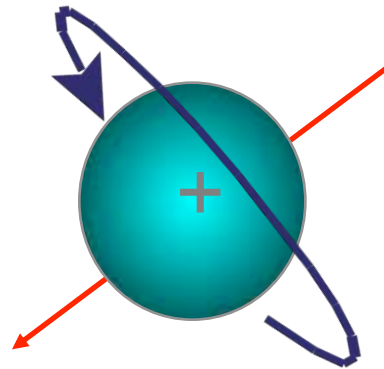
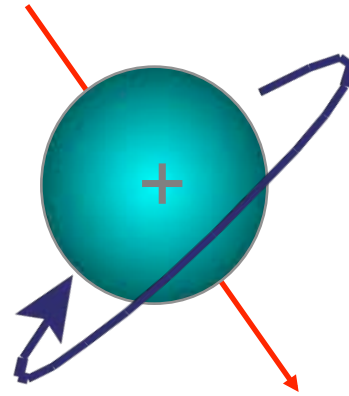
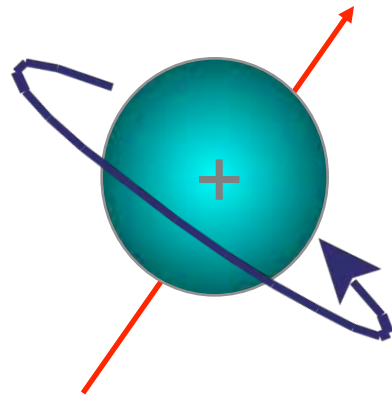
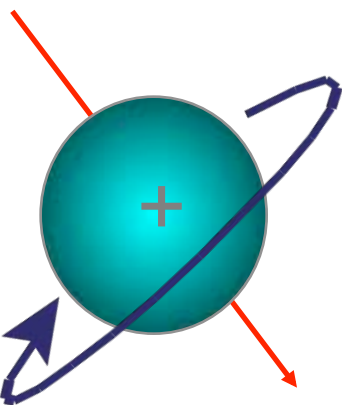
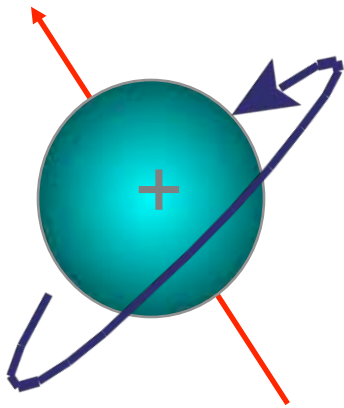
^{13}C is 1.1% at natural abundance

Nuclear Spin

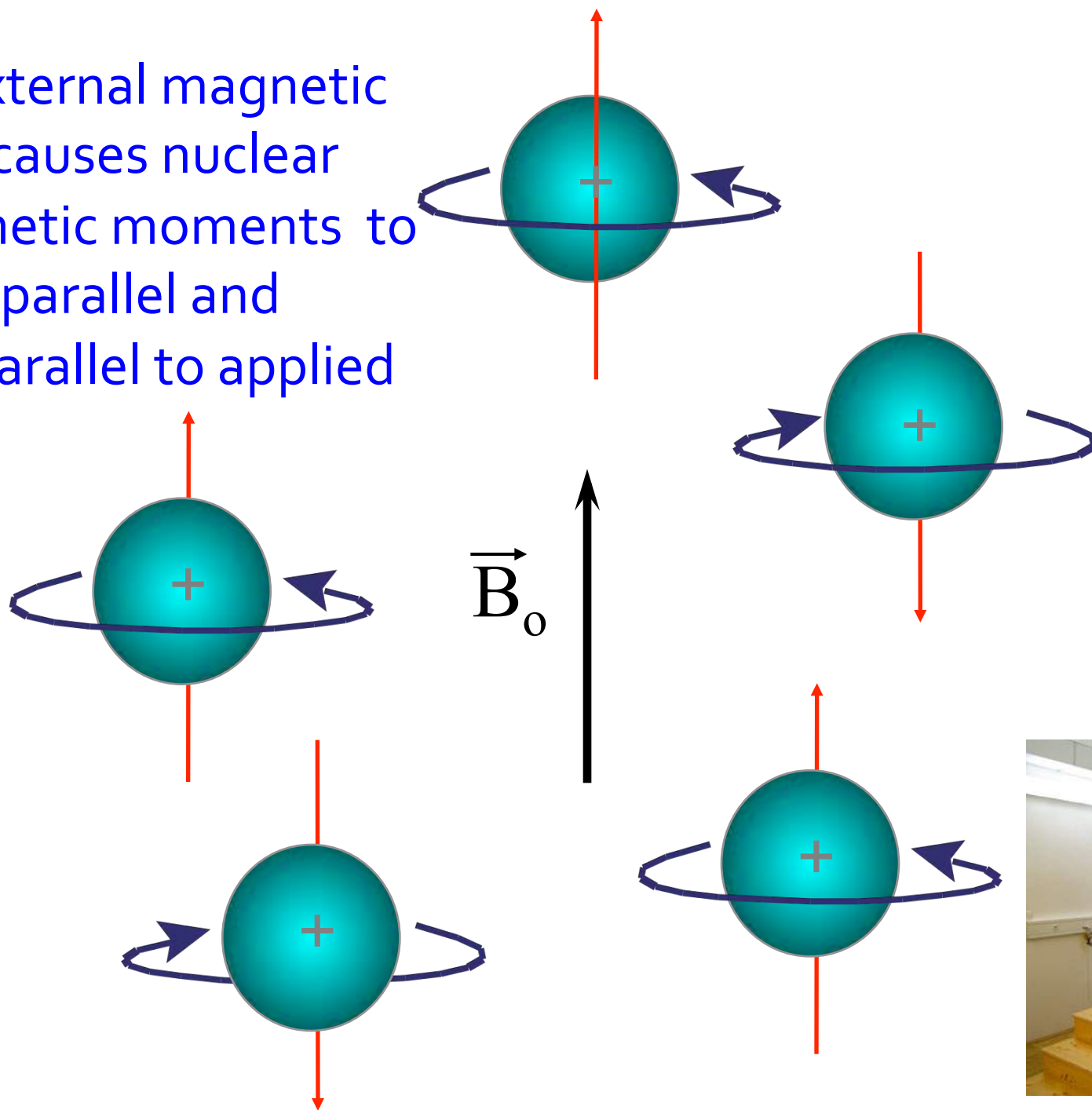


A spinning charge, such as the nucleus of ^1H or ^{13}C , generates a magnetic field. The magnetic field generated by a nucleus of spin $+1/2$ is opposite in direction from that generated by a nucleus of spin $-1/2$.

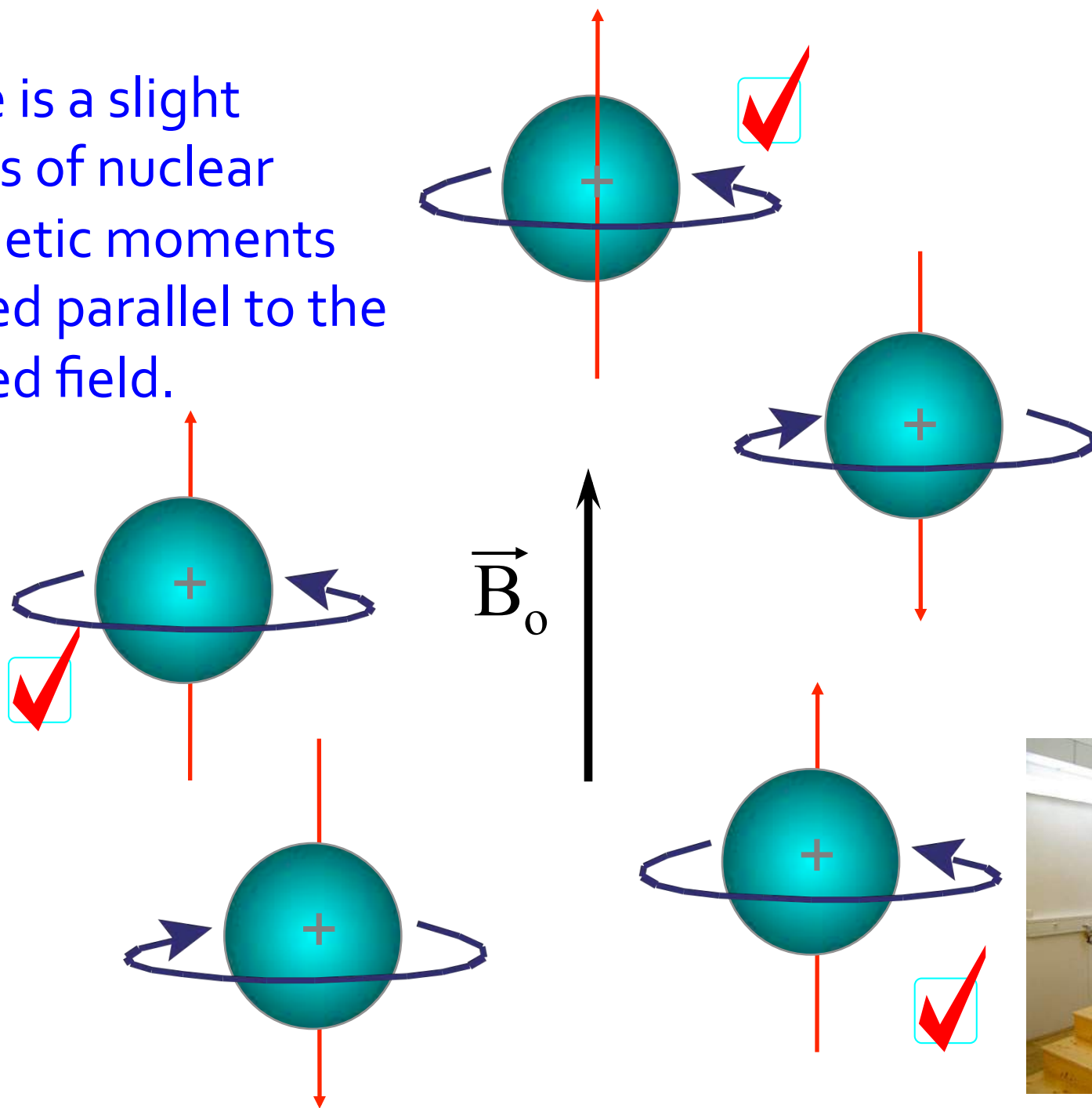
The distribution of nuclear spins is random in the absence of an external magnetic field.



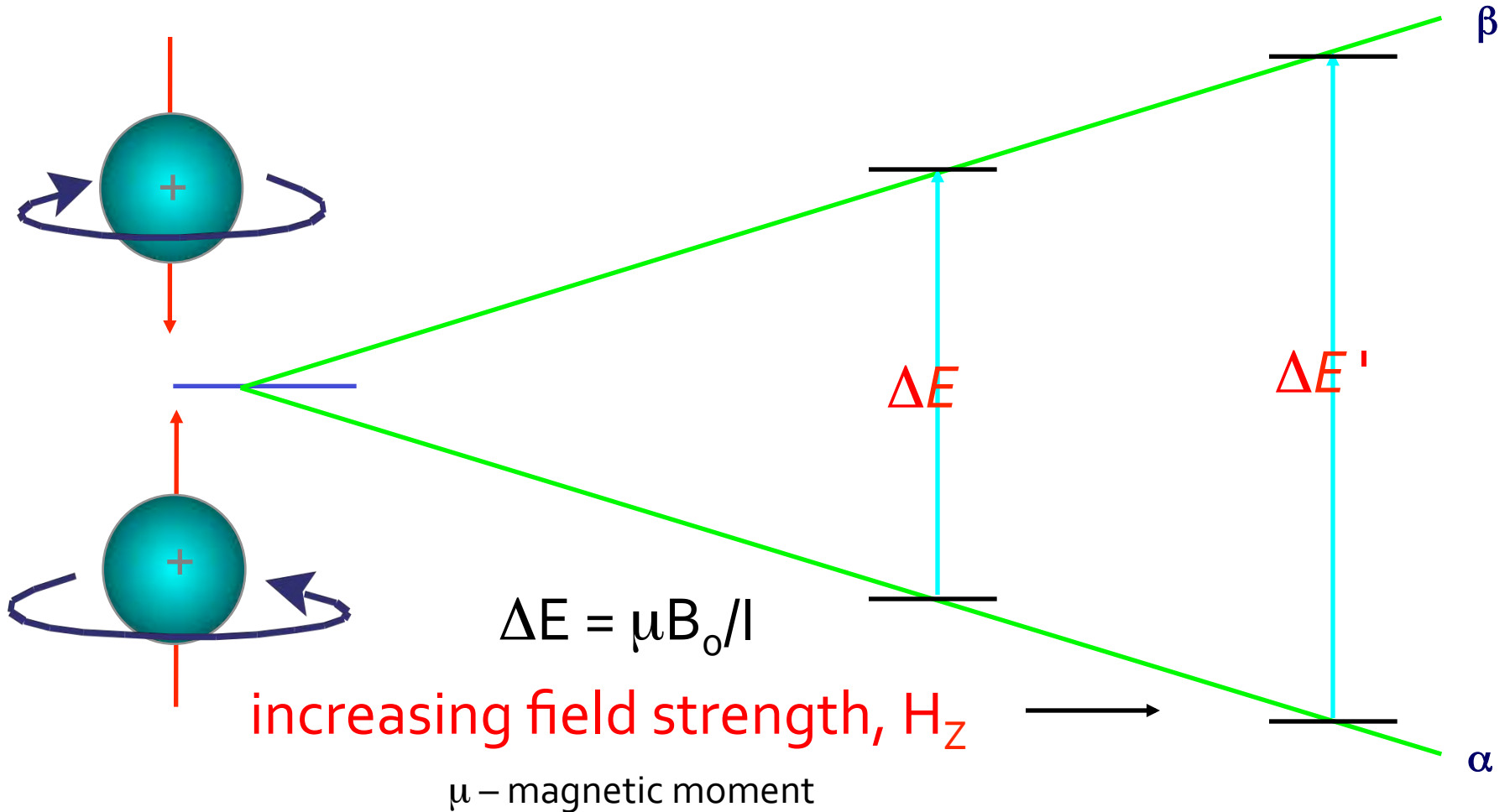
An external magnetic field causes nuclear magnetic moments to align parallel and antiparallel to applied field.



