Measurement techniques for bioinformatics, module IV: NMR spectroscopy

Hannu Maaheimo

Viikki 12. - 23.3.2007

NMR?

- eN yMmäRrä?
- Nyt Meni Rahat?
- No Meaningful Results?
- No Money for Research?

- Novel Multipurpose and Riskless?

NMR =

Nuclear Magnetic Resonance spectroscopy

Ydinmagneettinen resonanssi-spektroskopia

(MRI = Magnetic Resonance Imaging)

Examples of (biological) applications of NMR spectroscopy

- Determination of molecular structure
- Determination of molecular mobility and/or diffusion
- Determination of 3D structure of macromolecules, e.g. protein and nucleic acids
- Analysis of intermolecular binding and binding conformations of ligands like pharmaceuticals
- Determination of binding kinetics
- Metabolomics and identification of physiological states, e.g. diseases
- Real time follow up of chemical reactions (e.g. enzyme reactions)
- *In vivo* measurement of metabolite concentrations
- Determination of intra-cellular and compartmental pH of living cells
- Determination of energy state of living cells
- Real time *in vivo* analysis of diffusion over biological membranes
- *In vivo* NMR of living organisms like human beings
- Magnetic resonance imaging
- etc.

399673 Measurement techniques for bioinformatics (6 cr), module IV: NMR spectroscopy

Mon 12.3. 9-12 Lecture and laboratory practicals (NMR laboratory, Biocenter 3, Viikki)

- Lectures: Introduction to NMR spectroscopy, Basics of 1D NMR
- Laboratory practicals: Setting up and acquiring 1D 1H spectra

Wed 14.3. 8-12 Lecture and laboratory practicals (NMR laboratory, Biocenter 3, Viikki)

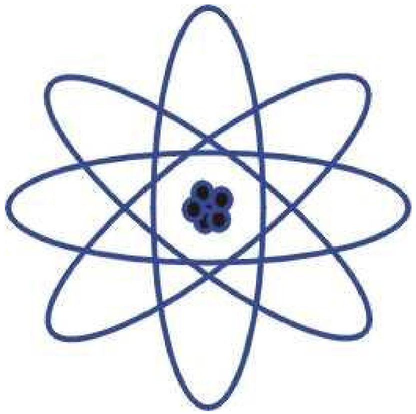
- Lectures: Interpretation of 1D NMR spectra, Different parameters measurable by NMR, Introduction to 2D NMR
- Laboratory practicals: Setting up and acquiring 2D NMR spectra

Mon 19.3. 9-12 Lecture and laboratory practicals (NMR laboratory, Biocenter 3, Viikki)

- Lectures: Solving protein sructures by NMR, Metabolomics by NMR
- Laboratory practicals: Working with spectrometer software, Interpretation of 2D NMR spectra

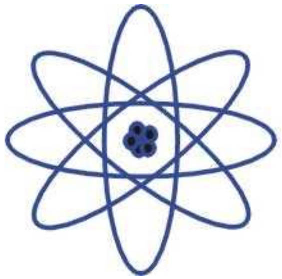
Wed 21.3. 8-12 Computer practicals (Room CK110, Exactum, Department of Computer Science, University of Helsinki)

- Lectures and practicals: Computer software for prosessing and analysing NMR spectra



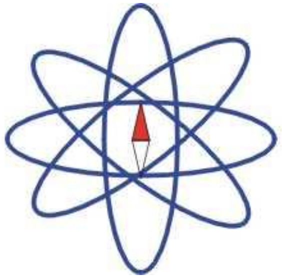
spin I = 0, ½, 1, 1½, ...

I = 0



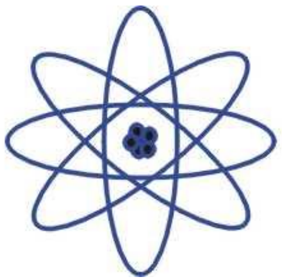
- the nucleus has no magnetic properties
  => no NMR signal
- e.g. ^{12}C, ^{16}O

I = ½



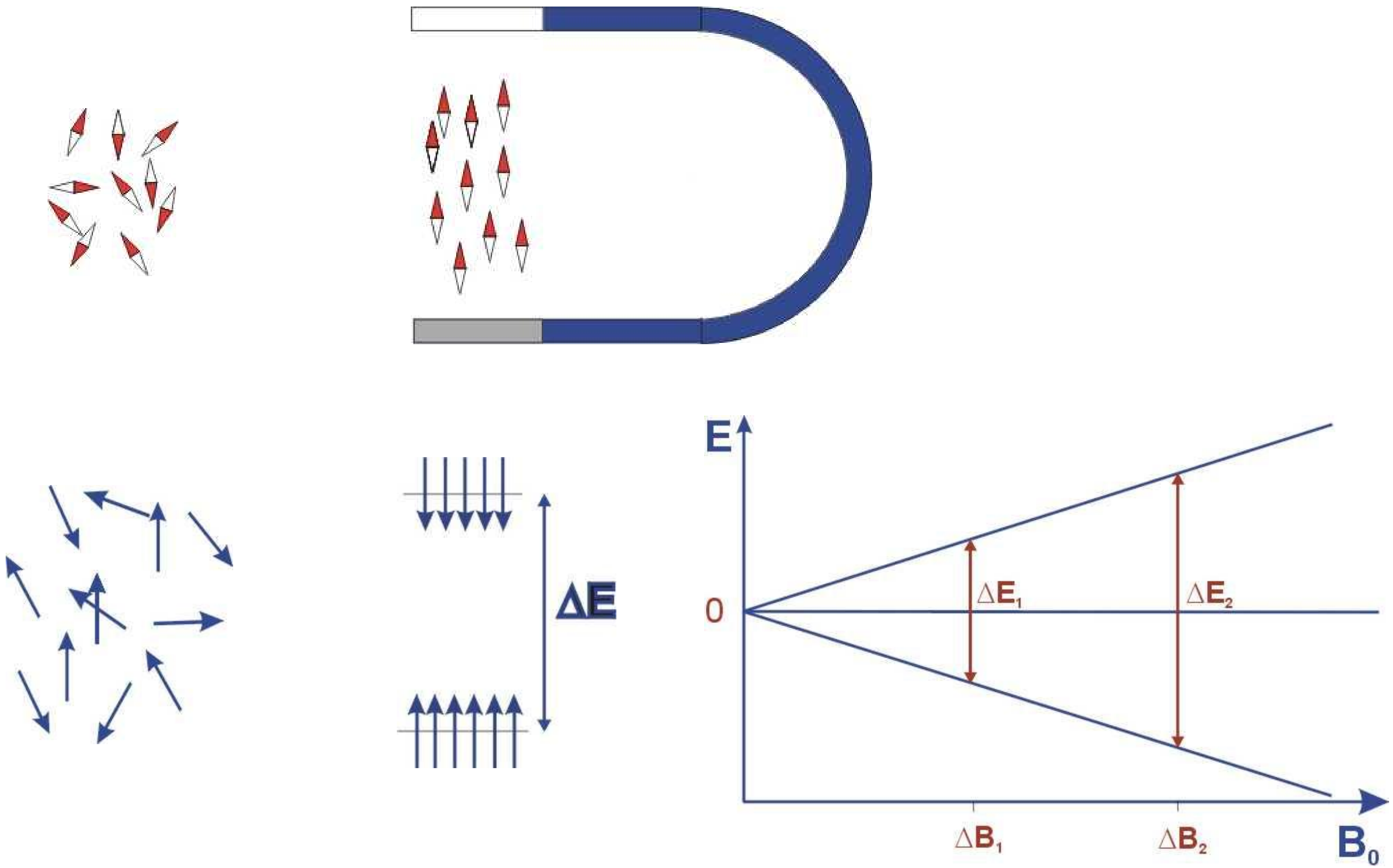
- when placed in a magnetic field, the nucleus behaves like a small bar magnet or compass needle
- e.g. ^{1}H, ^{13}C, ^{15}N, ^{31}P

