Solution State NMR

Autumn School FMP 2010

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AG Solution NMR

General aspects of NMR-spectroscopy

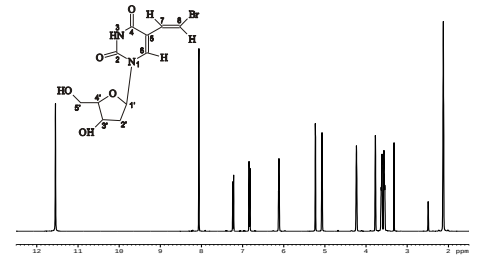
General aspects of NMR spectroscopy

Nuclear Magnetic Resonance

NMR-spectroscopy observes the resonance interaction of atomic nuclei with electromagnetic waves. The effect is only detectable in a strong magnetic field. Every atomic nucleus is observed separately and in addition interactions between nuclei can be visualized. NMR therefore corresponds well to the chemists view of a molecule as atoms connected by bonds.

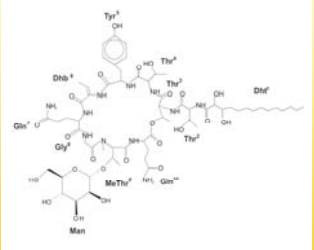
General aspects of NMR spectroscopy

Analytical method accompanying synthetic work



General aspects of NMR spectroscopy

Structure elucidation of natural compounds

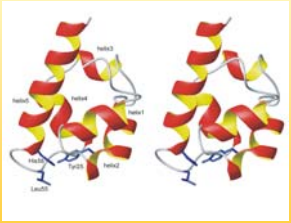


NMR is very powerful in the determination of the constitution of natural products

General aspects of NMR spectroscopy

Determination of the three-dimensional structure of proteins

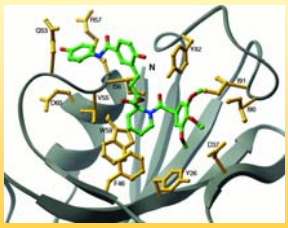
NMR can help to determine the 3D structure of proteins at atomic resolution, in solution as well as in the solid state



General aspects of NMR spectroscopy

Determination of molecular interactions

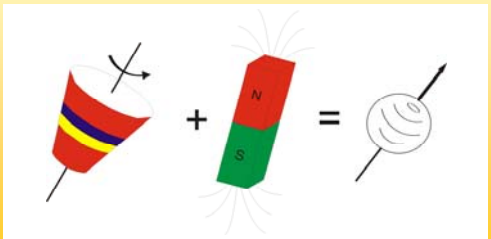
NMR can be used to detect the interaction between proteins and ligands



Basic principles of NMR-spectroscopy

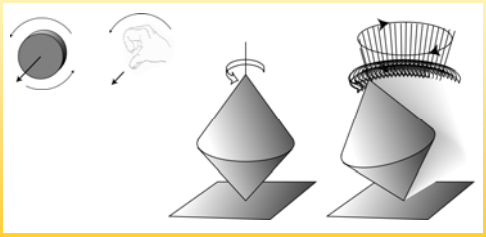
Basic principles of NMR-spectroscopy

Basis of the effect of nuclear magnetic resonance is the nuclear spin, that can be imagined as a mixture of gyroscope and magnetic needle



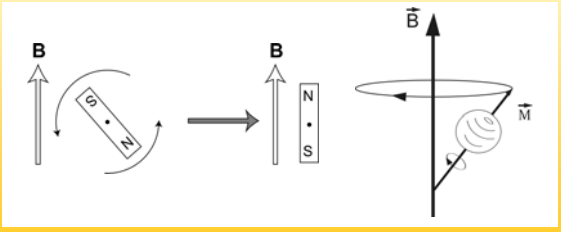
Basic principles of NMR-spectroscopy

A gyroscope has an angular momentum whose axis is stable in three-dimensional space