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



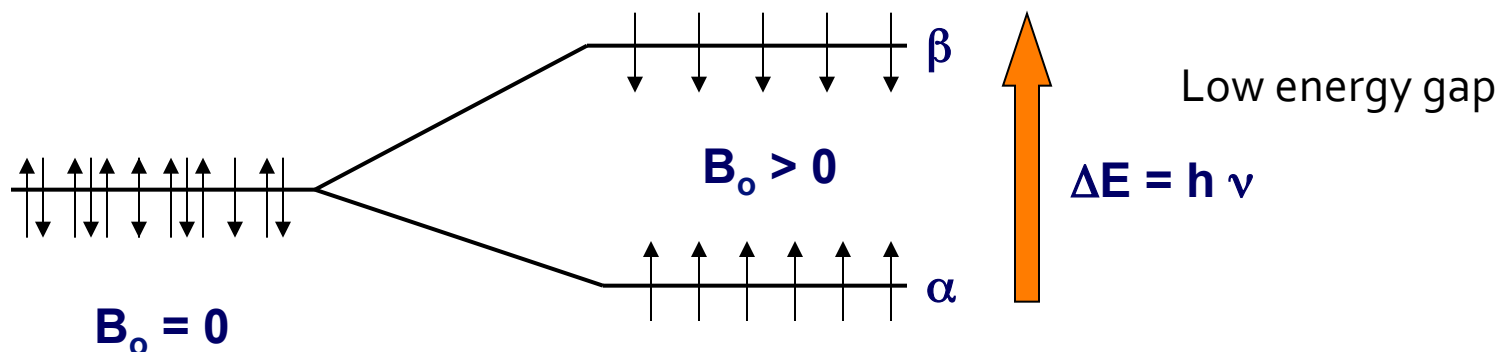
Energy Differences Between Nuclear Spin States



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

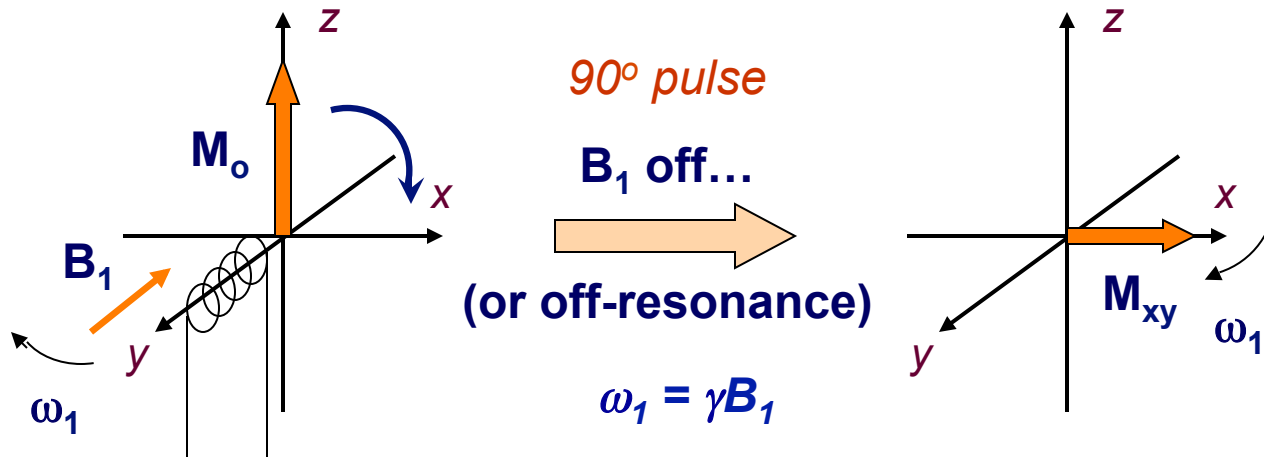
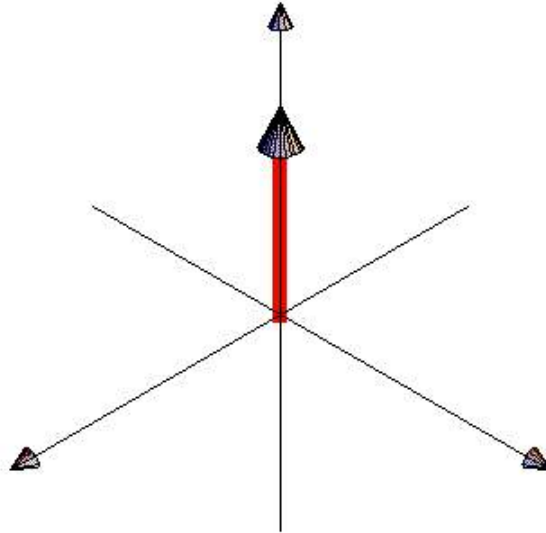


- The population (**N**) difference can be determined from the Boltzmann distribution and the energy separation between the α and β spin states:

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

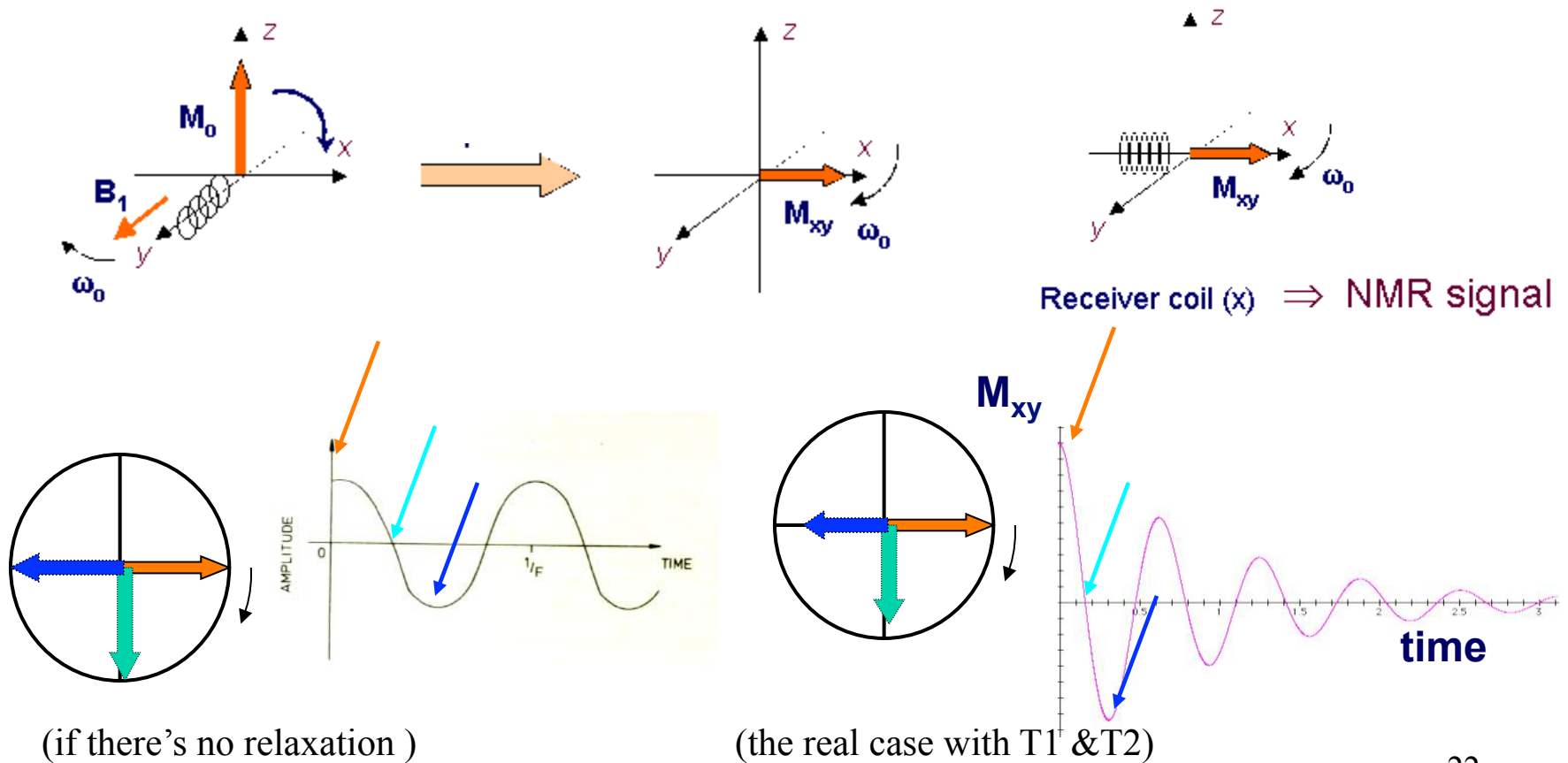
Classical Description

- *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)

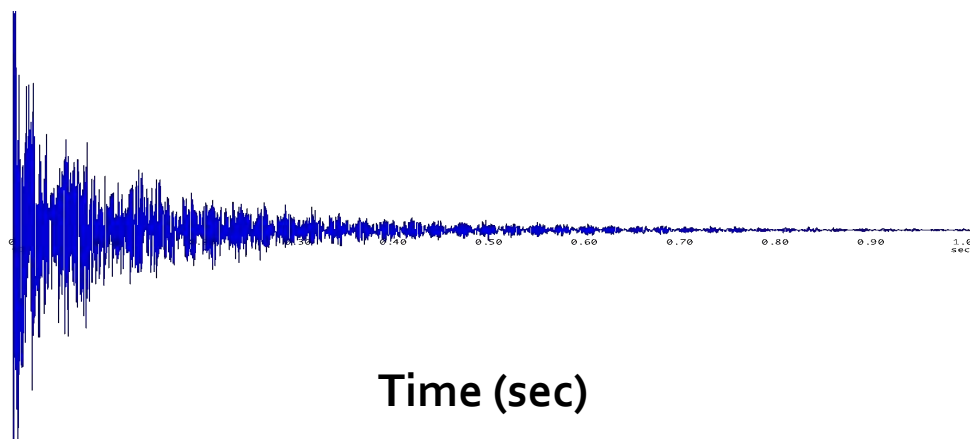


Collecting NMR signals

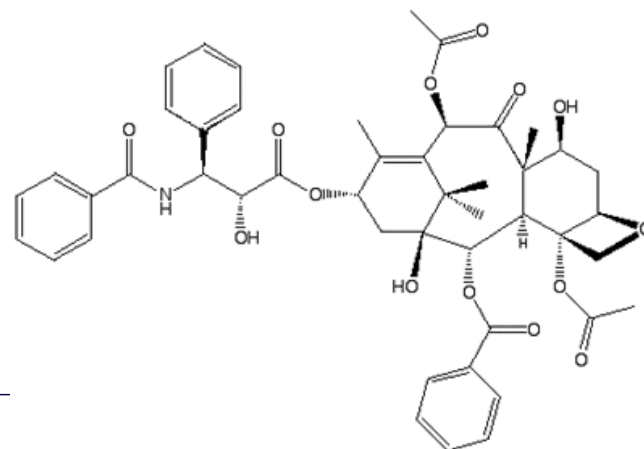
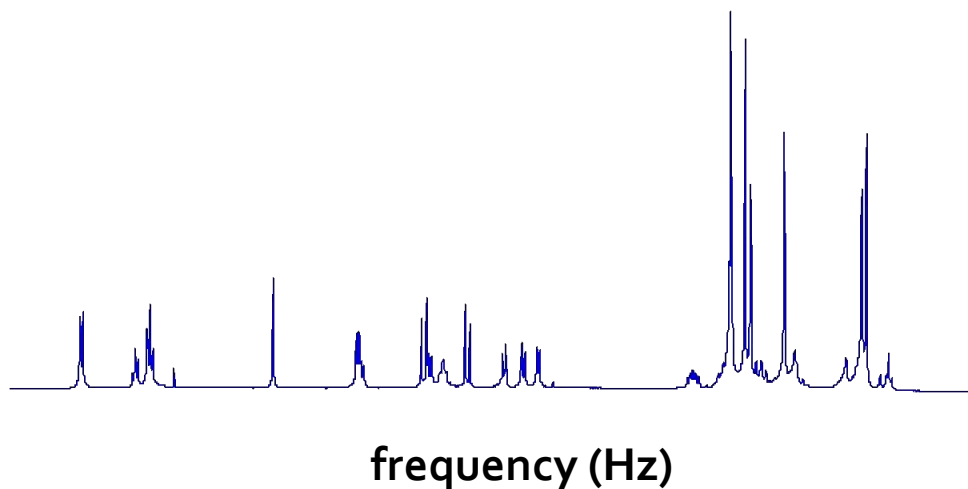
- The detection of NMR signal is on the xy plane. The oscillation of M_{xy} generate a current in a coil, which is the NMR signal.
- Due to the "relaxation process", the time dependent spectrum of nuclei can be obtained. This time dependent spectrum is called "free induction decay" (FID)



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



- The FID (free induction decay) is then **Fourier transform** to frequency domain to obtain each $\nu_{\text{precession}}$ (chemical shift) for different nuclei.



NMR Periodic Table

NMR "active" Nuclear Spin (I) = $1/2$:

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

biological and chemical relevance

Odd atomic mass

$$I = +1/2 \text{ \& \ } -1/2$$

NMR "inactive" Nuclear Spin (I) = 0:

^{12}C , ^{16}O

Even atomic mass & number

Quadrupole Nuclei Nuclear Spin (I) $> 1/2$:

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

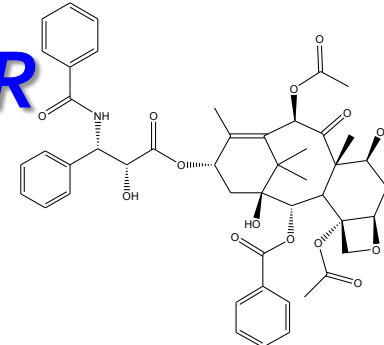
Even atomic mass & odd number

$$I = +1, 0 \text{ \& \ } -1$$

Table 1.1 Nuclear properties of some of the elements

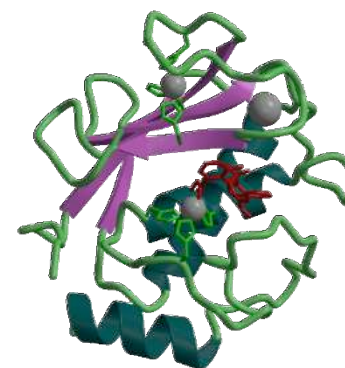
Element	Atomic mass	Spin I	Natural abundance (%)	Receptivity ($^{13}\text{C} = 1.00$)	Quadrupole moment (10^{30} m^2)	Resonant frequency (MHz) at 2.348 T
Hydrogen	1	1/2	99.985	5670	None	100.00
Deuterium	2	1	0.015	0.0082	0.287	15.35
Tritium	3	1/2	Radioactive	-	None	106.66
Helium	3	1/2	0.00014	0.0035	None	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



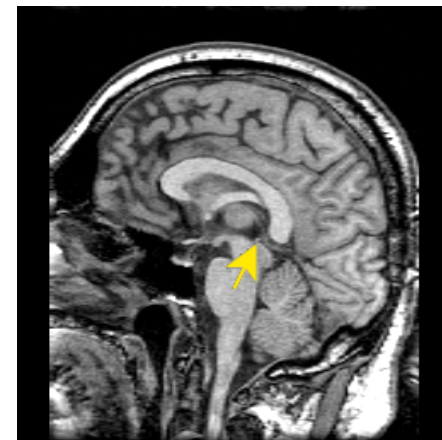
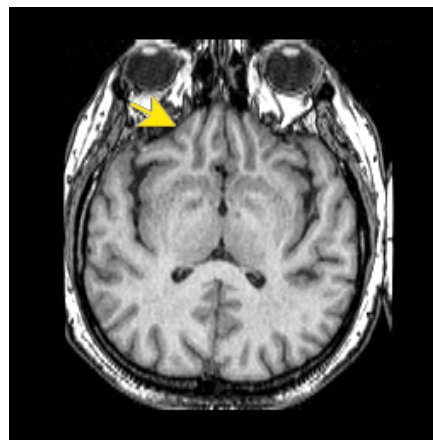
Taxol (natural product)

- 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
- 4.) Metabolomics
- 5.) Drug Design
 - , Structure Activity Relationships by NMR
- 6.) Medicine -MRI