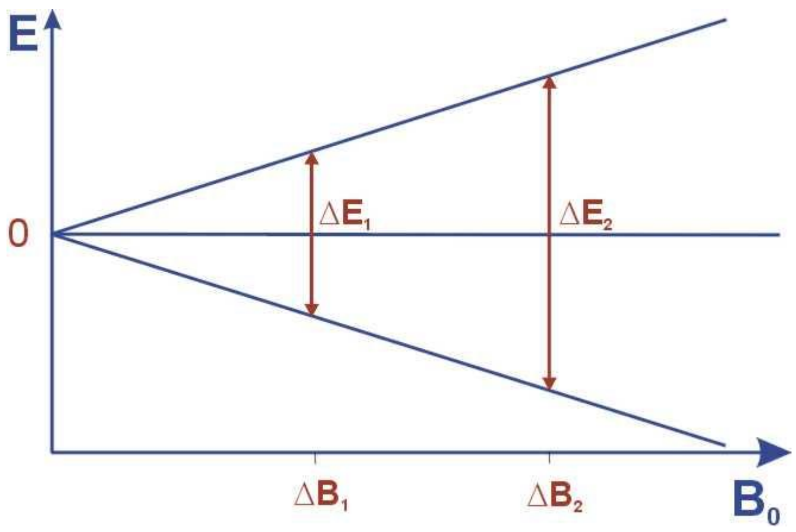
I = 1



- also "NMR-active", but the behavior in a magnetc field more complex way than spin ½ nucleus
- e.g. ^{2}H (=D)

"like a small compass needle"





$\Delta E = h\gamma B_0/2\pi$

$\nu = \gamma B_0/2\pi$

$\Delta E = h\nu$

γ = magnetogyric ratio,
a measure for the strength
of the nuclear magnet

^{1}H and ^{13}C resonance frequences at different magnetic flux densities

B_0 [T]	^{1}H [MHz]	^{13}C [MHz]
2.35	100	25.15
4.70	200	50.32
5.87	250	62.90
7.05	300	75.47
9.40	400	100.61
11.75	500	125.76
14.10	600	150.90
16.44	700	176.05
17.62	750	188.62
18.79	800	201.19
21.14	900	236.34

^{1}H and ^{13}C resonance frequences at different magnetic flux densities

B_0 [T]	^{1}H [MHz]	^{13}C [MHz]
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Very strong magnetic fields…

http://cc.oulu.fi/~nmrlab/nmrjaos/NMRkartta.JPG

Finnish NMR Discussion Group - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://cc.oulu.fi/~nmrlab/nmrjaos/fidgroup.htm

Finnish NMR Discussion Group

Finnish NMR Discussion Group

- About FiD
- Board Members
- NMR Groups
- NMR Map
- Meetings
- E-mail to all members (for members only)
- NMR-links

These pages were created by

Petri Ingman: manager
Elina Niskanen: artist
Kari Oikarinen: engineer

Last update 12-Feb-2007
Anu Kantola

Finnish NMR Discussion Group (FiD)

Welcome to the homepage of the Finnish NMR Discussion Group.

The Finnish NMR Discussion Group is part of the Association of Finnish Chemical Societies. The members of the Group are interested in Magnetic Resonance Spectroscopy in all its aspects. The board of the group consists of 8 members, Petri Ingman (University of Turku) being the chairman for this year.

The major event for sharing information and NMR debate is the Annual Finnish NMR Symposium. This two-day symposium includes lectures and posters of NMR studies in Finland. In addition 2-3 specialists from abroad are invited to give their contribution in the meeting. The board members for the next year will also be elected during the meeting. The Symposium is organized in the beginning of June in select resorts.

The following pages contain contact addresses of the Group members, more information about symposium and further information and links concerning NMR-Spectroscopy.

If you like to join the FiD, subscribe to the list in http://lists.oulu.fi/mailman/listinfo/nmr-jaos

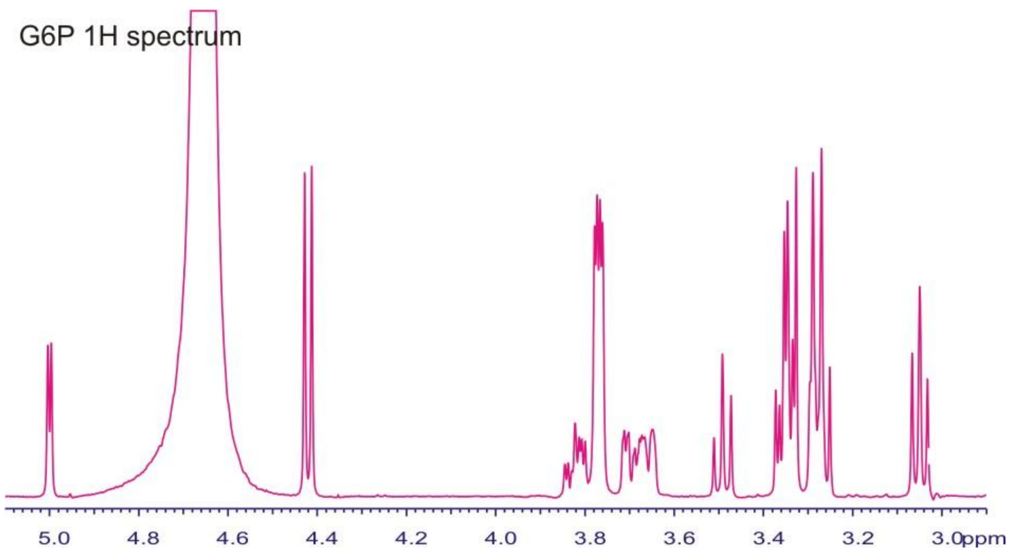
Comments (positive as well as negative), hints, suggestions for improvements and valuable links to other sources are also welcome and can be sent to Anu Kantola (Anu.Kantola (at) oulu.fi).

Properies of some nuclides of importance in NMR spectroscopy

Nuclide	Spin I	Natural abundance[a)] [%]	Magnetic Moment[b)] μ_z/μ_N	Electric quadrupole moment[a)] Q [10^{-30} m^2]	Magnetogyric ratio[a)] γ [10^7 rad T^{-1} s^{-1}]	NMR frequency[a)] [MHz] (B_0 = 2.3488 T)	Relative receptivity[c)]
^{1}H	1/2	99.9885	2.7928	–	26.7522	100.000	1.00
^{2}H	1	0.0115	0.8574	0.2860	4.1066	15.3506	9.65×10^{-3}
^{3}H[d)]	1/2	–	2.9790	–	28.5350	106.6640	1.21
^{6}Li	1	7.59	0.8220	–0.0808	3.9372	14.7161	8.50×10^{-3}
^{10}B	3	19.9	1.8006	8.459	2.8747	10.7437	1.99×10^{-2}
^{11}B	3/2	80.1	2.6887	4.059	8.5847	32.0840	1.65×10^{-1}
^{12}C	0	98.9	–	–	–	–	–
^{13}C	1/2	1.07	0.7024	–	6.7283	25.1450	1.59×10^{-2}
^{14}N	1	99.63	0.4038	2.044	1.9338	7.2263	1.01×10^{-3}
^{15}N	1/2	0.368	–0.2832	–	–2.7126	10.1368	1.04×10^{-3}
^{16}O	0	99.96	–	–	–	–	–
^{17}O	5/2	0.038	–1.8938	–2.558	–3.6281	13.5565	2.91×10^{-2}
^{19}F	1/2	100	2.6269	–	25.1815	94.0940	8.32×10^{-1}
^{23}Na	3/2	100	2.2177	10.4	7.0809	26.4519	9.27×10^{-2}
^{25}Mg	5/2	10.00	–0.8555	19.94	–1.6389	6.1216	2.68×10^{-3}
^{29}Si	1/2	4.68	–0.5553	–	–5.3190	19.8672	7.86×10^{-3}
^{31}P	1/2	100	1.1316	–	10.8394	40.4807	6.65×10^{-2}
^{39}K	3/2	93.258	0.3915	5.85	1.2501	4.6664	5.10×10^{-4}
^{43}Ca	7/2	0.135	–1.3176	–4.08	–1.8031	6.7301	6.43×10^{-3}
^{57}Fe	1/2	2.119	0.0906	–	0.8681	3.2378	3.42×10^{-5}
^{59}Co	7/2	100	4.627	42.0	6.332	23.7271	2.78×10^{-1}
^{119}Sn	1/2	8.59	–1.0473	–	–10.0317	37.2906	5.27×10^{-2}
^{133}Cs	7/2	100	2.5820	–0.343	3.5333	13.1161	4.84×10^{-2}
^{195}Pt	1/2	33.832	0.6095	–	5.8385	21.4968	1.04×10^{-2}