Basic principles of NMR-spectroscopy

An alignment of the "magnetic needle" with an external magnetic field is prevented by the properties of a gyroscope, a precession begins



Basic principles of NMR-spectroscopy

The resonance frequency of the spins is determined by the magnetic field, as is the sensitivity and the resolution of the spectra

B_0 [Tesla]	ν_0 [MHz]
1.4	60
5.9	250
9.4	400
14.1	600
21.2	900

Basic principles of NMR-spectroscopy

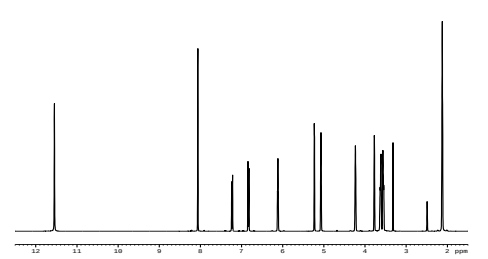
Magnetic properties of relevant nuclei

nucleus		natural abundance	gyrom. ratio
^{1}H	1/2	99.98 %	26.75
^{12}C	0	98.89 %	0
^{13}C	1/2	1.11 %	6.73
^{14}N	1	99.63 %	1.93
^{15}N	1/2	0.37 %	-2.71
^{19}F	1/2	100 %	25.18
^{31}P	1/2	100%	10.84
^{113}Cd	1/2	12.26 %	-5.96

Parameters in NMR-spectroscopy

Parameters in NMR-spectroscopy

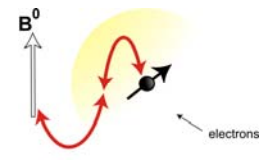
Each atom in the molecule gives rise to a resonance line



Parameters in NMR-spectroscopy

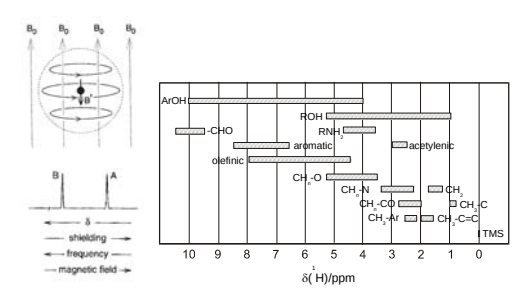
Chemical shift

Electrons around the nucleus shield it from the external magnetic field, the more electrons the weaker the field



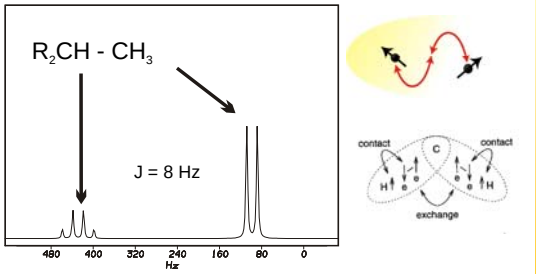
Parameters in NMR-spectroscopy

The chemical shift depends on the chemical environment



Parameters in NMR-spectroscopy

Scalar coupling splits the signals according to the number of neighboring nuclei



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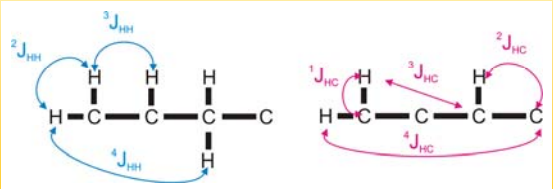
Parameters in NMR-spectroscopy

Coupling constants can either be homonuclear (between like nuclei) or heteronuclear (between different nuclei) and can either be direct (one-bond) or long-range (multiple bonds)



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Parameters in NMR-spectroscopy

Direct couplings are usually one order of magnitude larger than the so-called long-range couplings