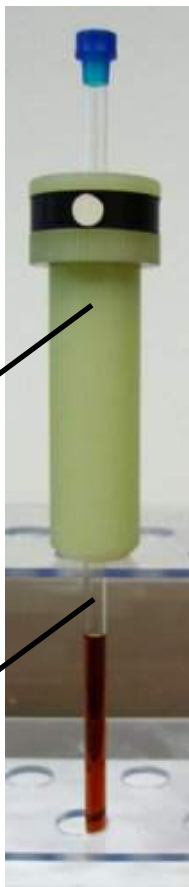
NMR Structure of MMP-13 complexed to a ligand

MRI images of the Human Brain



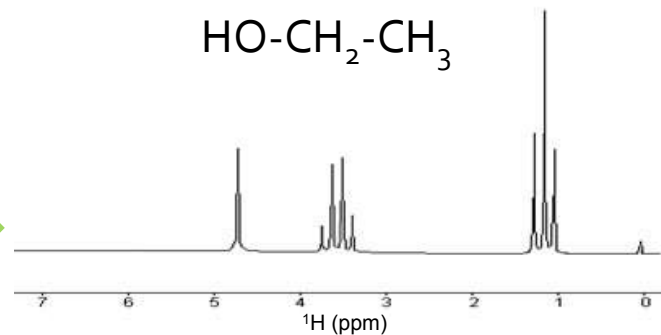
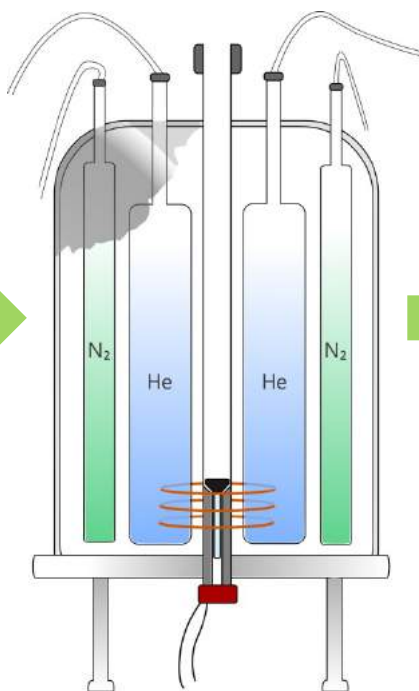
NMR is a widely used structural tool

Liquid-state NMR



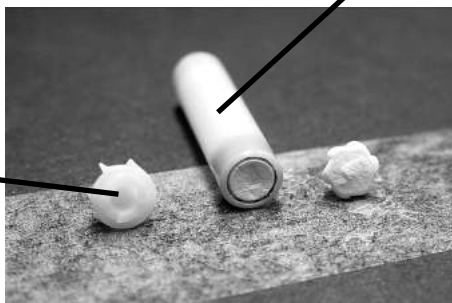
Spinner

Sample Tube



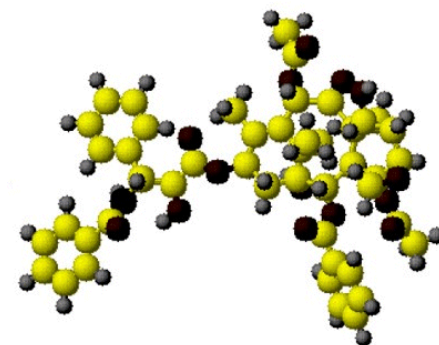
- Chemical shift
- Signal intensity
- Scalar couplings

Solid-state NMR



Rotor

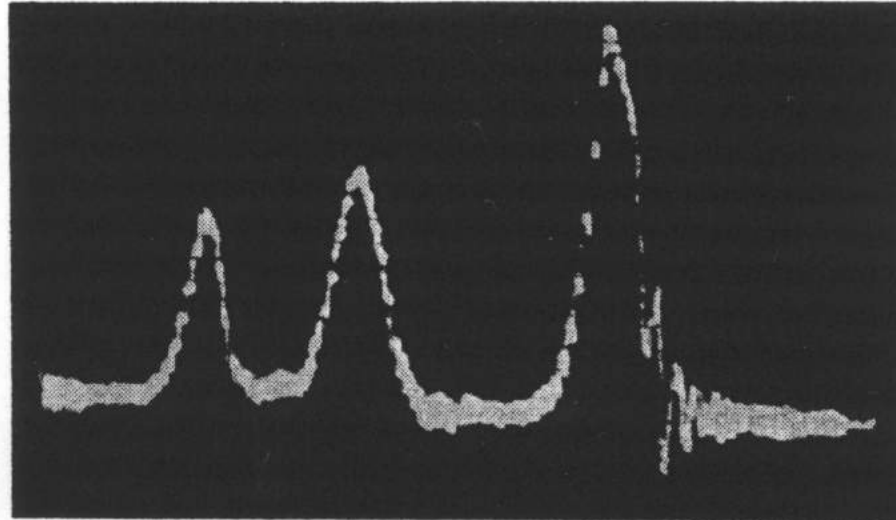
Turbine



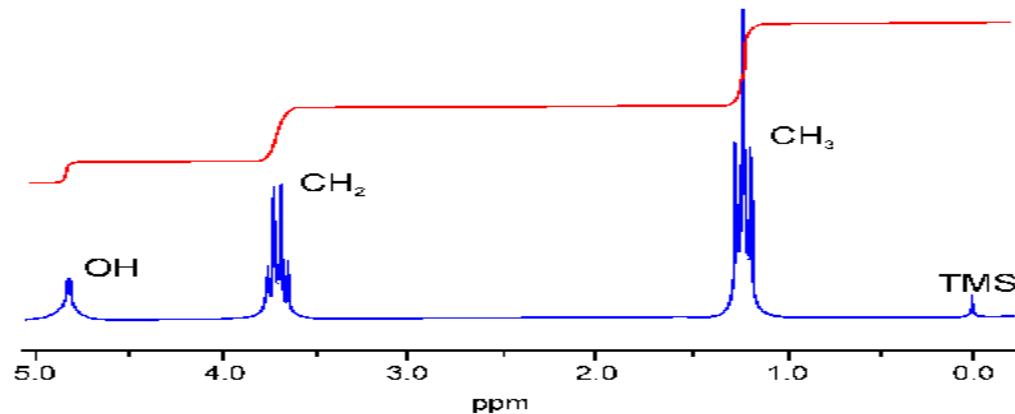
NMR History

First Observation of the Chemical Shift

^1H NMR spectra ethanol



Modern ethanol spectra

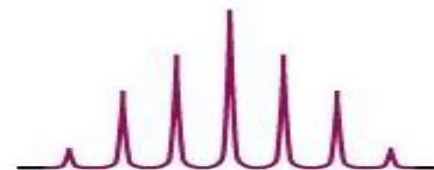
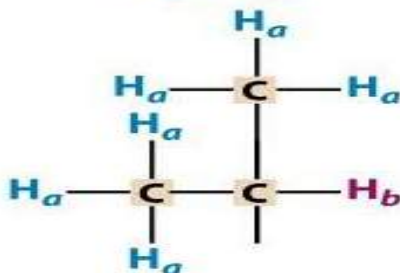
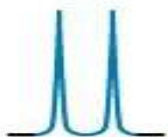
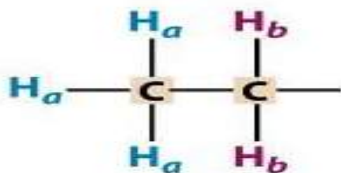
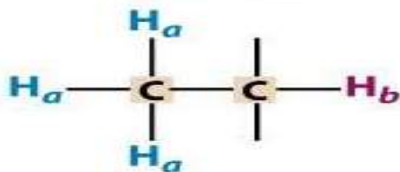
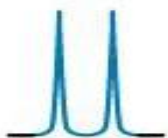
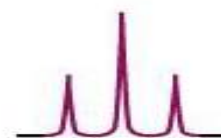
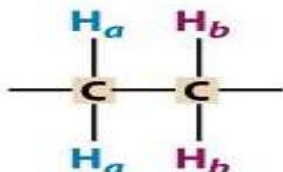
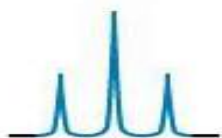
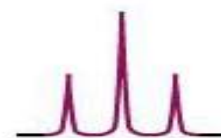
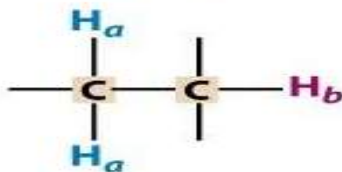
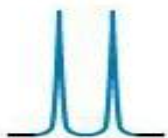
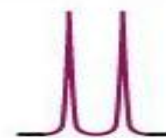
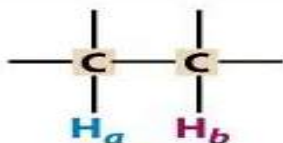
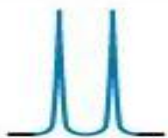


Some common splitting patterns

Splitting pattern
for H_a

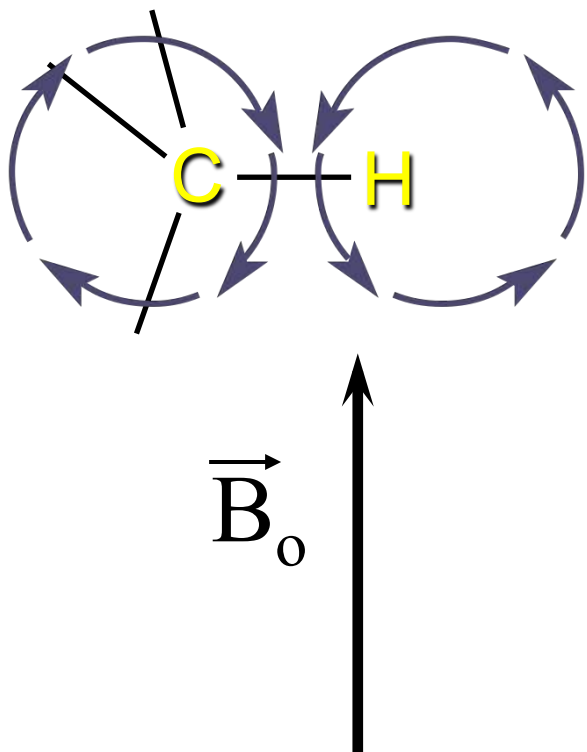
Structure

Splitting pattern
for H_b



Chemical shift

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

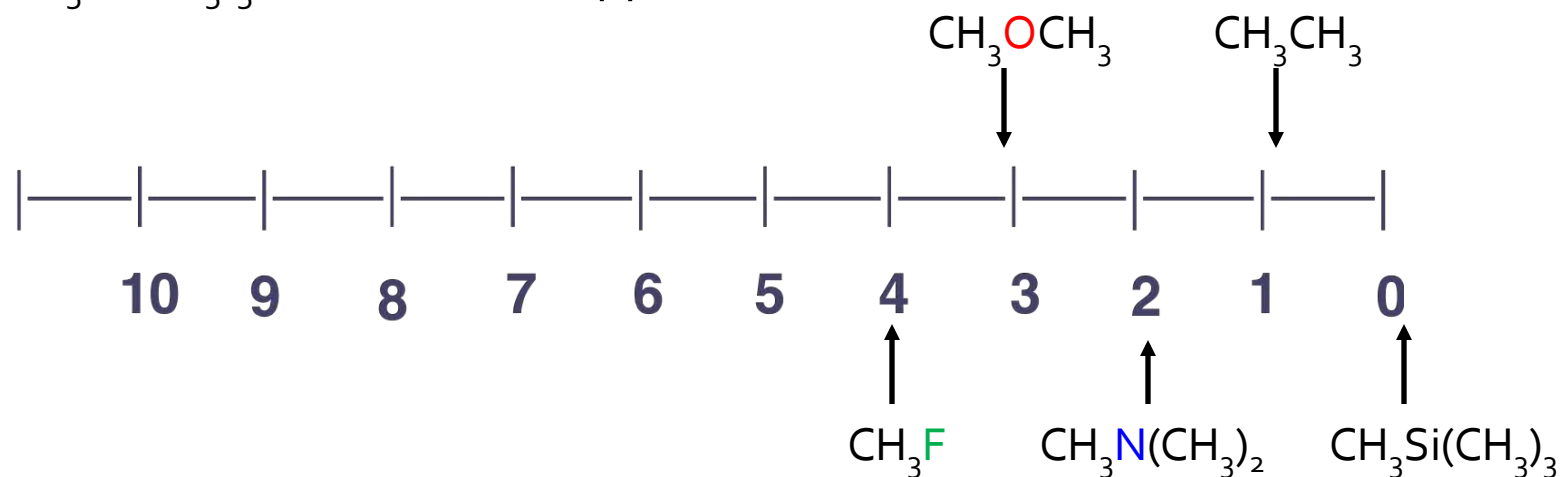


Chemical shift is a measure of the degree to which a nucleus in a molecule is shielded.

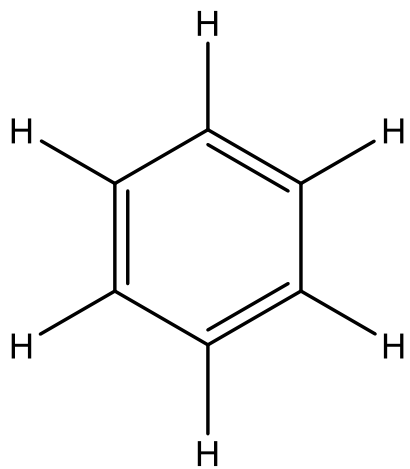
Protons in different environments are shielded to greater or lesser degrees; they have different chemical shifts.

Electronegative substituents decrease the shielding of methyl groups

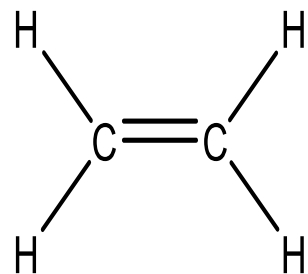
CH_3F	δ 4.3 ppm
CH_3OCH_3	δ 3.2 ppm
$\text{CH}_3\text{N}(\text{CH}_3)_2$	δ 2.2 ppm
CH_3CH_3	δ 0.9 ppm
$\text{CH}_3\text{Si}(\text{CH}_3)_3$	δ 0.0 ppm



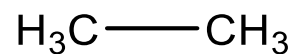
Protons attached to sp^2 hybridized carbon are less shielded than those attached to sp^3 hybridized carbon



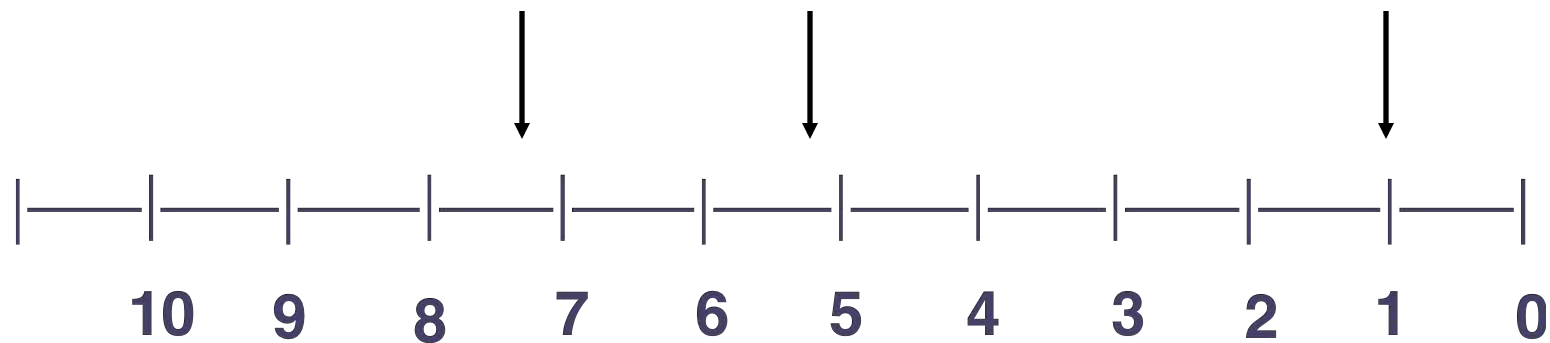
δ 7.3 ppm



δ 5.3 ppm

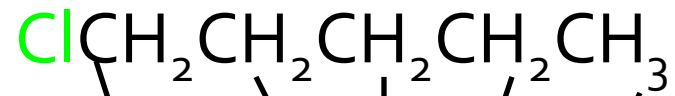


δ 0.9 ppm

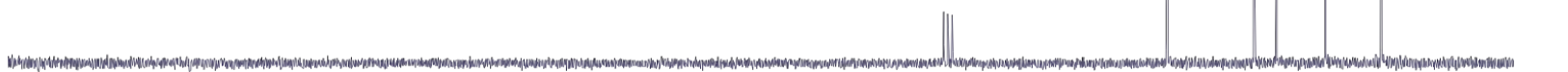


^{13}C NMR spectrum

Five carbon atoms exhibit signals at different chemical shifts.