Chemical shift

- The nuclei have different resonance frequences depending on their chemical environment
- The electrons around the nucleus slightly modify the magnetic field the nucleus experiences



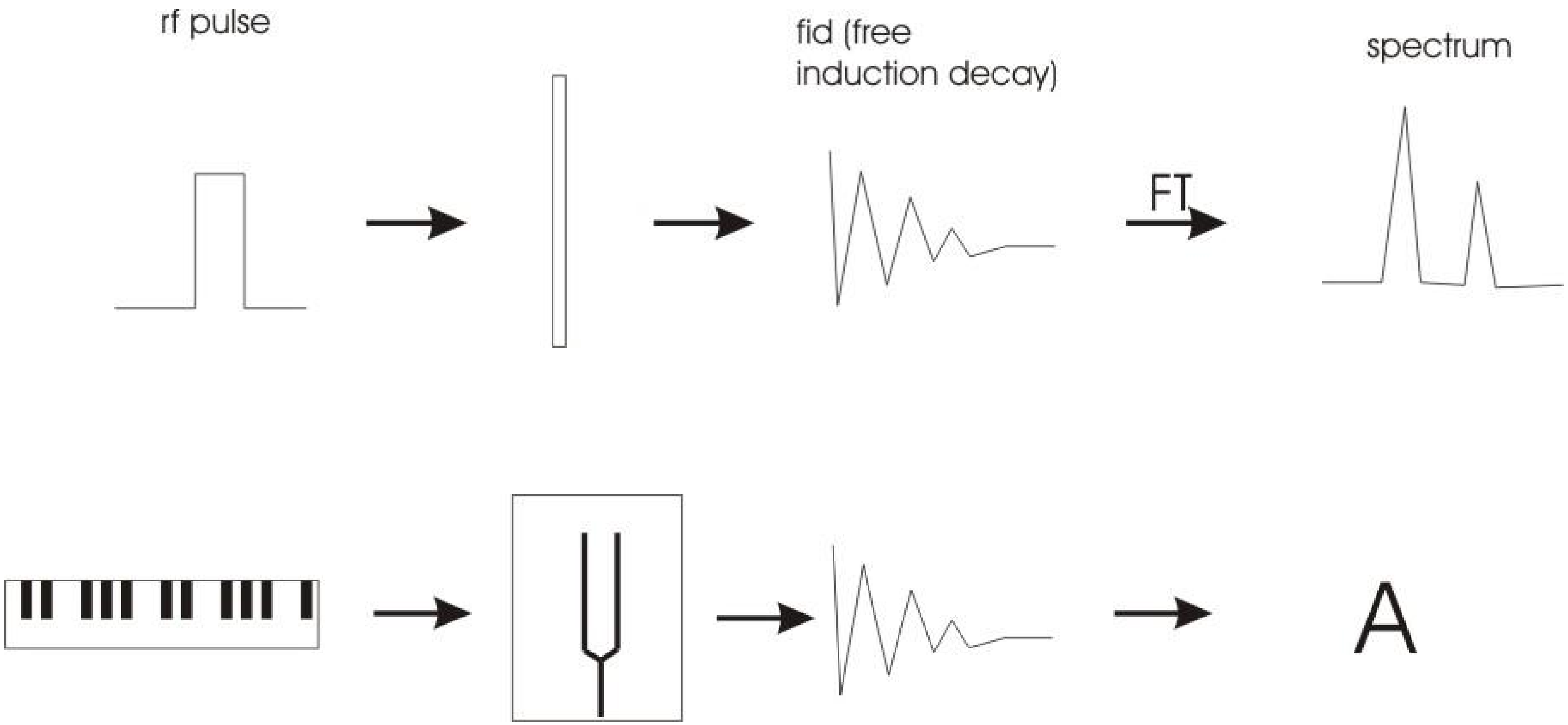
- chemical shift
  - place of the signal
  - local magnetic filed of the nucleus determined by shielding by the electrones (= chemical structure)
  - given as relative value in ppm - independent on external field strength

$$\delta_{sample}\ [\text{ppm}] = \frac{\nu_{sample} - \nu_{reference}\ [\text{Hz}]}{\nu_{reference}\ [\text{MHz}]}$$

Methods to record an NMR spectrum

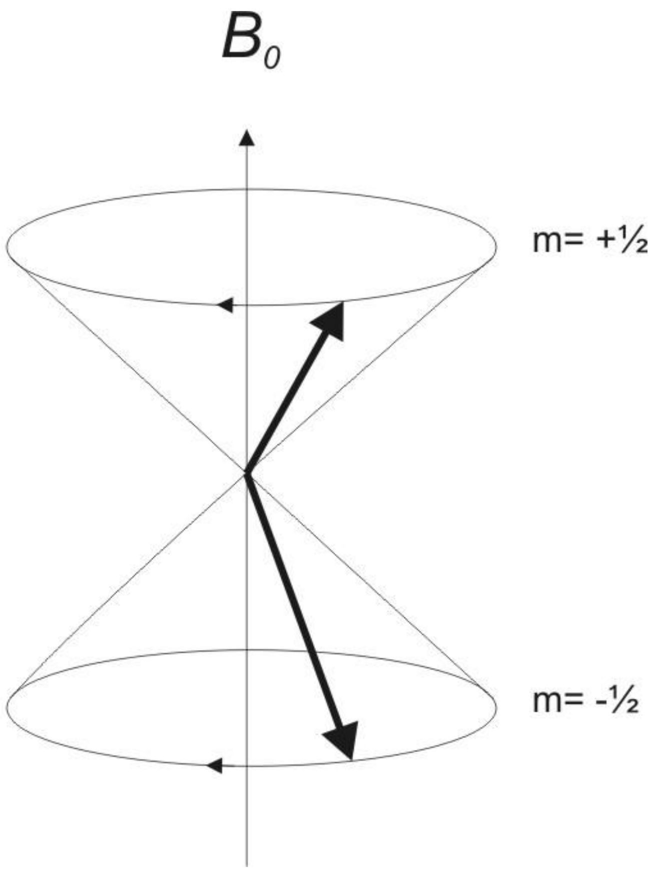
- continuous wave (CD)
  - sweep the frequency (or magnetic field) and record the spectrum
  - slow
  - only basic 1D spectra
  - prevaling method until late 1960's
- pulsed NMR
  - very short (μs) radio frequency (rf) pulses to exite and manipulate the spin states of the nuclei
  - fast, sensitive (data accumulation)
  - endless possibilities for designing experiments
  - the shorter the pulse, the broader range of rf present (and vice versa)