$^1J_{HH}$ = 276 Hz	$^1J_{HC}$ = 125 .. 200 Hz	$^1J_{HN}$ = 60 .. 100 Hz
$^2J_{HH}$ = 0 .. 30 Hz $^3J_{HH}$ = 0 .. 20 Hz $^4J_{HH}$ = 0 .. 3 Hz	$^2J_{HC}$ = 0 .. 20 Hz $^3J_{HC}$ = 0 .. 15 Hz $^4J_{HC}$ = 0 .. 2 Hz	$^2J_{HN}$ = 0 .. 15 Hz $^3J_{HN}$ = 0 .. 8 Hz $^4J_{HN}$ = 0 .. 1 Hz

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Parameters in NMR-spectroscopy

Dipolar coupling

The nuclei interact directly through space via a dipol-dipol interaction

In solution NMR this interaction is averaged to zero due to the fast isotropic movement of the molecules but it is still a source of relaxation

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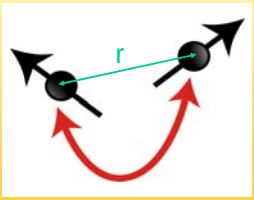
Parameters in NMR-spectroscopy

One aspect of relaxation is the NOE-Effect, that depends on the distance between two nuclei

$I_{NOE} \sim 1/r^6$

Since the intensity drops quickly with increasing distance the effect can only be observed up to 500 pm



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Multidimensional NMR-spectroscopy

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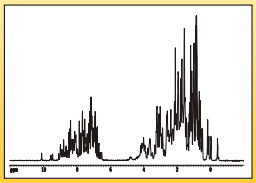
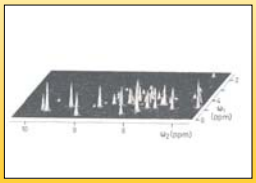
Multidimensional NMR-spectroscopy

1D-NMR:
2 axis
intensity vs. frequency

2D-NMR:
3 axis
intensity vs. frequency (1)
vs. frequency (2)





Multidimensional NMR-spectroscopy

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The two major advantages of multidimensional NMR are:
Improved resolution: Signals are spread over a surface (2D) or in a three-dimensional space (3D, 4D)
Magnetization transfer: Signals result from the interaction between nuclei. That can be interactions through bond (via J-coupling) or through space (via NOE).

Taken together this eases the interpretation and the assignment of the spectra considerably

Multidimensional NMR-spectroscopy

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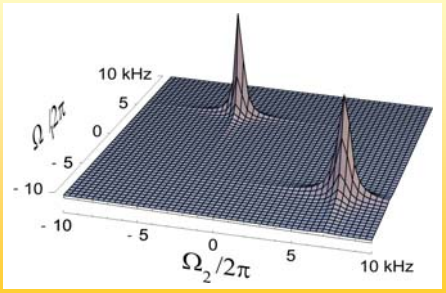
2D spectra have two frequency axes



Multidimensional NMR-spectroscopy

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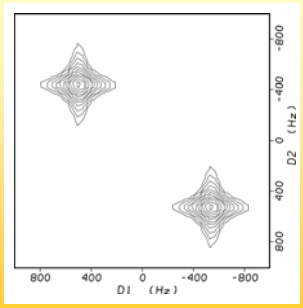

For analysis of the data the spectra are converted in contour plots

Multidimensional NMR-spectroscopy

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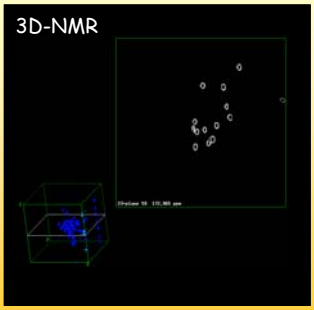

Multidimensional NMR-spectroscopy

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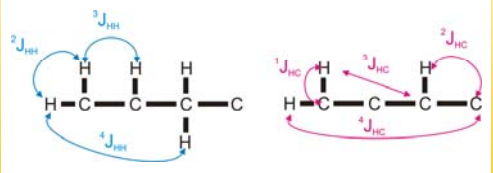
The most important part of a 2D experiment is the transfer of magnetization from one nucleus to another taking place between the recording of the two frequencies, in most cases scalar coupling is used, sometimes also the NOE-effect