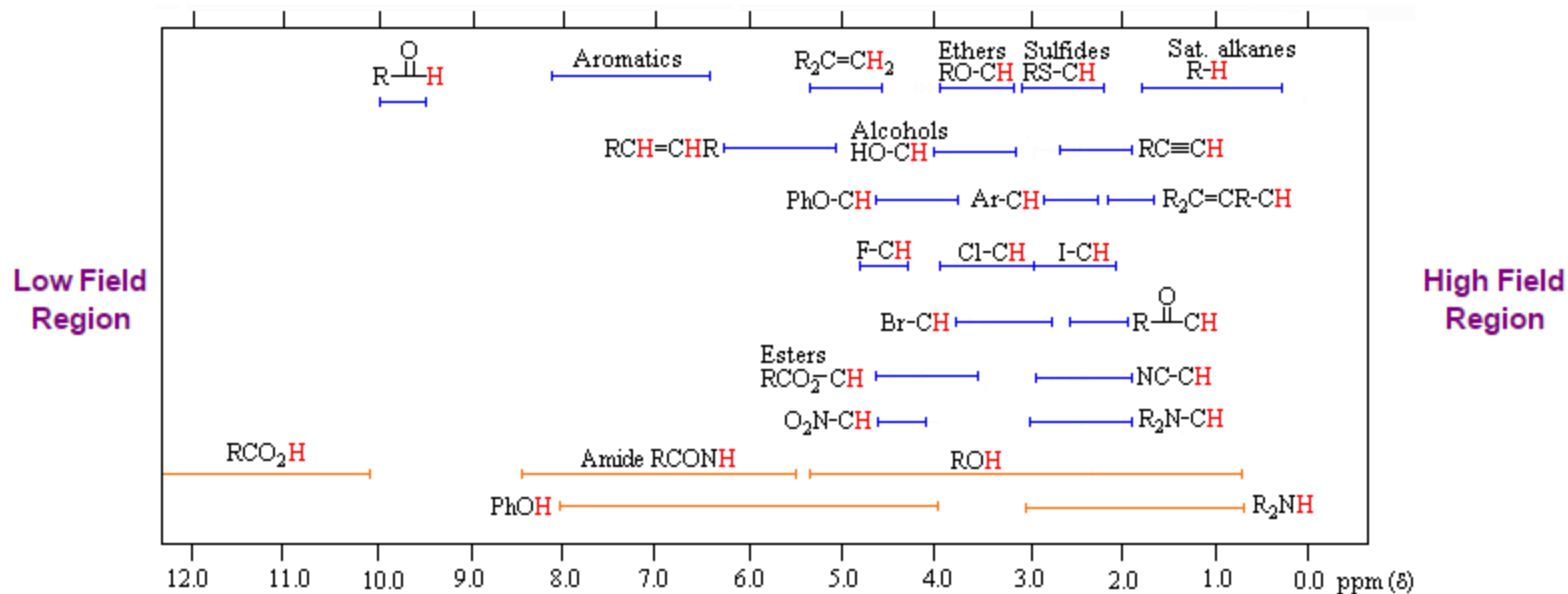
Solvent
 CDCl_3



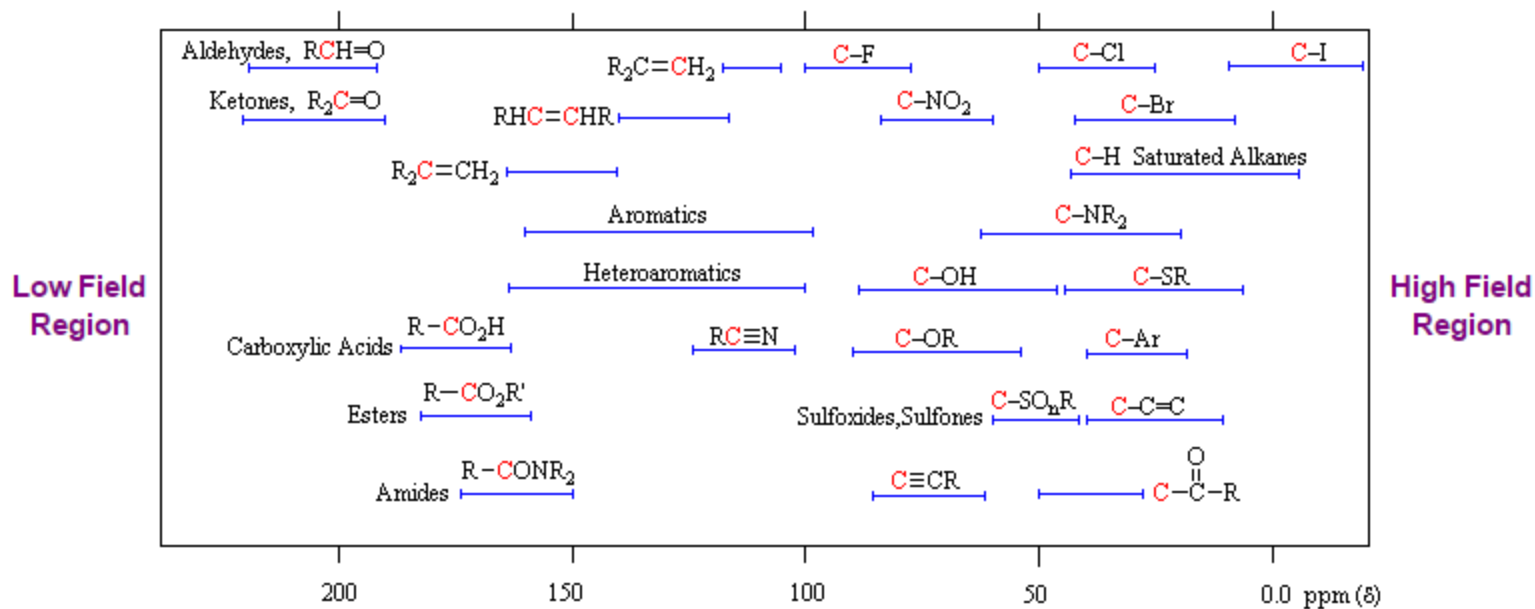
200 180 160 140 120 100 80 60 40 20 0

Chemical shift (δ , ppm)

Proton chemical shift ranges



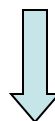
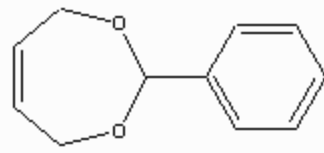
Carbon chemical shift ranges



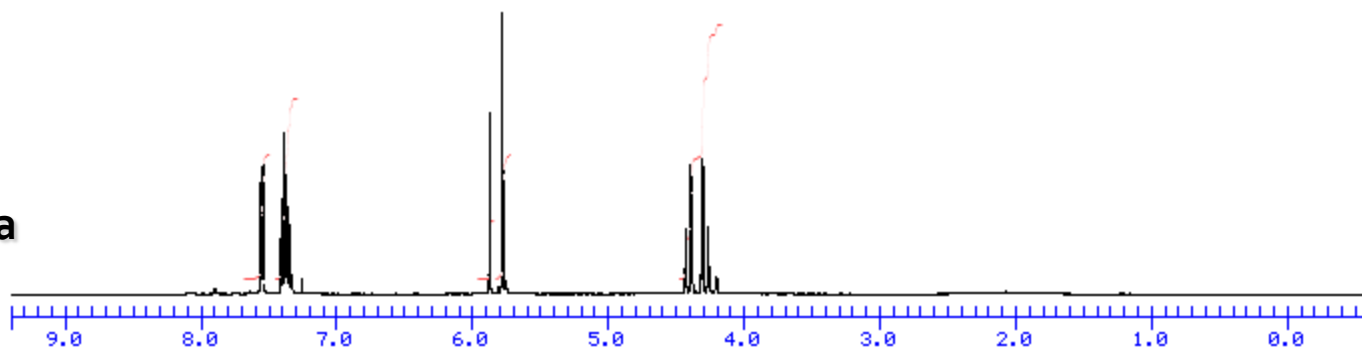
Each NMR observable nuclei yields a peak in the spectra