Basic operation of an NMR spectrometer



Precession of nuclear dipoles with spin I = 1/2

- Larmour frequency



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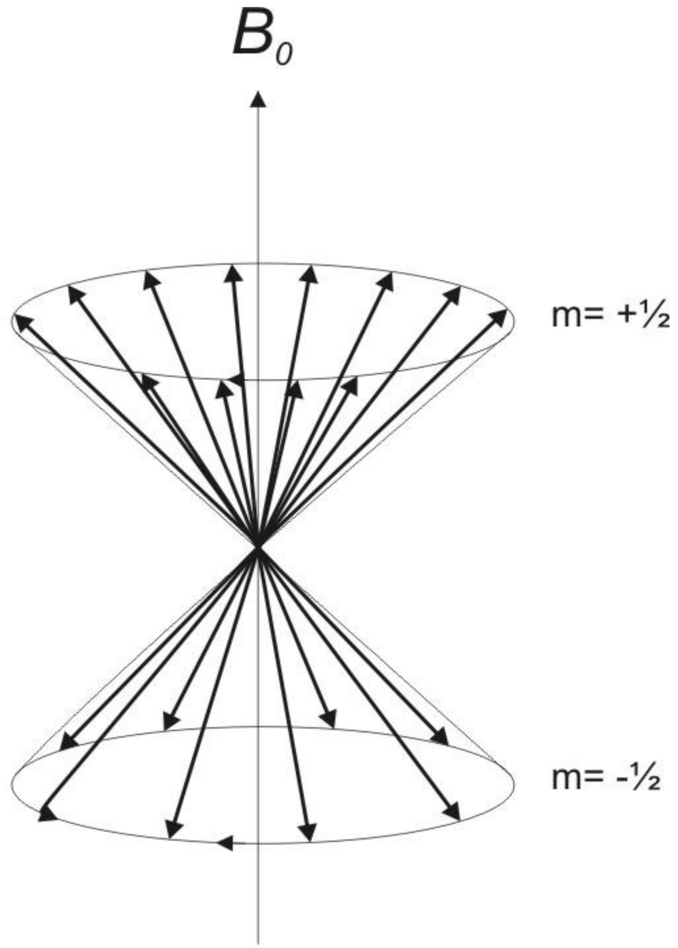
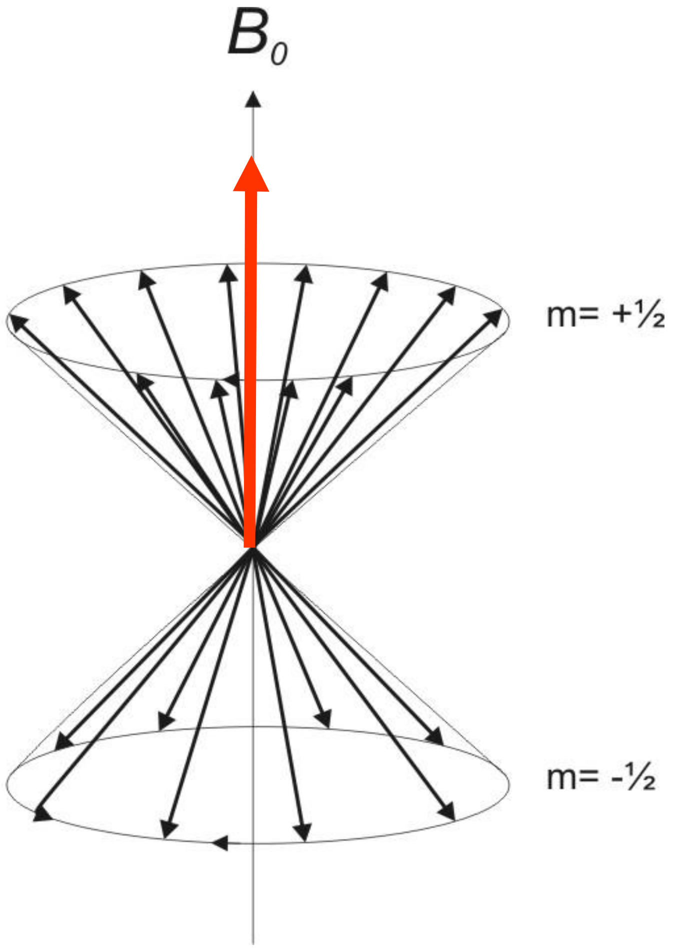
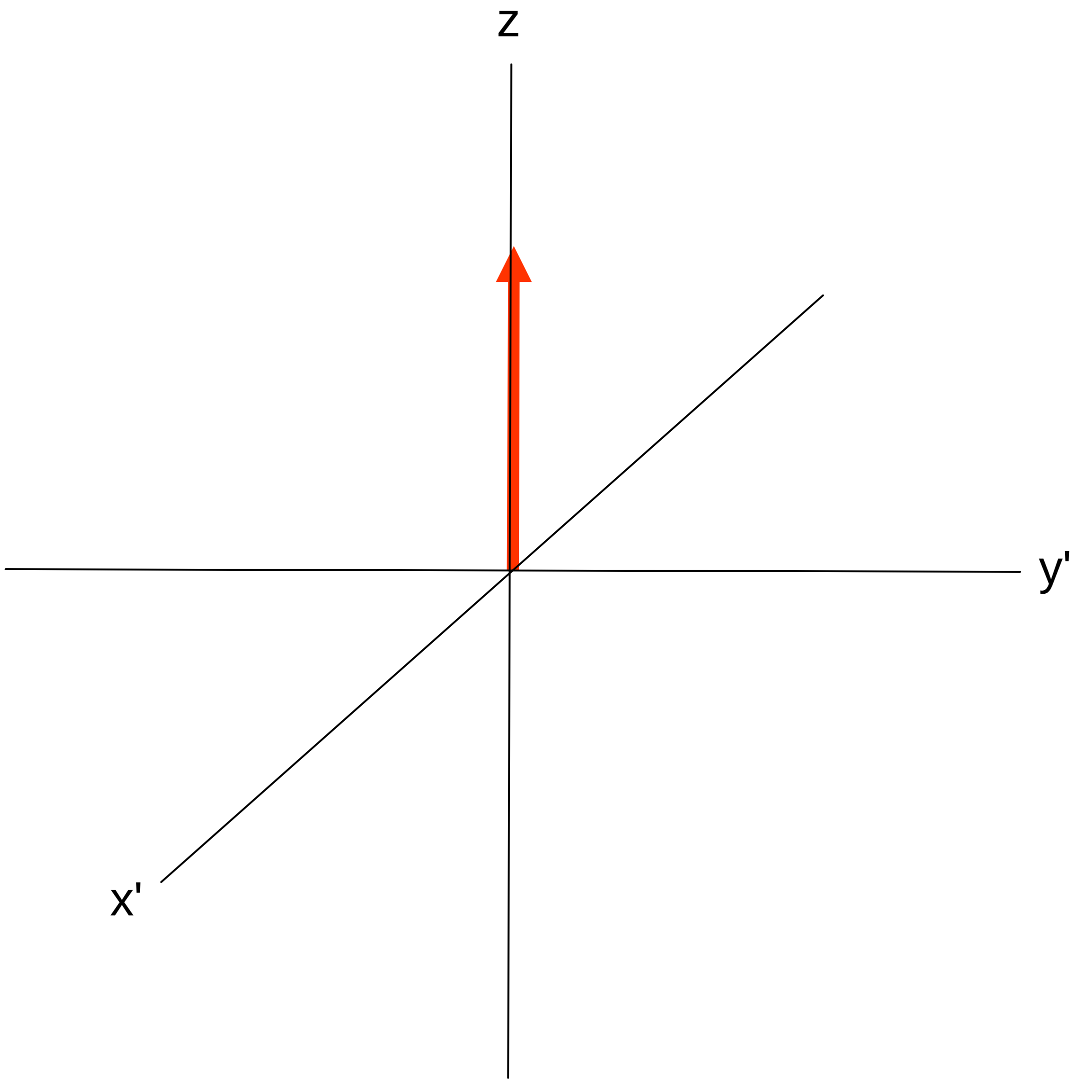