Multidimensional NMR-spectroscopy

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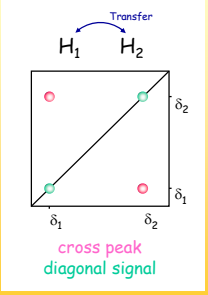
homonuclear spectra

Transfer of magnetization takes place between like nuclei. Both axis exhibit the chemical shift of the same type of nucleus. If a transfer has taken place, the signal has different frequencies in the two dimensions:
cross peak
If no transfer has taken place, the shifts are the same in both dimensions:
diagonal signal

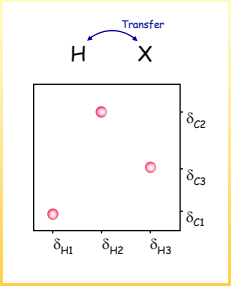


cross peak
diagonal signal

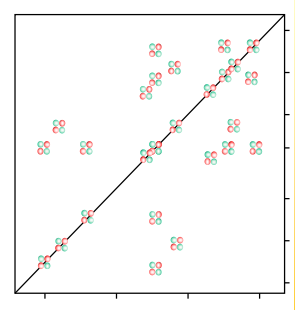
Multidimensional NMR-spectroscopy

heteronuclear spectra

Transfer of magnetization takes place between nuclei of different types. The two axis show the chemical shift of the respective type of nucleus. If a transfer has taken place, a signal appears at the intersection of the two frequencies, without a transfer there is no signal.



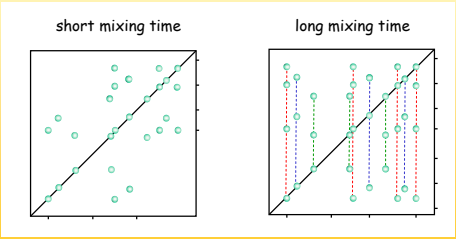
Multidimensional NMR-spectroscopy



A DQF-COSY is a homonuclear experiment and accomplishes a transfer via scalar coupling, usually via not more then three bonds

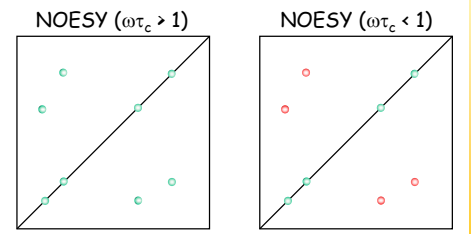
Multidimensional NMR-spectroscopy

A TOCSY also accomplishes a transfer via scalar coupling through whole spin systems. But if there is no coupling there is no transfer (e.g. more than three bonds between protons)



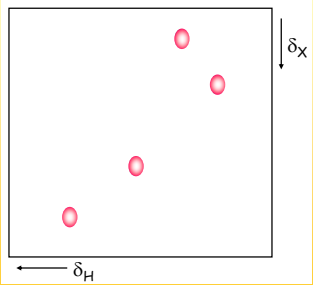
Multidimensional NMR-spectroscopy

Besides the transfer via scalar coupling there is the possibility to transfer via the NOE-effect, i.e. via a distance dependent interaction through space



Multidimensional NMR-spectroscopy

HMQC = Heteronuclear MultipleQuantum Correlation

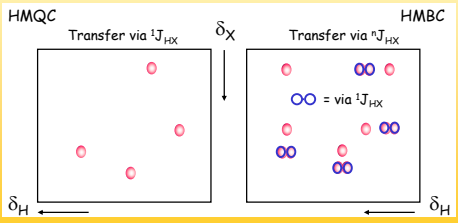


A signal indicates a direct bond between the proton and the heteronucleus

Multidimensional NMR-spectroscopy

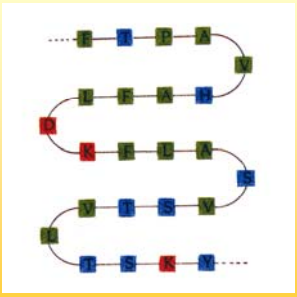
HMBC = Heteronuclear MultipleBond Correlation

A signal indicates a correlation via two, three or four bonds between the proton and the heteronucleus.