"fingerprint" of the structure

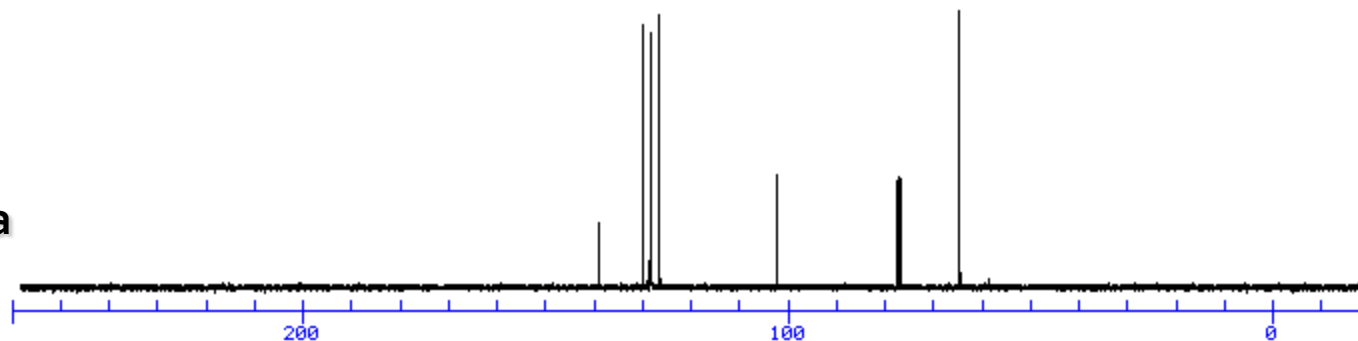
2-phenyl-1,3-dioxep-5-ene



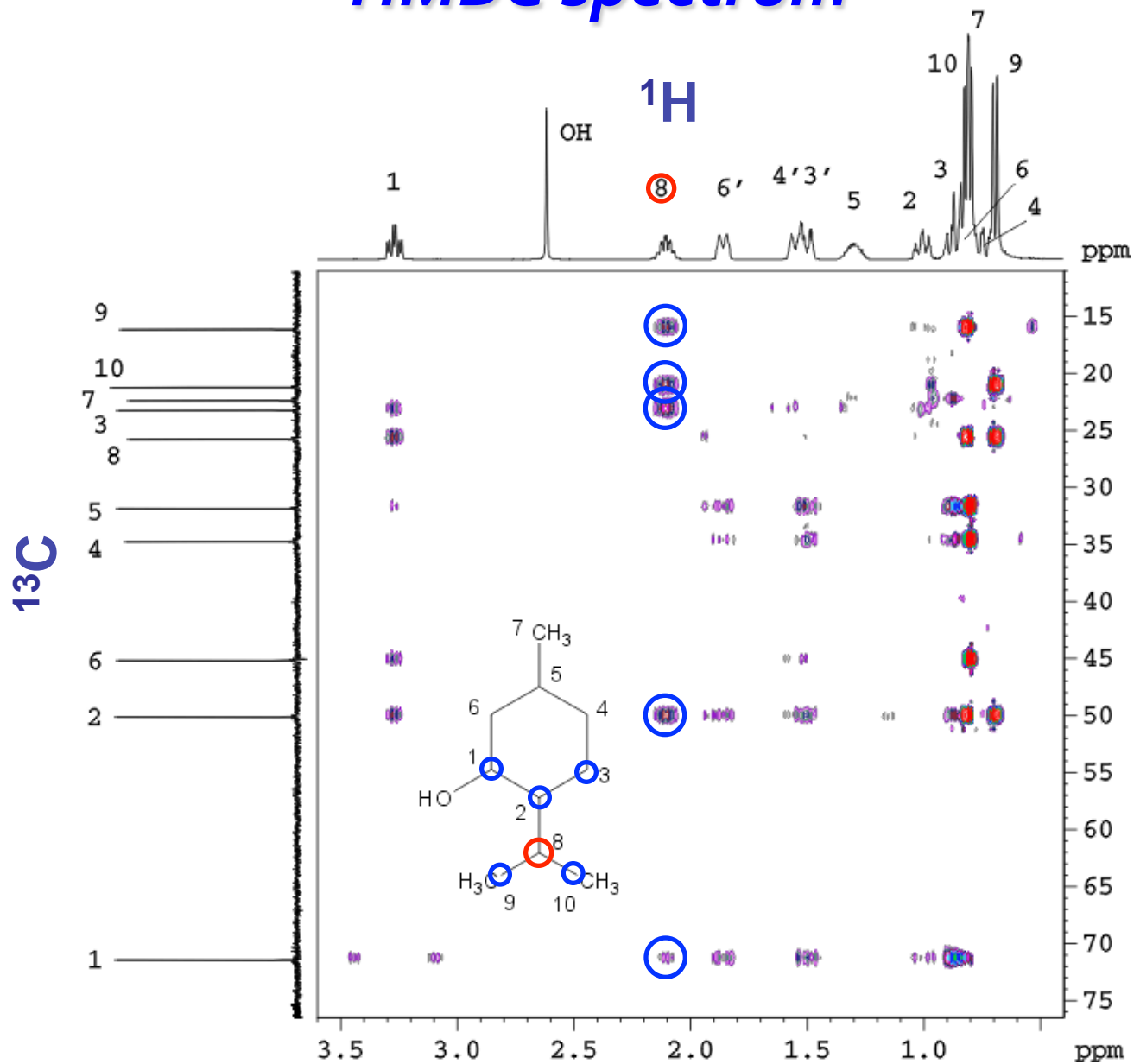
^1H NMR spectra



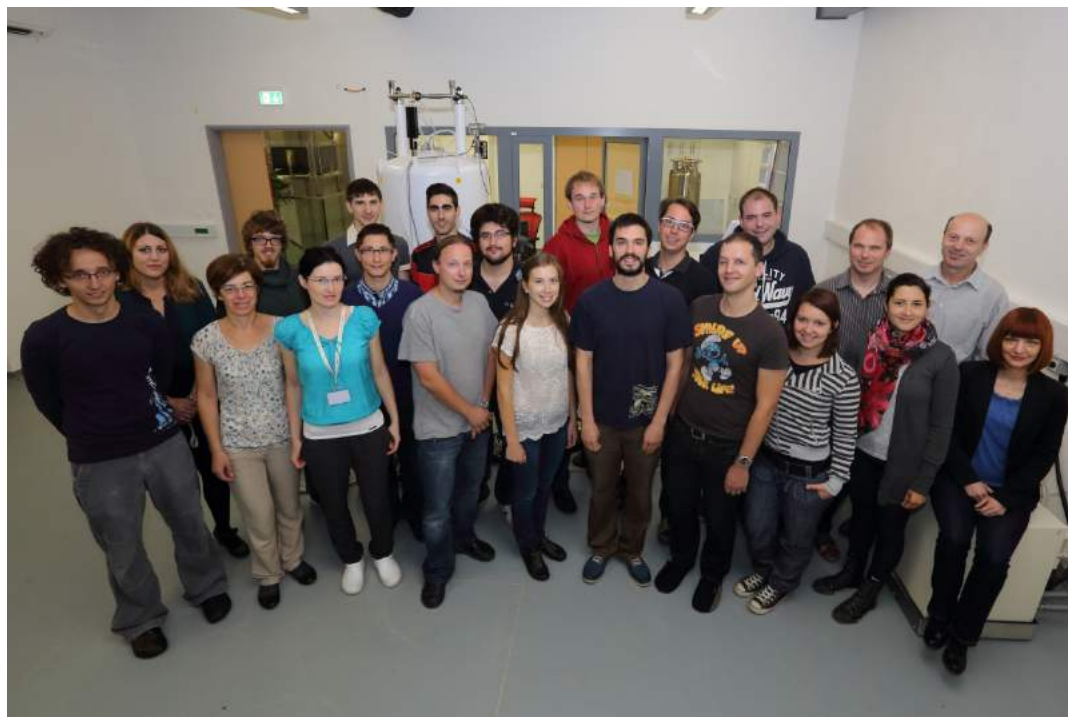
^{13}C NMR spectra



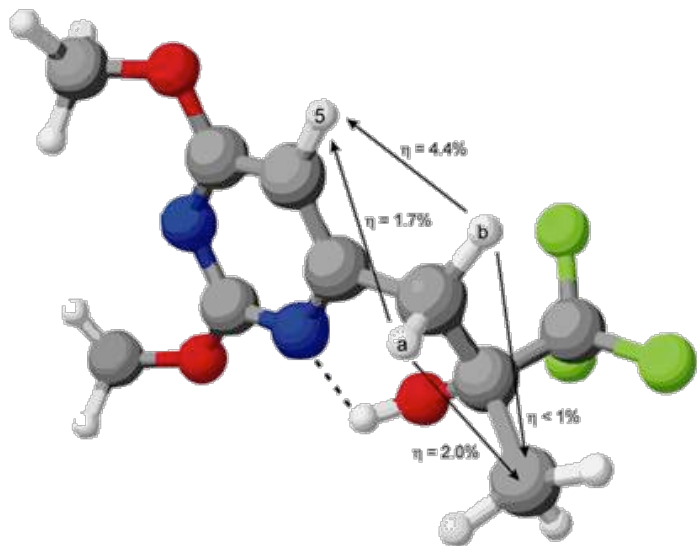
HMBC spectrum



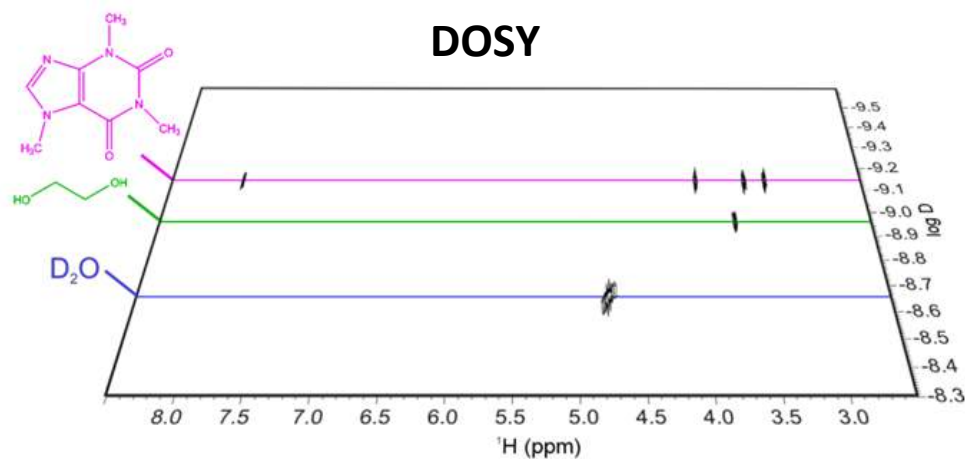
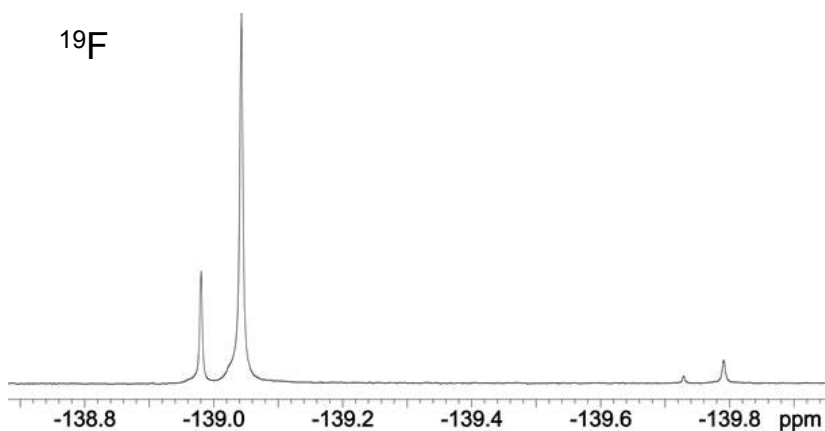
Liquid state NMR



Analysis of organic compounds in solution

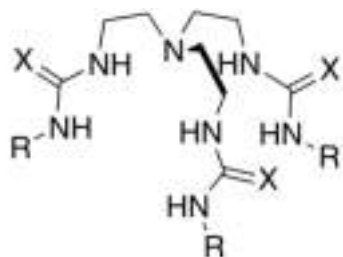
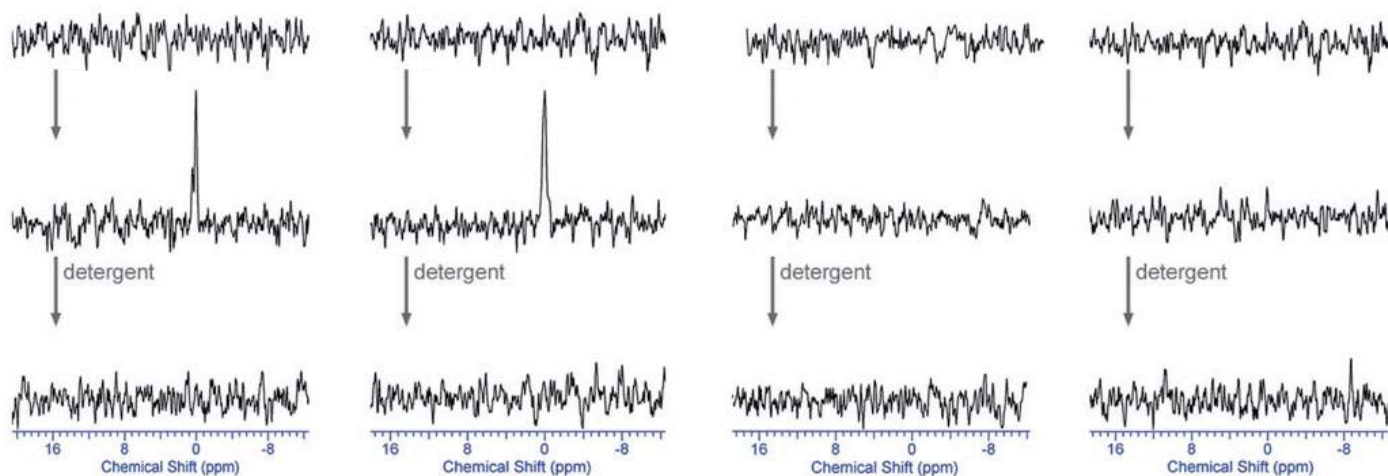


- Complete structure determination
- Study of dynamics of molecule (flexibility)
- Determination of kinetics
- Determination of structures inside mixtures

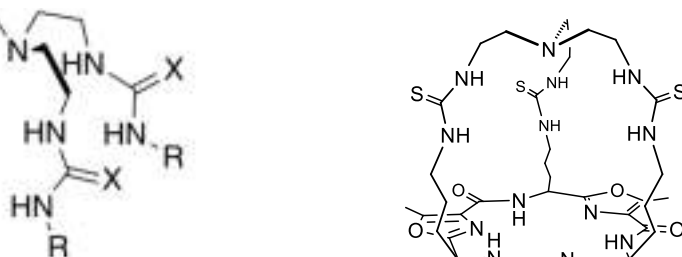


Chloride/sulfate transport

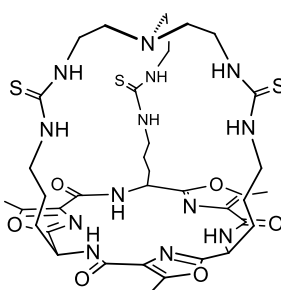
^{33}S NMR experiments



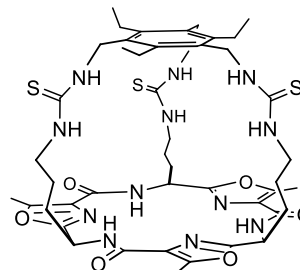
$\text{X}=\text{O}$, $\text{R}=\text{C}_6\text{F}_5$



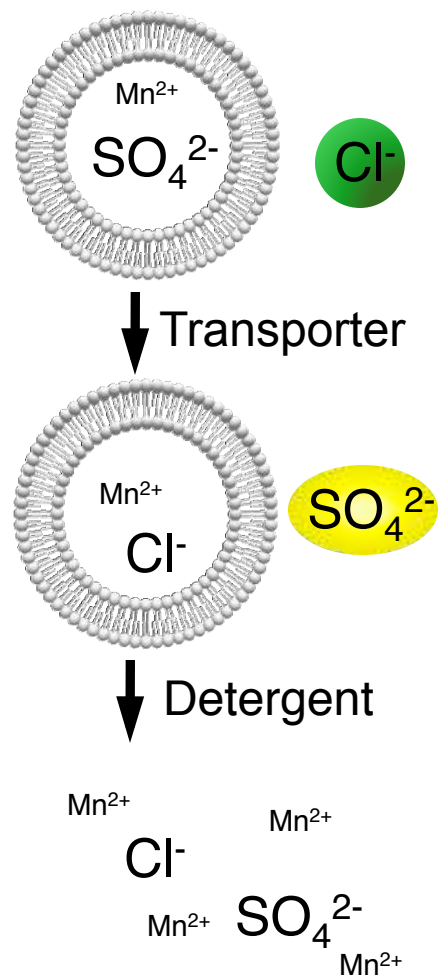
$\text{X}=\text{S}$, $\text{R}=\text{C}_6\text{F}_5$