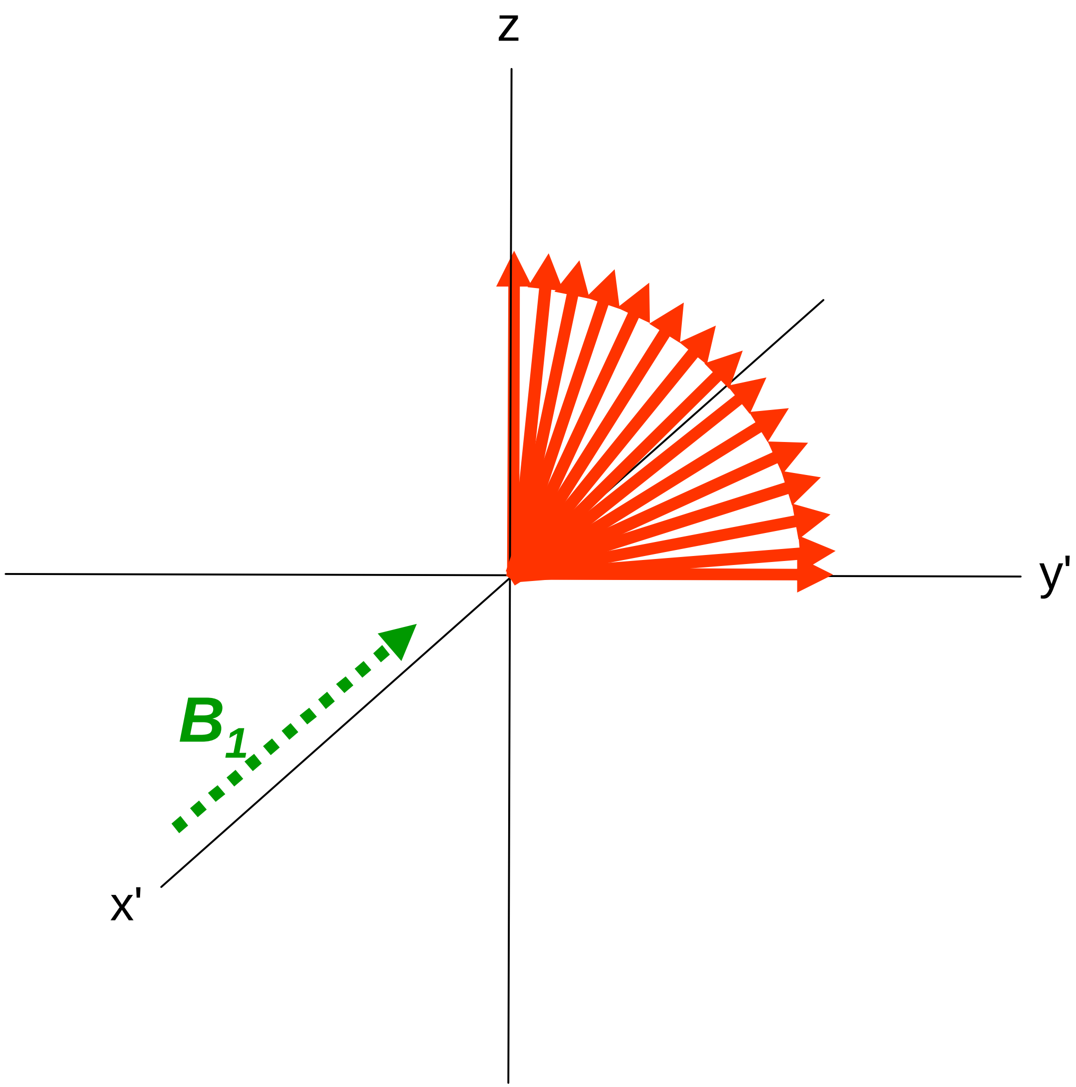
Macroscopic magnetisation



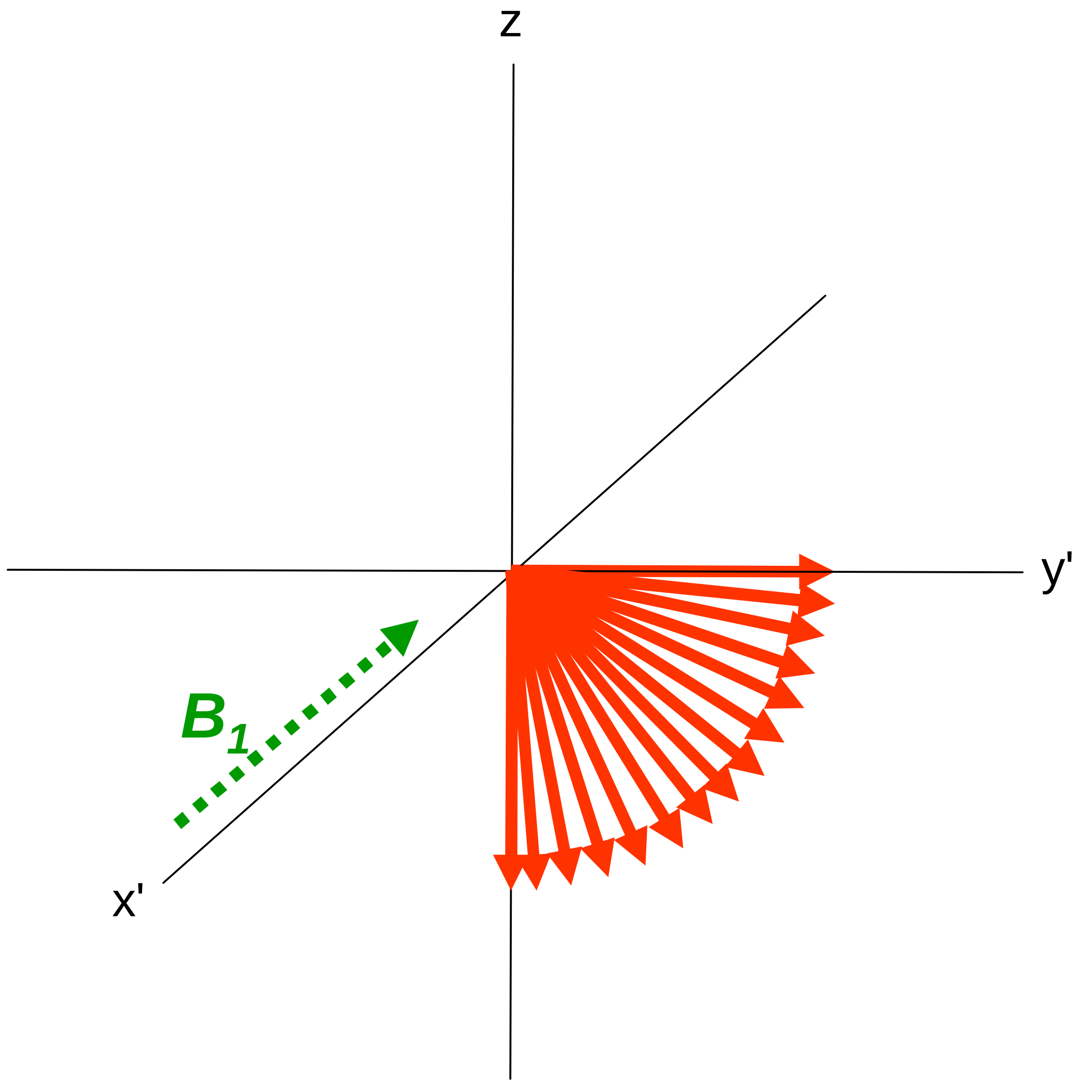
Vector model of macroscopic of pulsed NMR experiments