NMR-spectroscopy of proteins

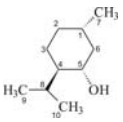
NMR-spectroscopy of proteins



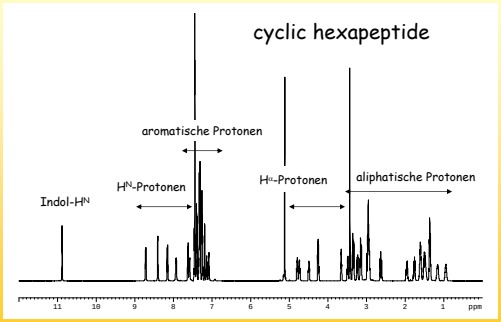
The major problem of protein NMR results from the fact that proteins are polymers, i.e. the repetition of almost identical subunits

NMR-spectroscopy of proteins

(-)-Menthol

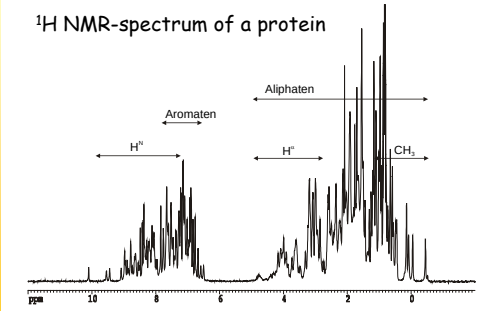


NMR-spectroscopy of proteins



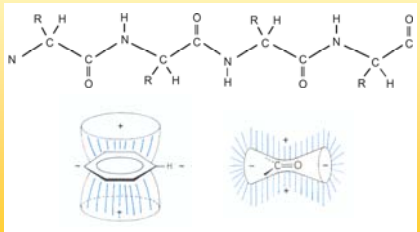
NMR-spectroscopy of proteins

^{1}H NMR-spectrum of a protein



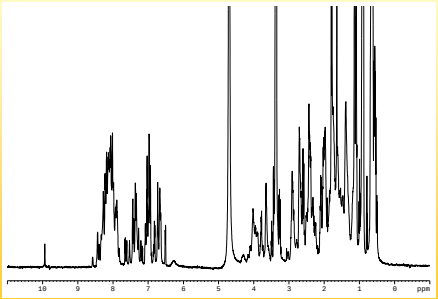
NMR-spectroscopy of proteins

Differences in chemical shifts can be produced by structure and the accompanying anisotropy effect

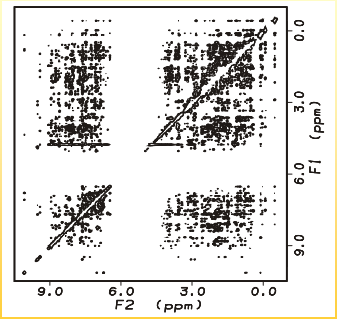


NMR-spectroscopy of proteins

^{1}H NMR-spectrum of an unfolded protein

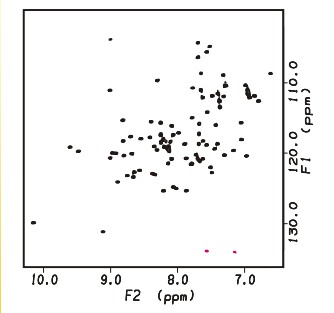


NMR-spectroscopy of proteins



Obviously multidimesional spectra are mandatory for proteins, either homonuclear (e.g. NOESY)....

NMR-spectroscopy of proteins



....or heteronuclear (e.g. HSQC)

That's it

www.fmp-berlin.de/schmieder/teaching/autumn_school_2010.htm