PY34



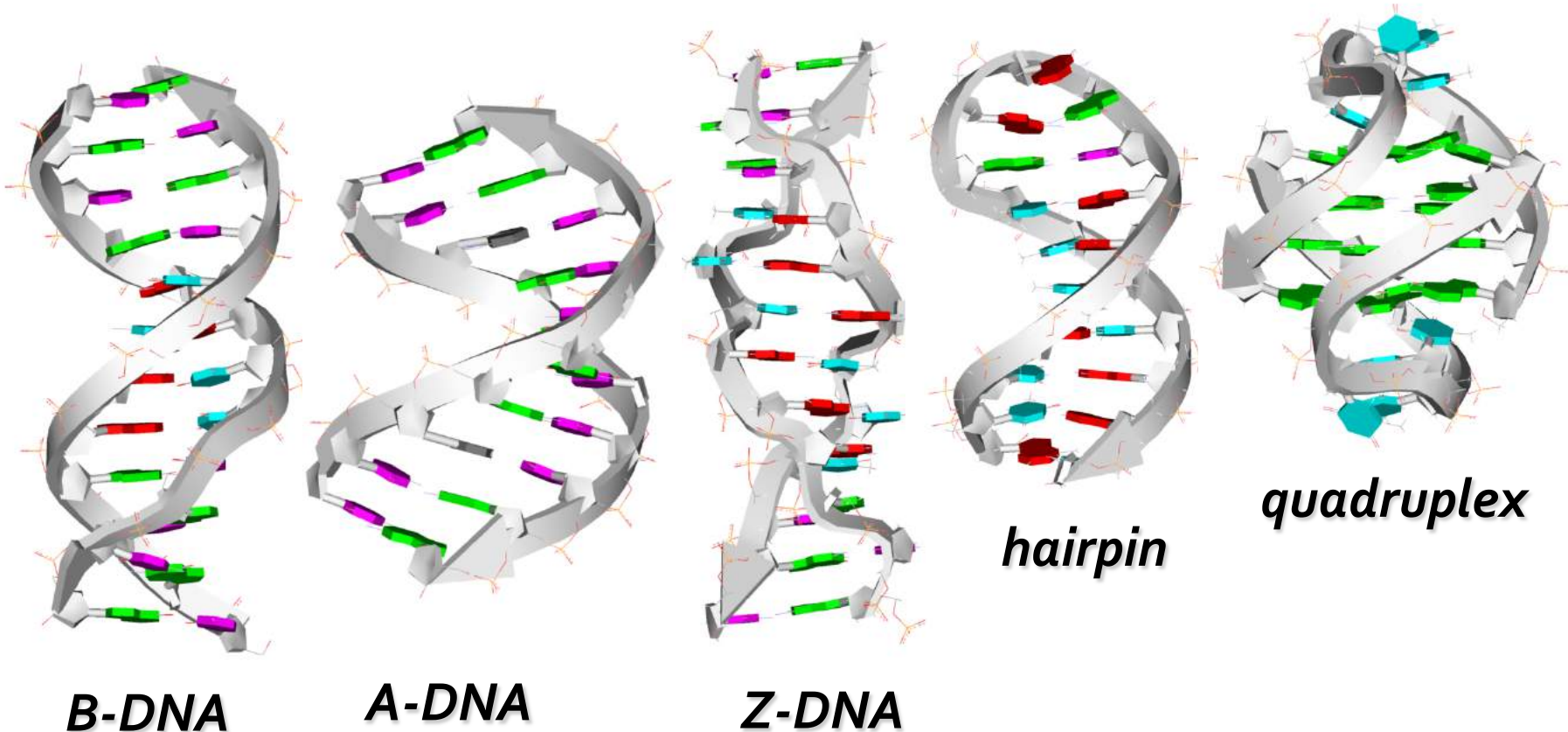
PY45



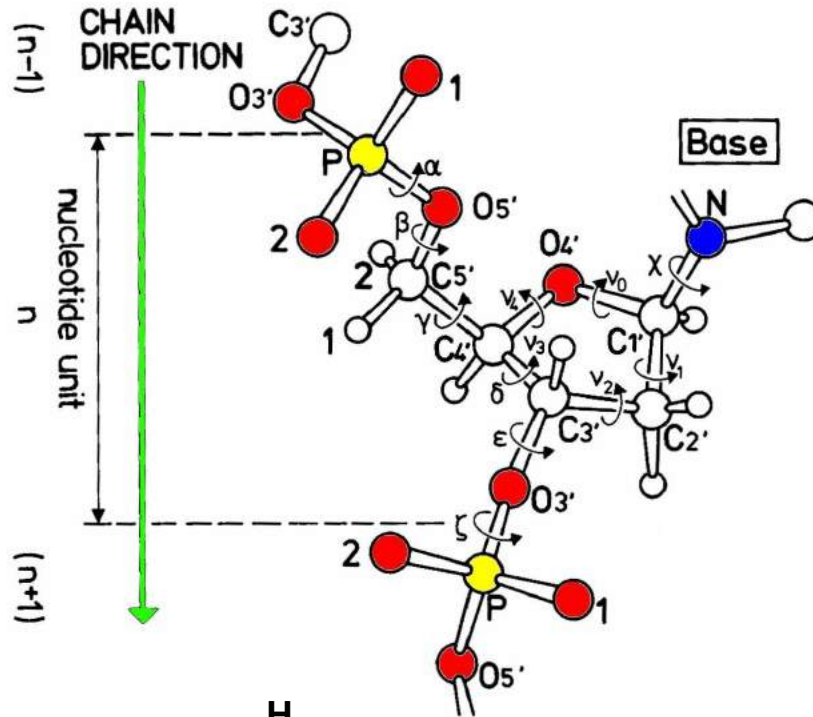
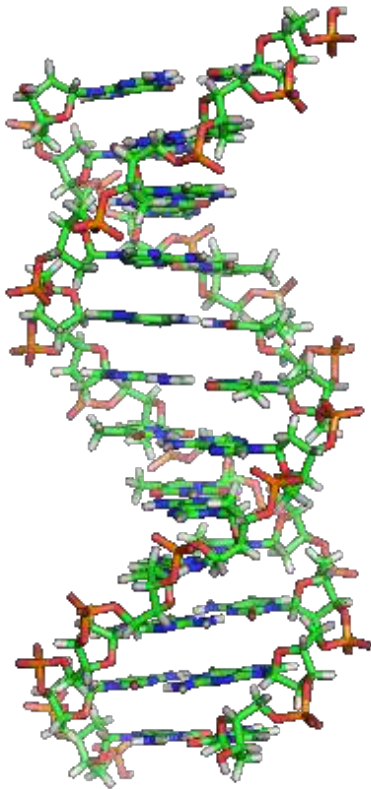
Nucleic acids

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

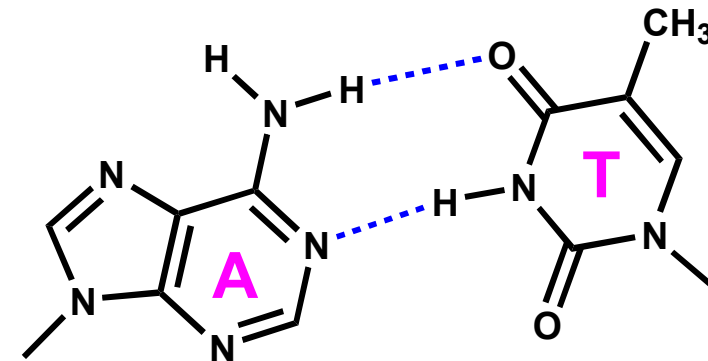
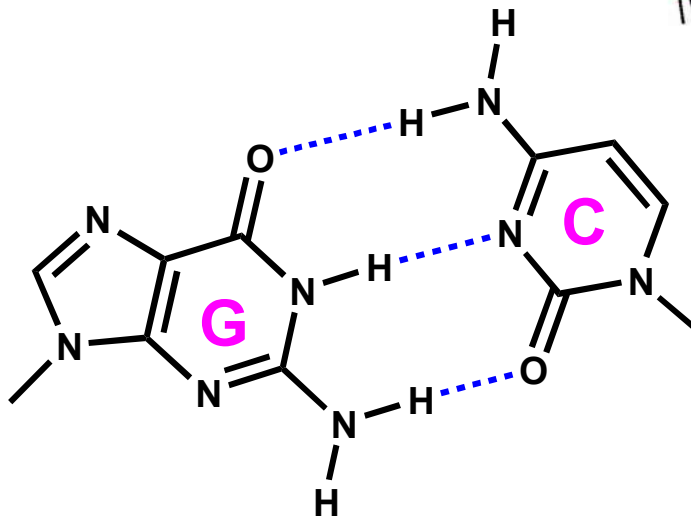
Together with proteins, nucleic acids form the most essential macromolecules.



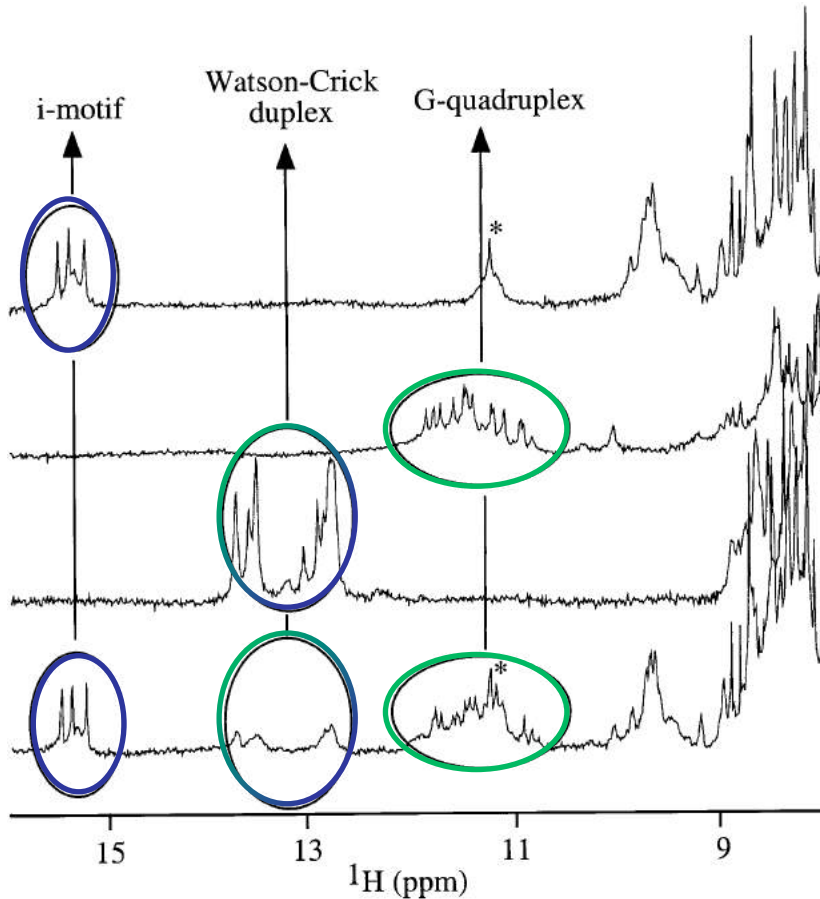
DNA - double-helix, Watson-Crick base pairs, ...



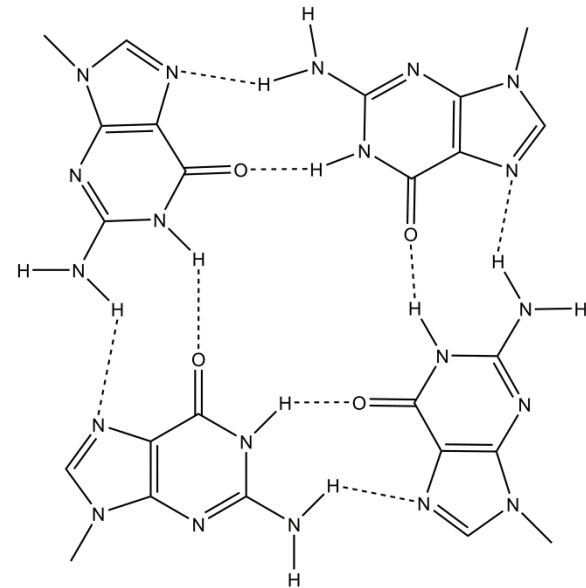
B-DNA



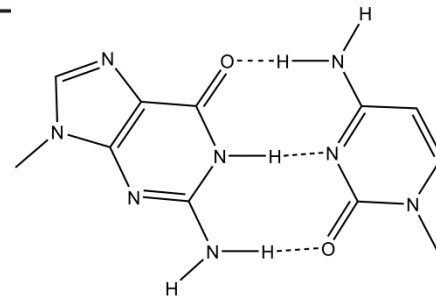
NMR easily distinguishes different structures



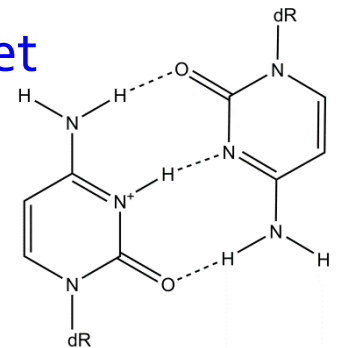
A.T. Phan and J.L. Mergny, NAR, 2002, 30, 21)



G-quartet



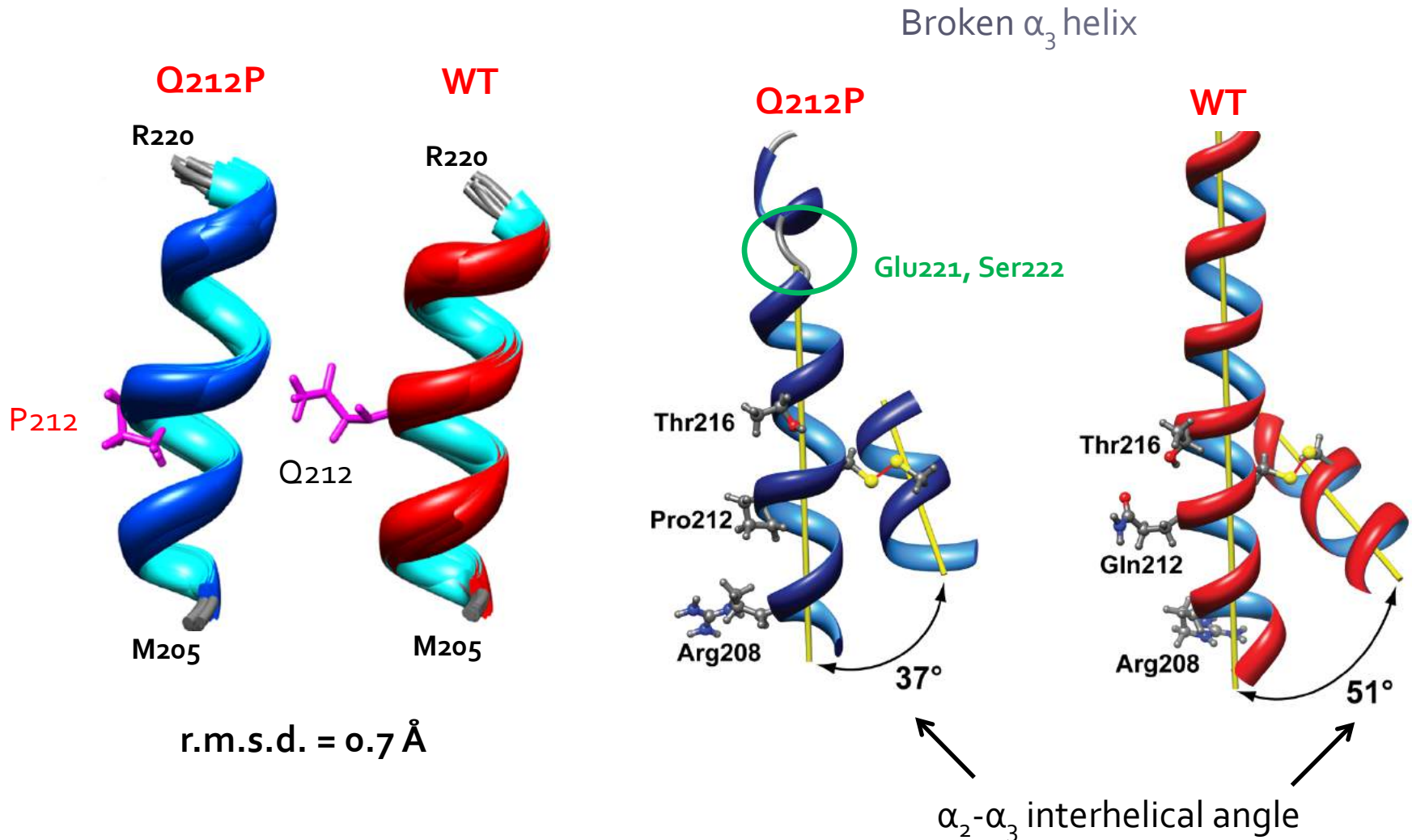
GC base pair



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.