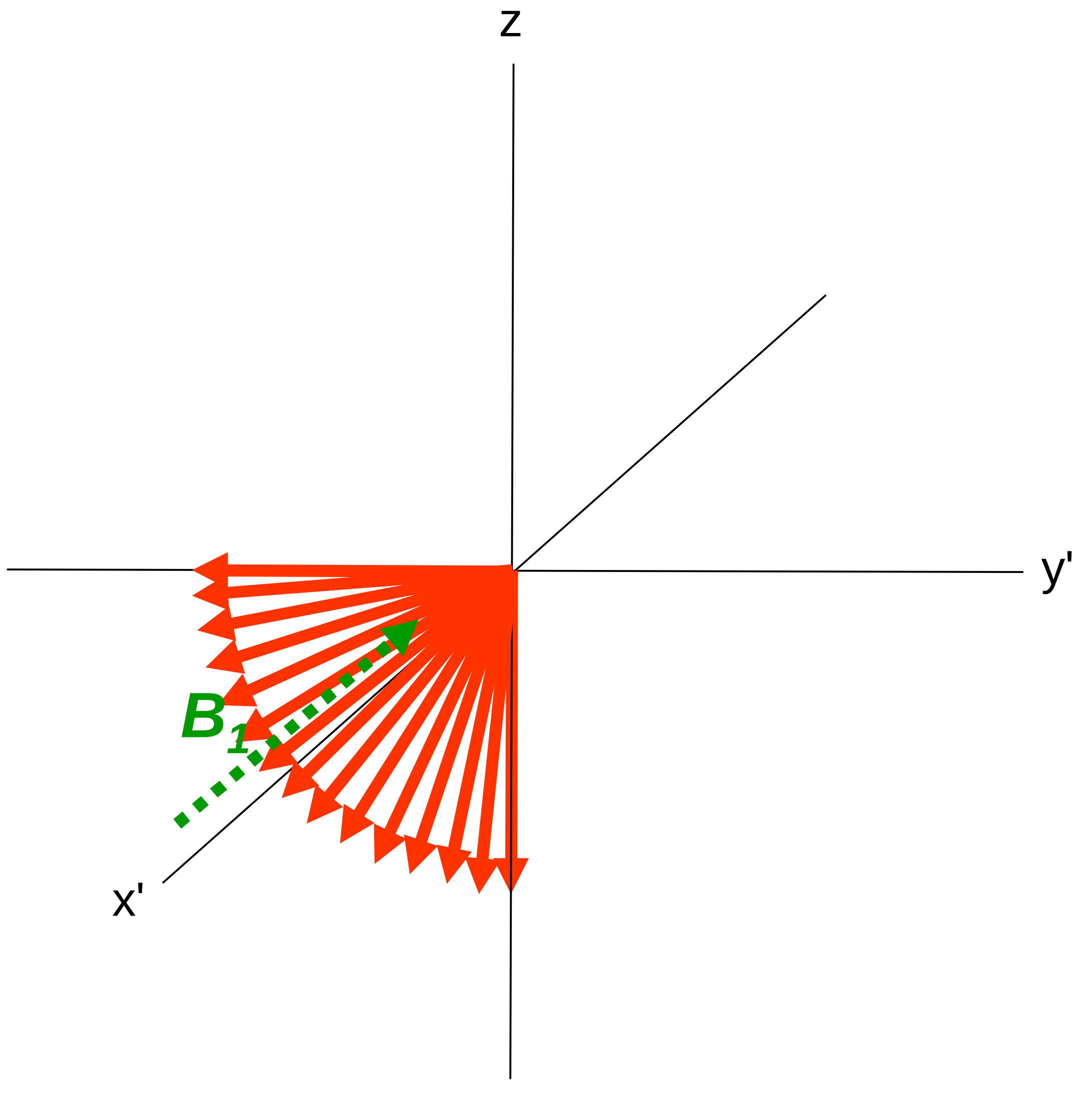
90° pulse



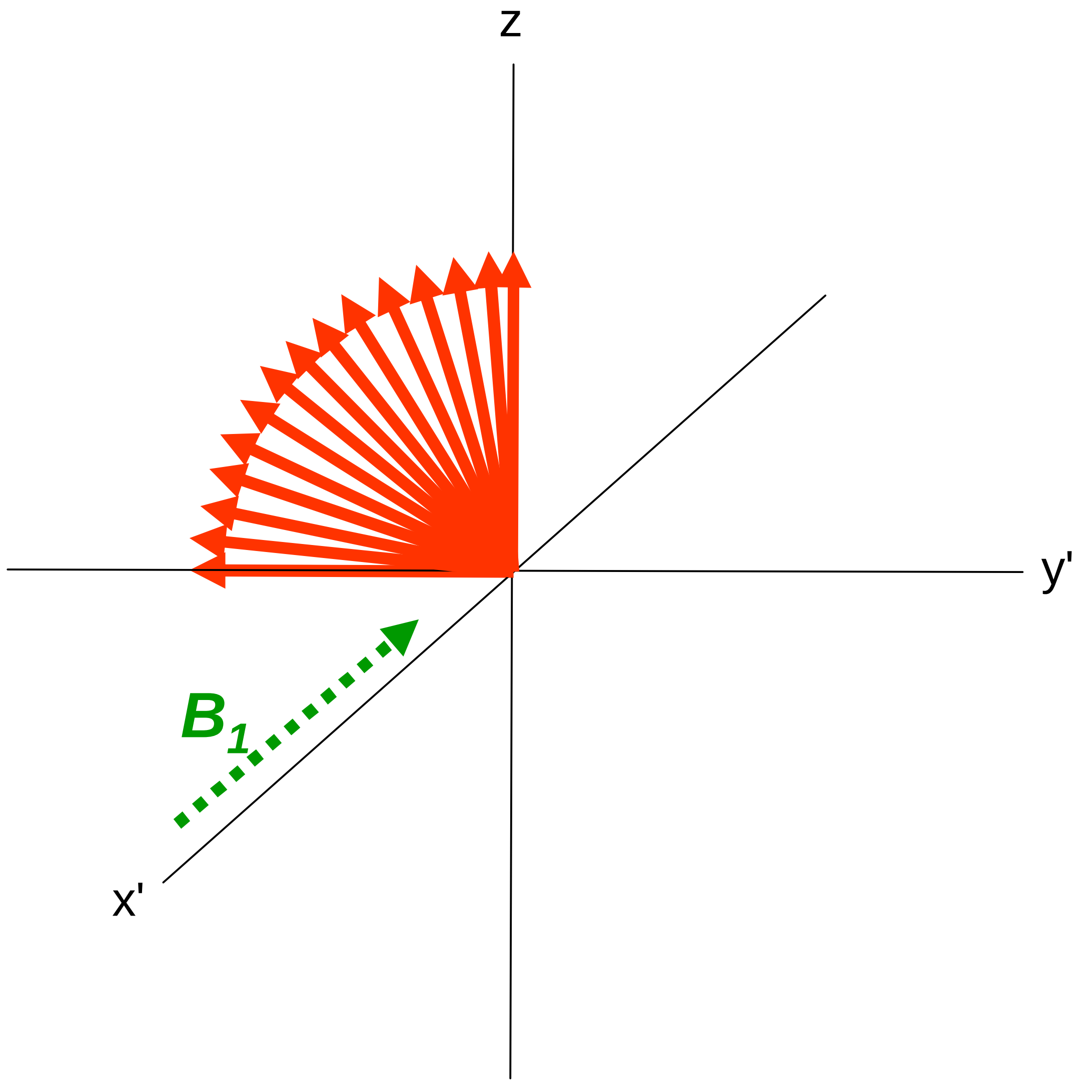
180° pulse



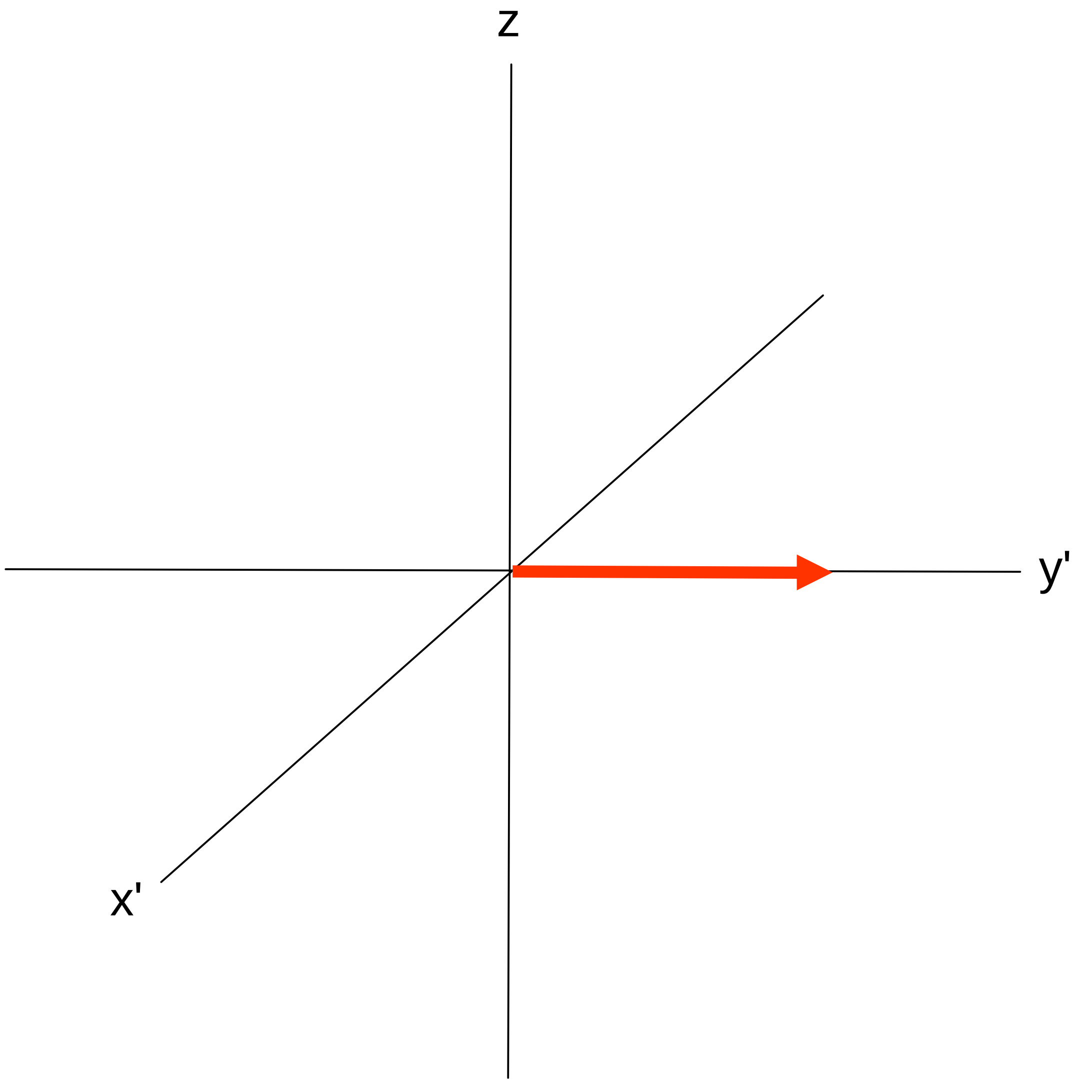
270° pulse



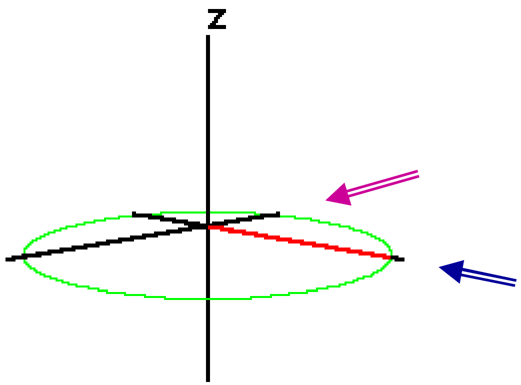
360° pulse

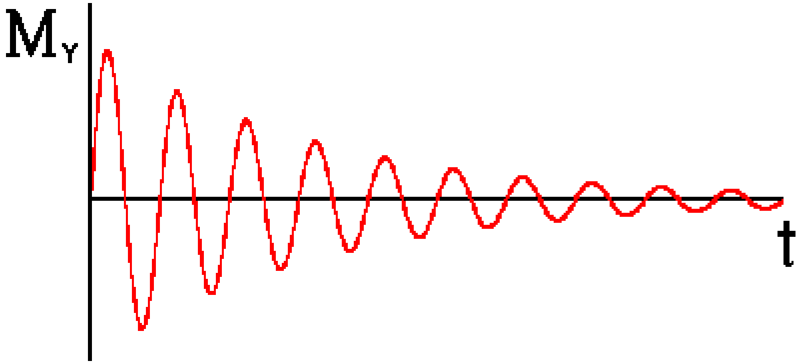


90° pulse



Origin of the NMR signal





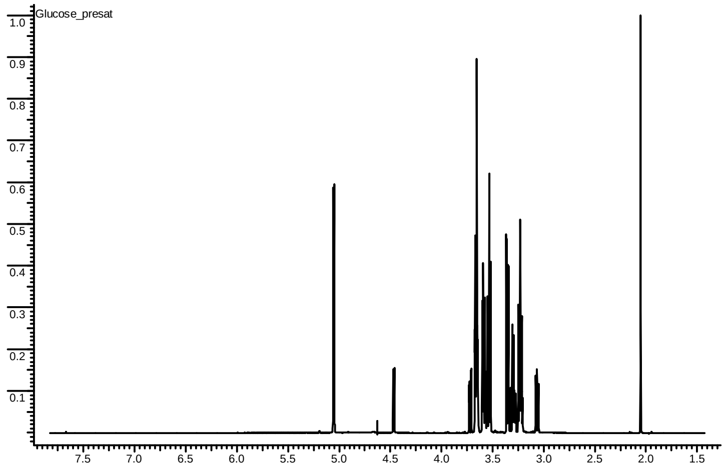
Free induction decay
FID

Quadrature detection:
- two phase sensitive detectors