Solid state NMR

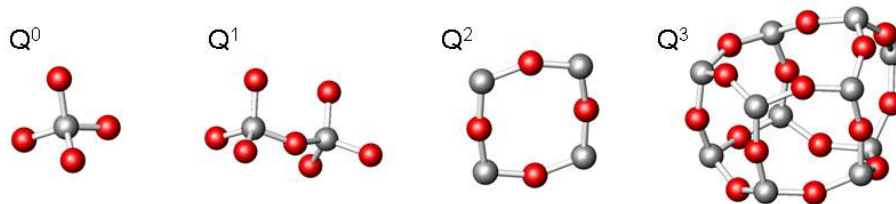
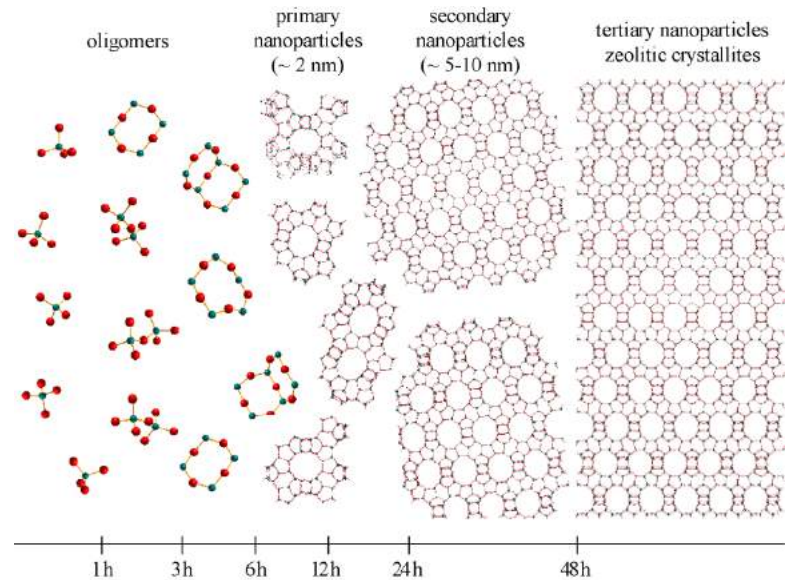
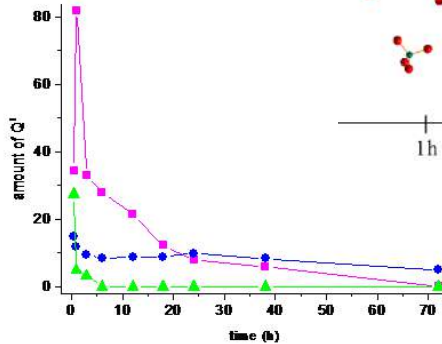
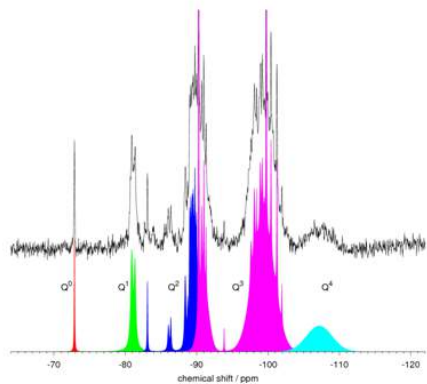


NMR spectroscopy of porous materials

- **formation of porous materials**

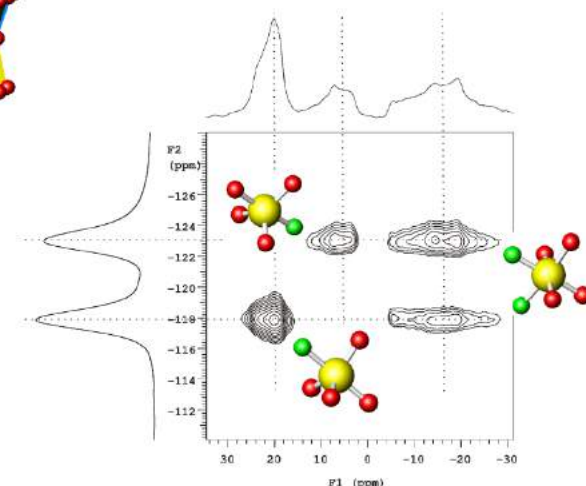
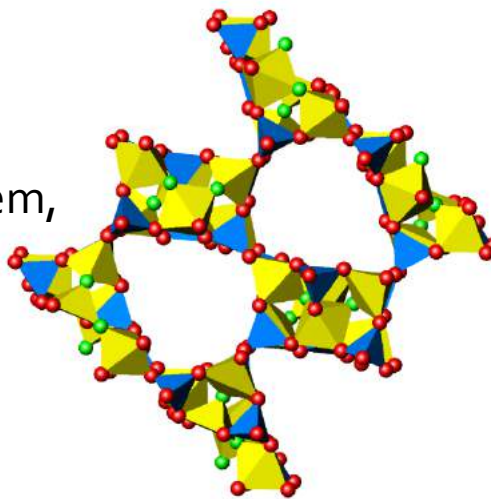
micro- and mesoporous silicates, aluminophosphates, metal-organic frameworks

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



- **structure of porous materials**

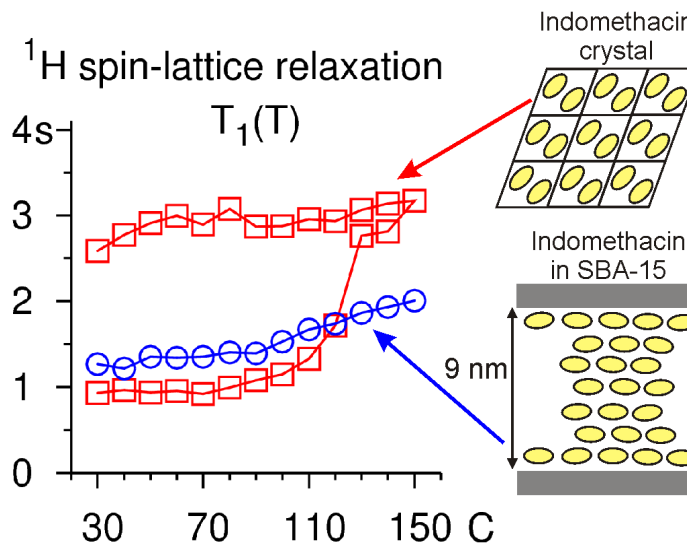
inequivalent sites, connectivities among them,
motifs that do not exhibit long-range order



- **molecules embedded within the pores**

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

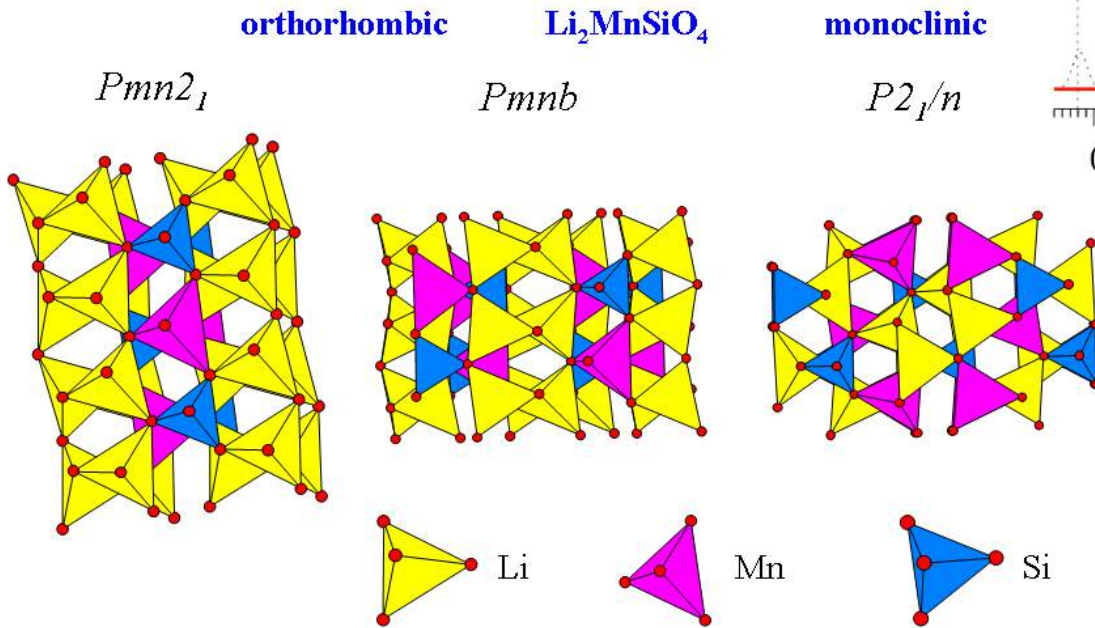
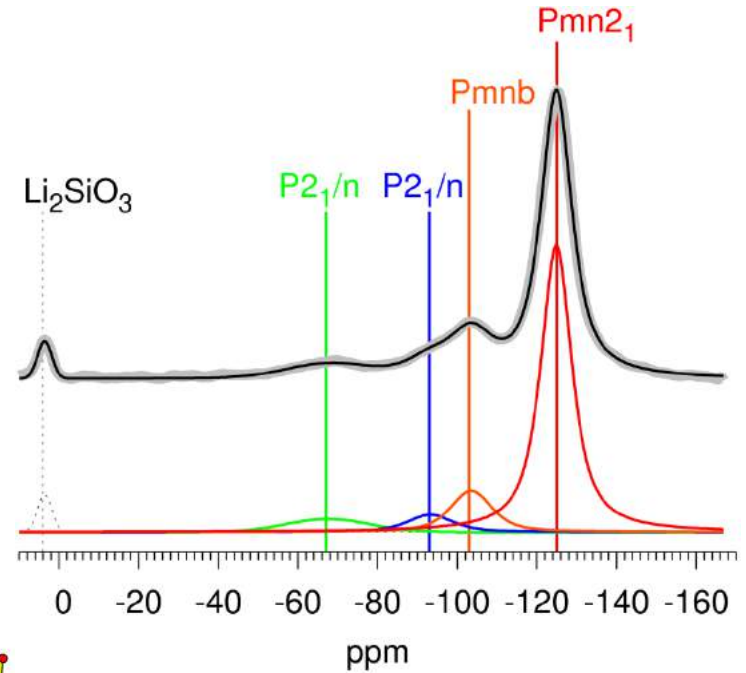
structural information, dynamics,
interactions



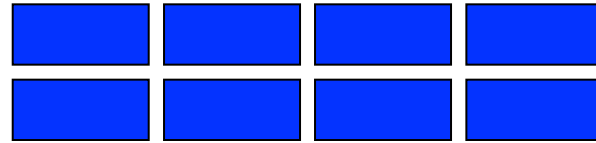
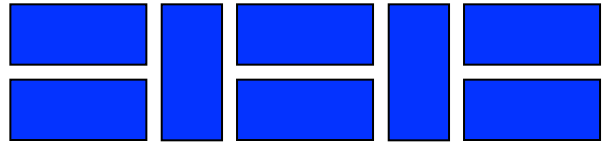
New materials for Li-ion batteries

- **composition of materials**

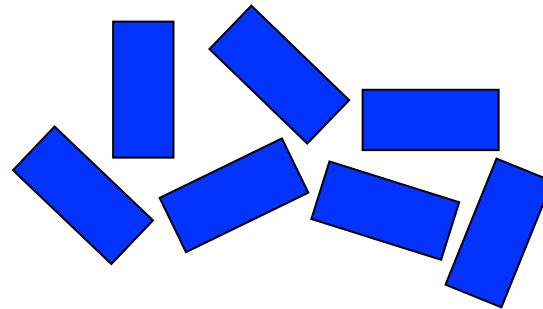
impurities, mixtures of polymorphs



Polymorphism



Polymorphs



Amorphous



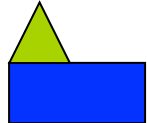
Molecule



Solvent molecule



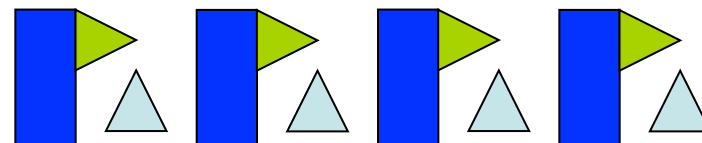
Deprotonated acid



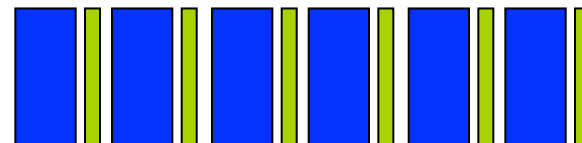
Protonated molecule



Molecule, that is solid at room temperature



Salt



Co-Crystal



Solvate

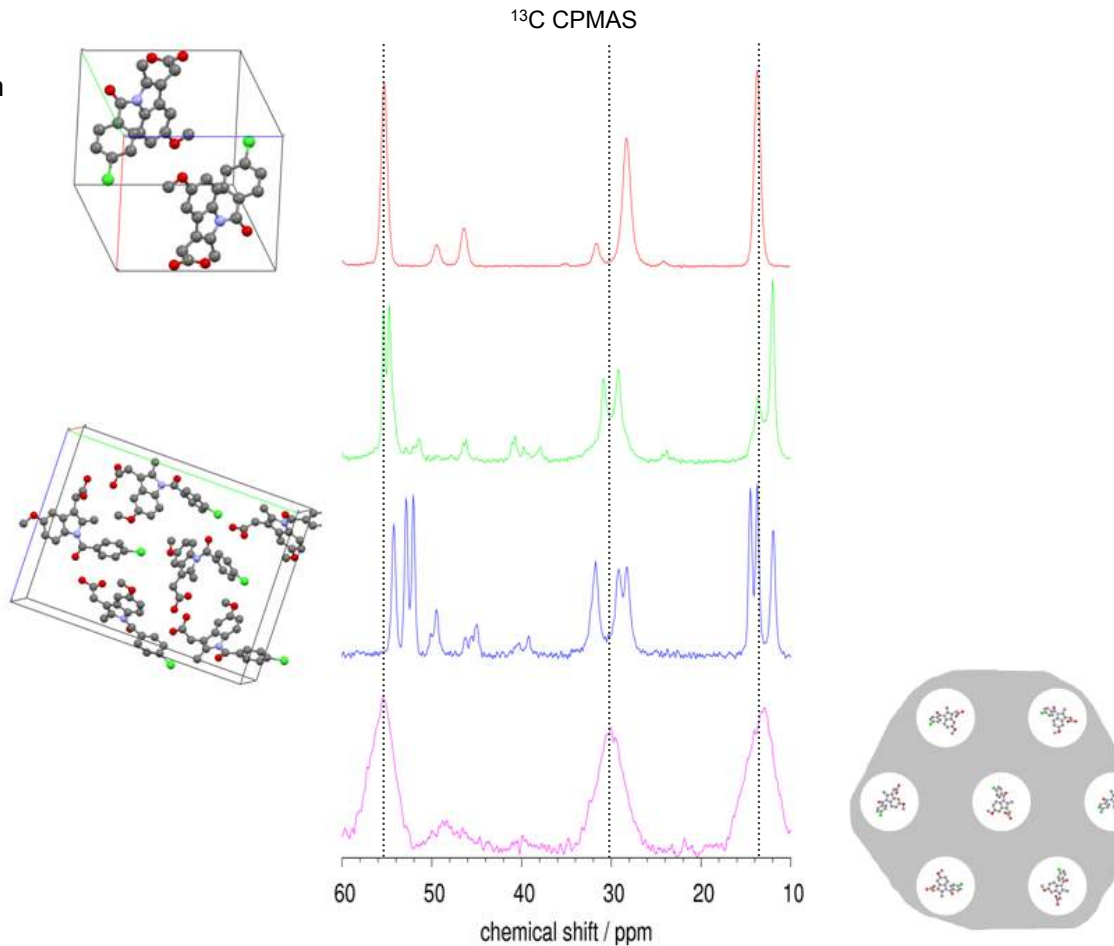


Pseudopolymorph

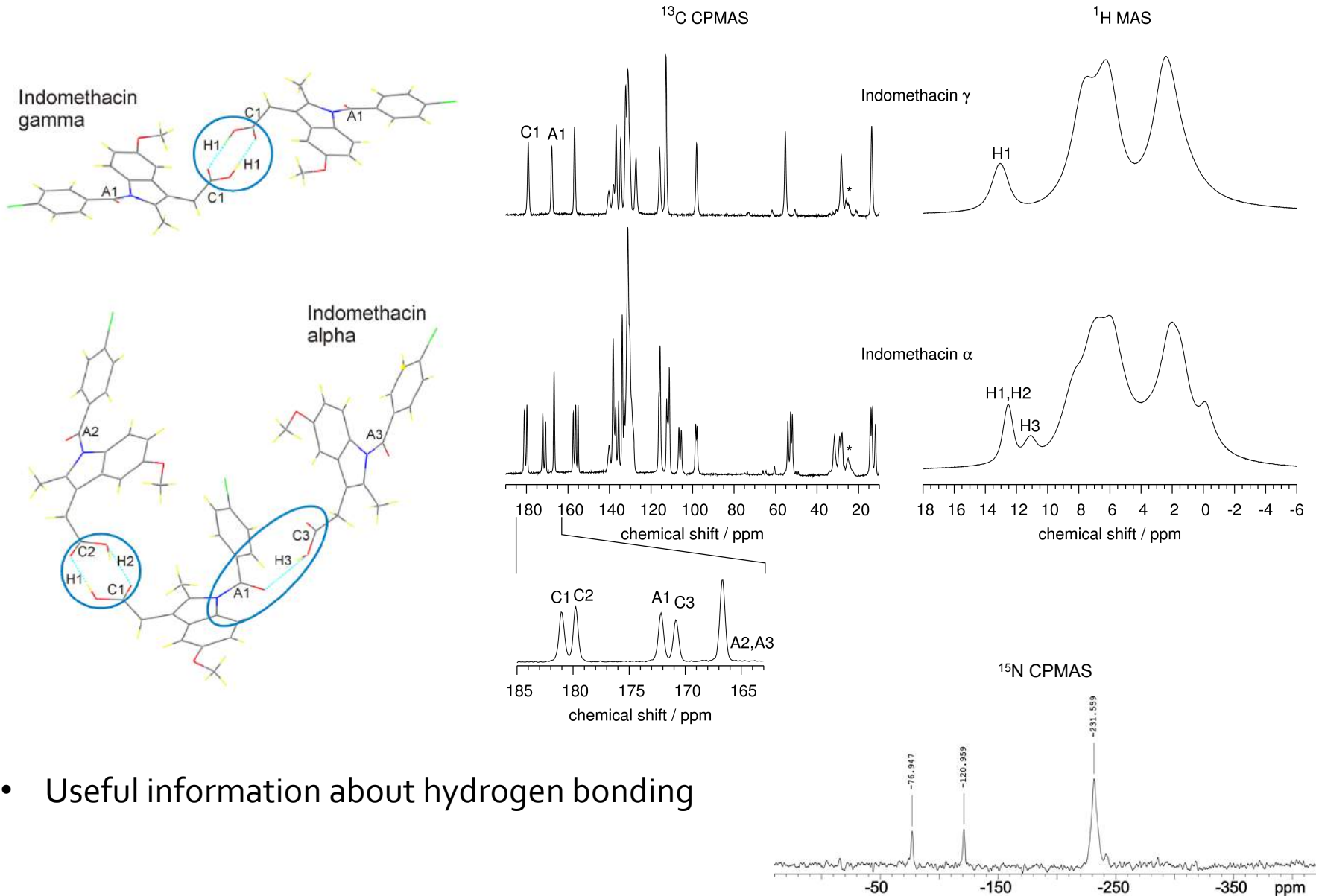
Structure of polymorphs

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

Indomethacin



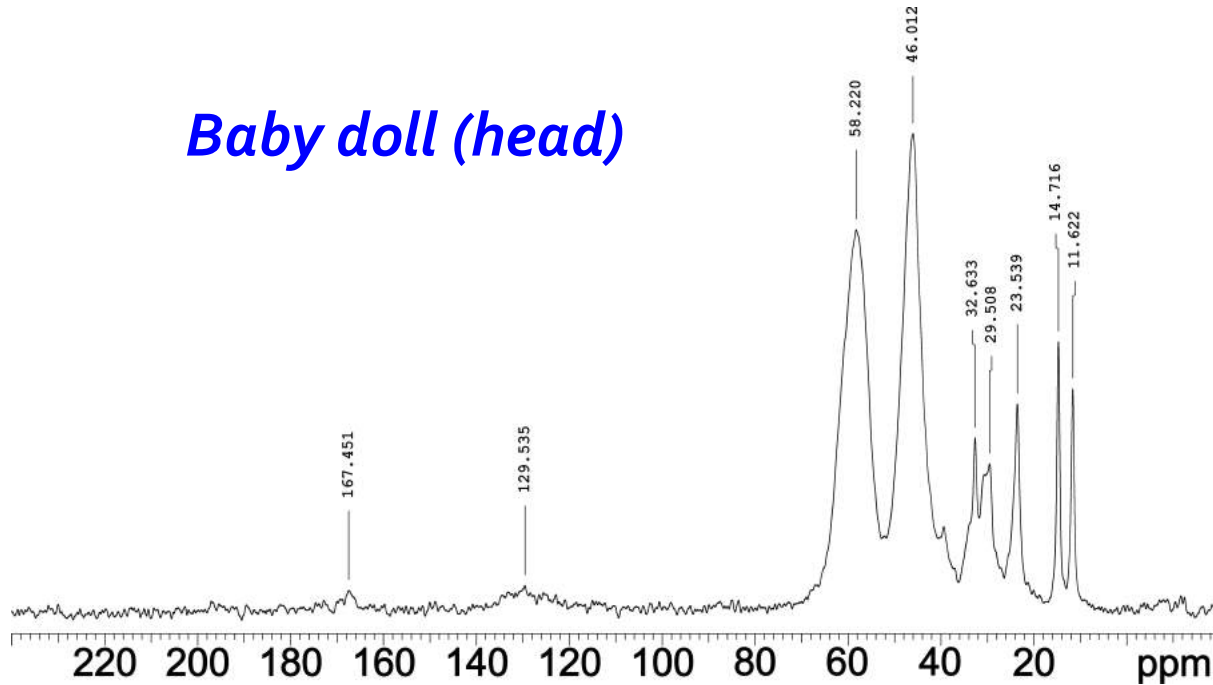
Interactions between molecules



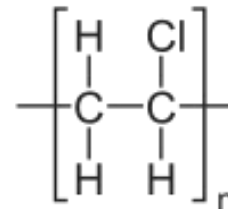
- Useful information about hydrogen bonding

HC-CPMAS NMR spectra of different plastics

Baby doll (head)

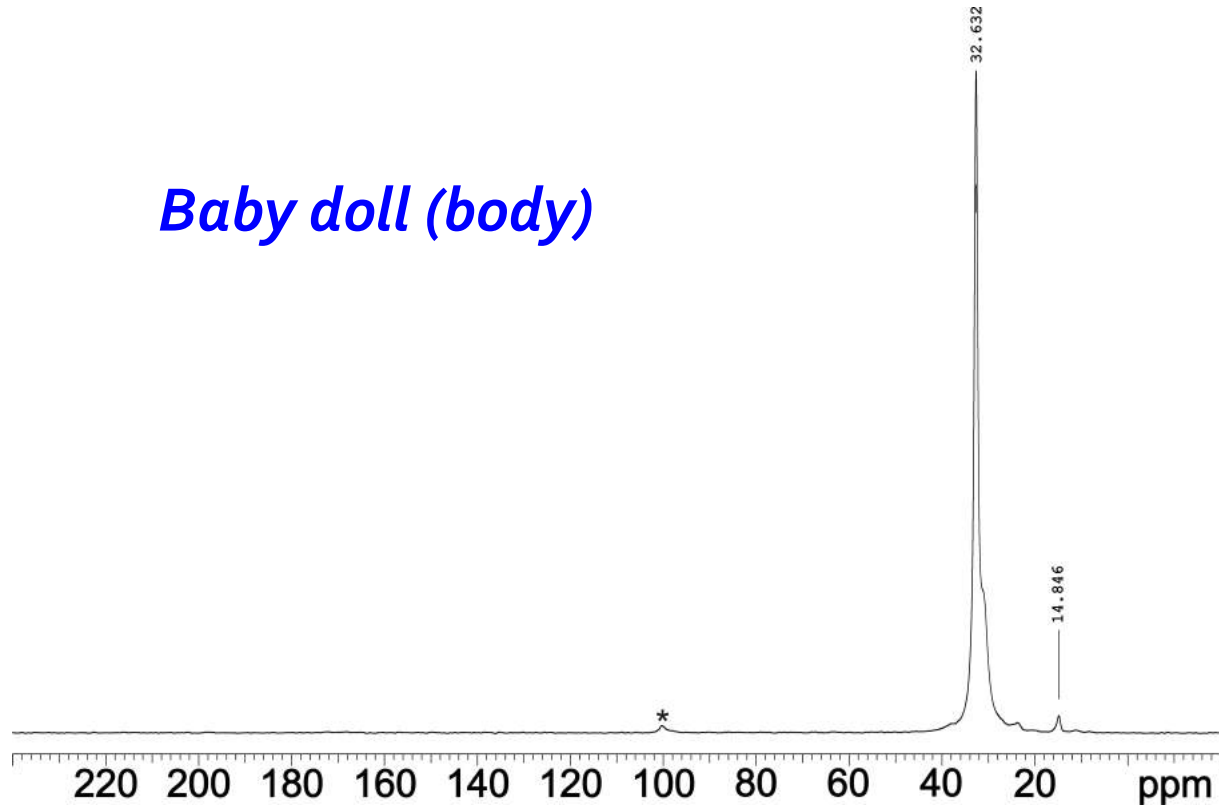


polyvinylchloride (PVC)

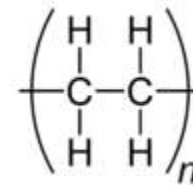


HC-CPMAS NMR spectra of different plastics

Baby doll (body)

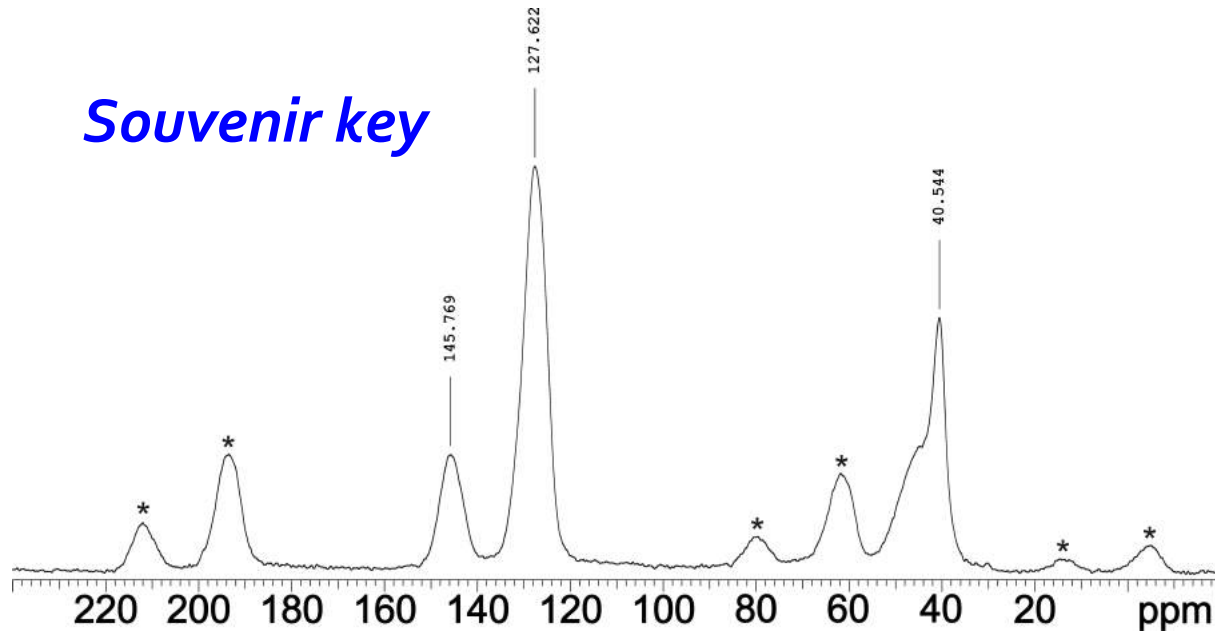


polyethylene (PE)

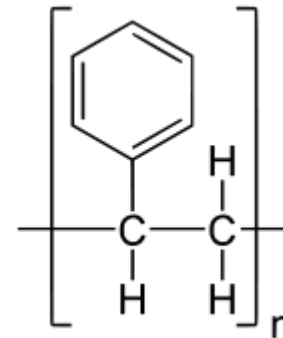


HC-CPMAS NMR spectra of different plastics

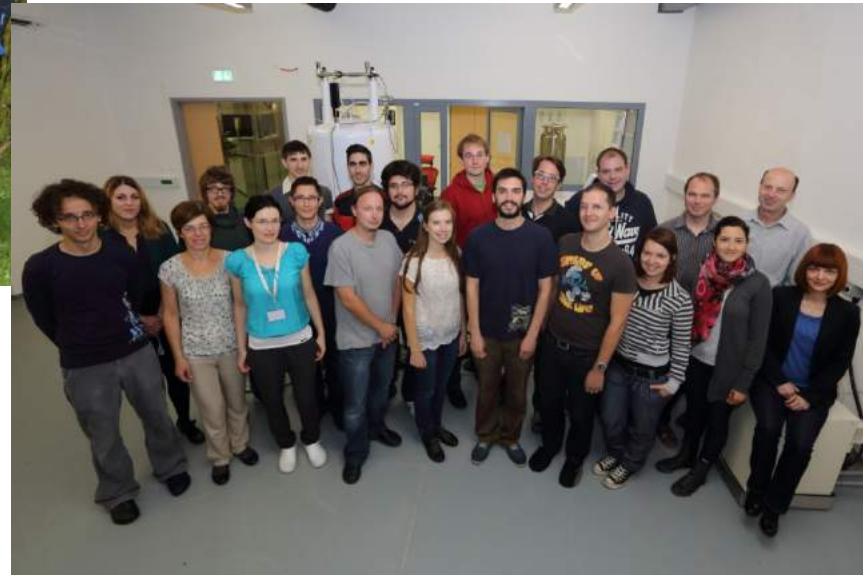
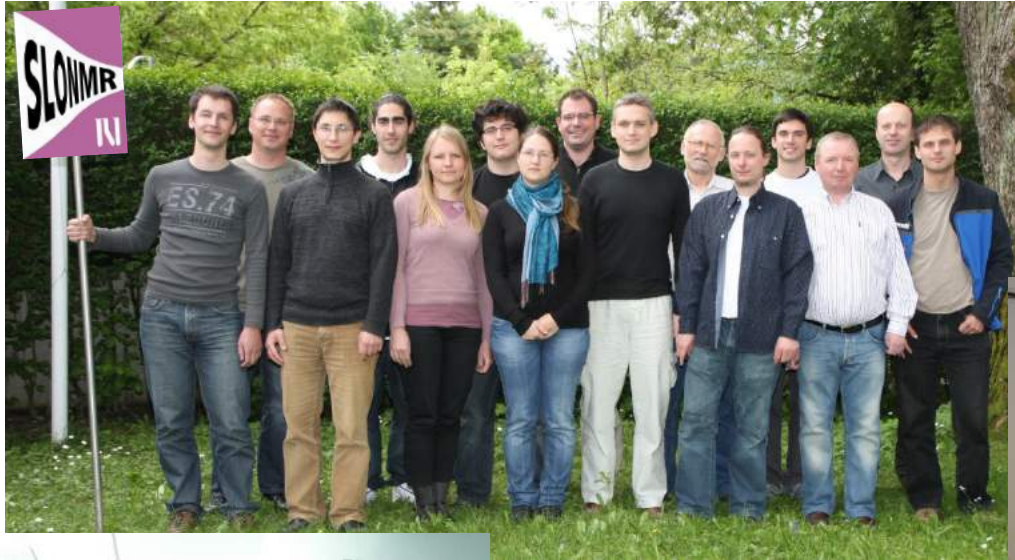
Souvenir key



polystyrene (PS)



Acknowledgments



SLOVENIAN RESEARCH AGENCY



**Thank you for your
attention**