- makes it possible to distinquish between frequencies that are above and below the reference frequency

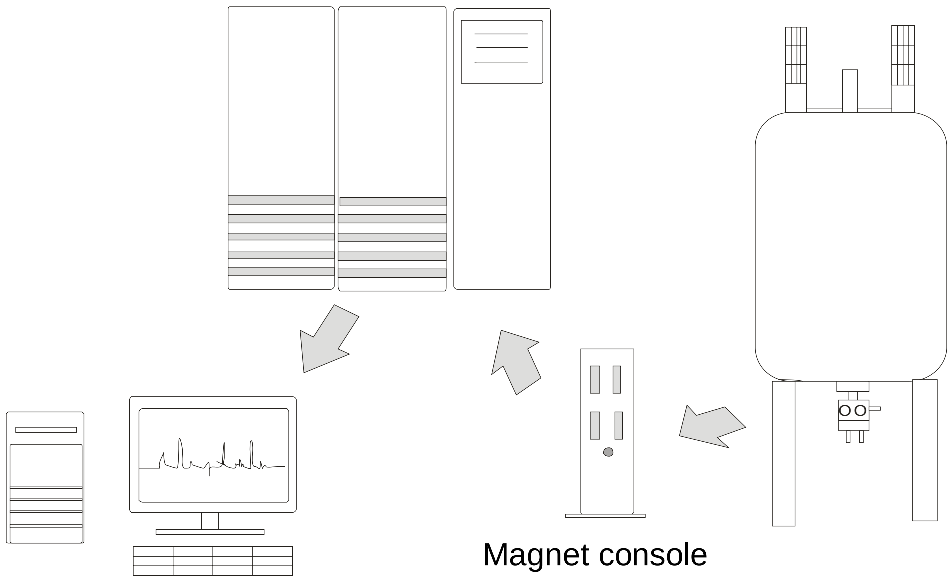
Figures from http://www.cis.rit.edu/htbooks/nmr/inside.htm

Fourier transformation


Glucose_presat
0.5
0
-0.5
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0


Glucose_presat
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Console
- acquisition computer
- radio frequency generators
- radio transmitters and receivers
- power supplies
- etc.

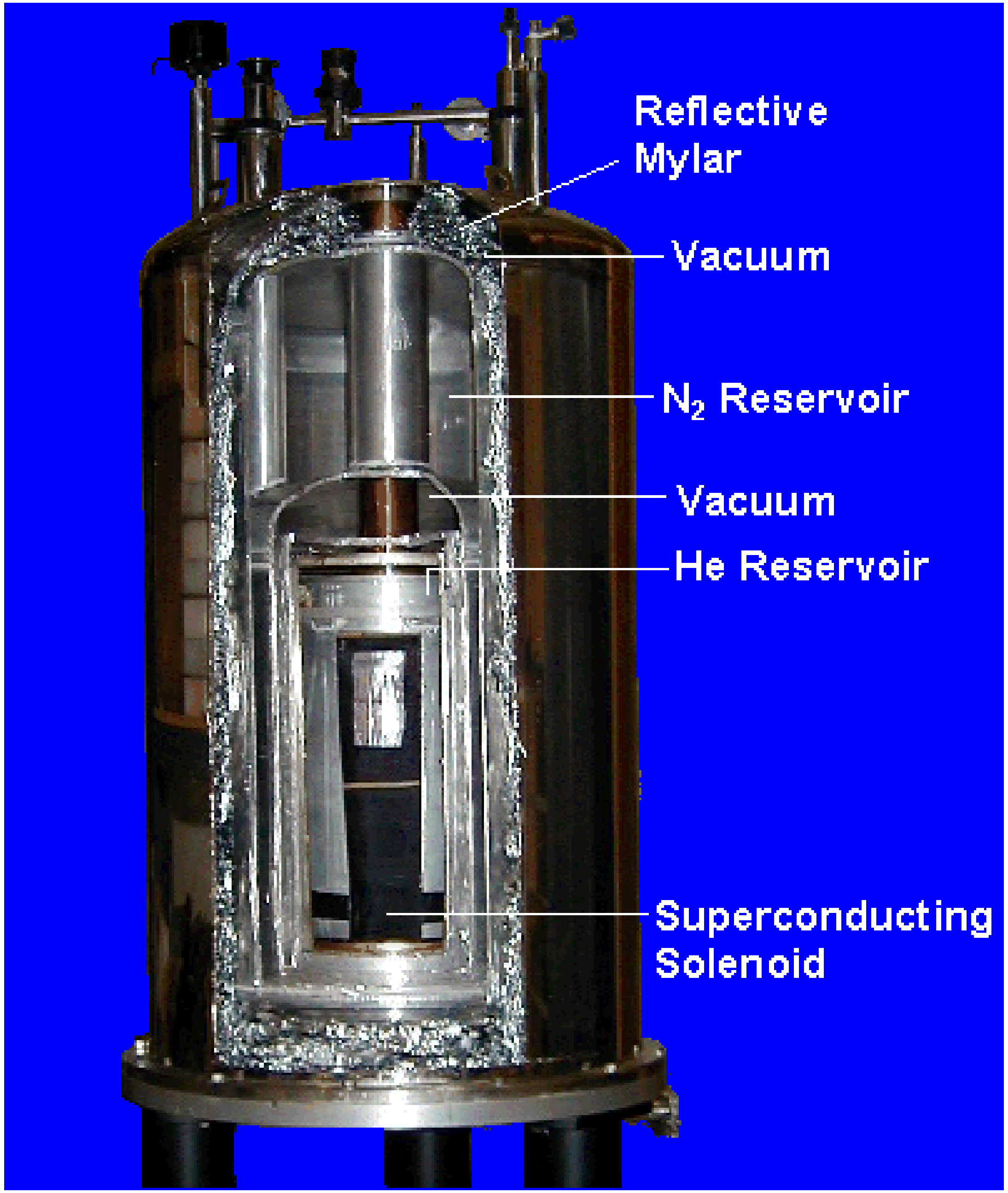


Superconducting magnet

Probe
- different probes for different nuclei
- cold probes

Magnet console

Workstation
- user interface
- data storage
- data processing



Reflective Mylar
Vacuum
N_2 Reservoir
Vacuum
He Reservoir
Superconducting Solenoid

Sample preparation

- (pre-deuterate)
- dissolve in deuterated solvent (may need time)
  - D_2O is very hygroscopic!
- add chemical shift reference
  - TMS, TSP, acetone, phosphoric acid, ...
  - typically few µl
- if any particles (or dust...), filtter
  - moving particles problem, particles sticking to the walls or precipitate in bottom not necessarily
- if desired, remove dissolved oxygen
- check that the NMR tube is clean and unbroken
- transfer to NMR tube
- wipe the tube from outside

Setting up an NMR experiment

- Prepare the sample
- Set the sample temperature
- Insert the sample to the magnet
- WAIT for the sample temperature to settle (might take up to 30 min)
- Tune the probe
- Lock the spectrometer, adjust lock power, gain and phase
- Set the parameters for shimming and for the real experiment (e.g. load a previous parameter set)
  - Acquisition time (number of points)
  - Pulse width (may need to be calibrated, see below)
  - Receiver gain
  - Number of transients (scans, repetitions,…)
  - Number of steady state (dummy) pulses
- Shim the sample, i.e. adjust the field homogenity
- Calibrate the pulses, if desired
- Start the experiment
- Wait and see the result of the first transients

Calibration of the 90º pulse width

- Try different pulse widths to find out where the signal changes sign = 180º pulse
- 90º = 180
- "array" command in Varian software (Vnmr)


HENGENVAARA
Erittäin voimakkaita magneettikenttiä
Metalliesineet voivat yllättäen lennähtää kohti magneettia erittäin suurella voimalla. Kellot, magneettikortit ja elektroniset laitteet saattavat tuhoutua.
PÄÄSY KIELLETTY
LIVSFARA
Mycket starka magnetfält.
Magneten drar oförutsätt till sig metalföremål med mycket stor kraft. Klockor magnetkort och elektroniska apparater kan förstöras.
TILLTRÄDE FÖRBJUDET
DANGER
STRONG MAGNETIC AND RADIO-FREQUENCY FIELDS ARE PRESENT
Pacemaker and Metallic Implant Hazard
Strong magnetic and radio-frequency fields are present that could cause serious injury or death to persons with implanted or attached medical devices, such as pacemakers and prosthetic parts.
Such persons must not go closer to the magnet than the 5-GAUSS WARNING signs until safety at a closer distance is identified by a physician or medical device manufacturer.
Magnetic Media and ATM/Credit Cards
Strong magnetic fields are present that could erase magnetic media such as floppies and tapes, disable ATM and credit cards, and damage some watches.
Do not take such objects closer to the magnet than the 5-GAUSS WARNING signs.
Tools and Equipment
Strong magnetic fields are present that could make some magnetic items suddenly fly towards the magnet body, which could cause personal injury or serious damage.
Do not take tools, equipment, or personal items containing steel, iron, or other magnetic materials closer to the magnet than the 10-GAUSS WARNING signs.
varian

Very strong magnetic fields!!!

- no entrance for persons with pace makers or metallic implants
- no metallic objects/tools
- no watches
- no banking/credit cards
- no electronic devises (e.g. mobile phones, calculators, computers...)

You are <u>NOT ALLOWED</u> to open the door to the NMR lab to <u>ANYBODY</u> you don't know!!