

Basic principles of multidimensional NMR in solution

21.06.2006

Peter Schmieder
AG Solution NMR

The program

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- General aspects
- Basic principles
- Parameters in NMR spectroscopy
- Multidimensional NMR-spectroscopy
- Protein structures
- NMR-spectra of proteins
- Sequence specific assignment
- Protein structure determination
- Ligand-screening



Basic principles of multidimensional NMR spectroscopy

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General aspects of NMR-spectroscopy

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General aspects of NMR spectroscopy

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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.



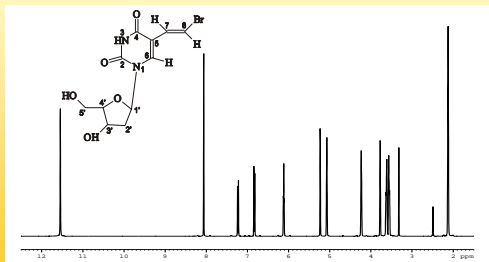
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General aspects of NMR spectroscopy

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Analytical method accompanying synthetic work



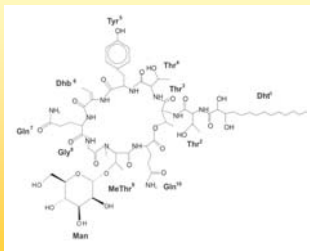
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Structure elucidation of natural compounds



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



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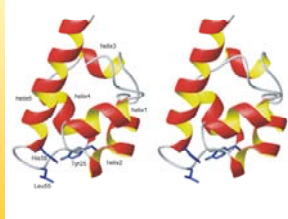
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Determination of the three-dimensional structure of proteins

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



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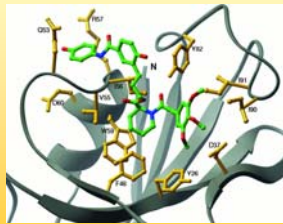
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Determination of molecular interactions

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



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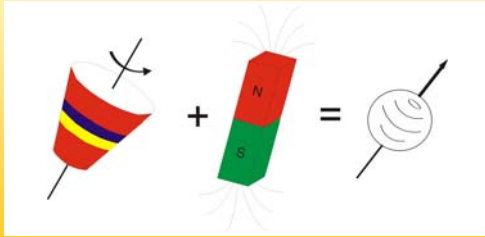
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Basis of the effect of nuclear magnetic resonance is the nuclear spin, that can be imagined as a mixture of gyroscope and magnetic needle



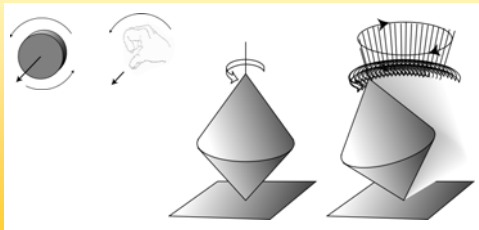
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A gyroscope has an angular momentum whose axis is stable in three-dimensional space



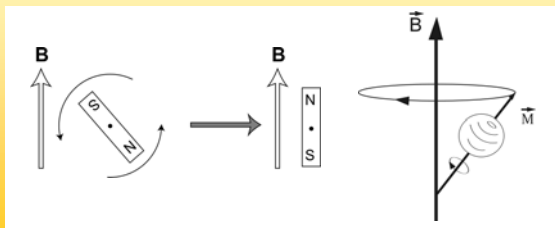
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An alignment of the "magnetic needle" with an external magnetic field is prevented by the properties of a gyroscope, a precession begins



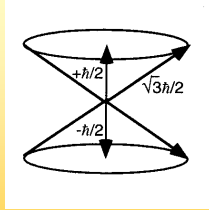
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In case of the nuclear spin we have a „quantum mechanic gyroscope“ and not all orientations of the angular momentum are allowed, in case of high resolution NMR there are only two, named α and β state.



Symbolic representation of the two states of the spin



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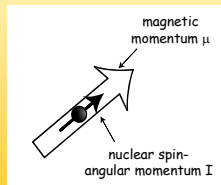
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Connected to the nuclear angular momentum via the gyromagnetic ratio is a magnetic momentum, that is detectable by an NMR spectrometer

$$\mu = \gamma I$$

The bigger the gyromagnetic ratio the bigger the magnetic moment and hence the detectable effect



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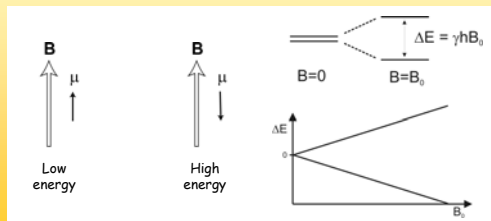
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The energy of the two possible states is not equal:

$$E = -\mu B$$

$$\Delta E = \hbar \gamma B_0$$



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The resonance frequency of the spins can be derived from the energy difference between the two states α and β

$$\Delta E = h \nu_0$$
$$\nu_0 = \gamma B_0 / 2\pi$$
$$\omega_0 = 2\pi \nu_0 = \gamma B_0$$

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



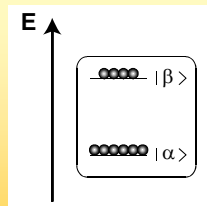
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The population of the two energy levels can also be derived from the energy difference, it will be a Boltzmann distribution



$$N_\beta / N_\alpha = \exp(-\Delta E / kT) = \exp(-\gamma h B_0 / 2\pi kT)$$



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At 600 MHz, ambient temperature and assuming hydrogen as the nucleus the difference is quite small

$$N_\beta / N_\alpha = 0.999904$$

The small difference is the reason for the low sensitivity of NMR spectroscopy and also for desire to have bigger magnets (B_0 !)



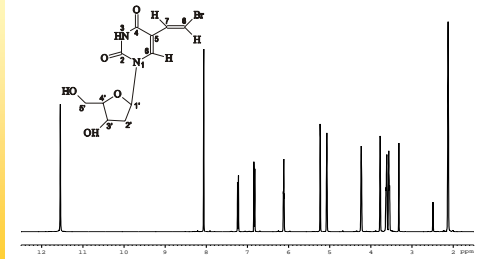
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Parameters in NMR-spectroscopy

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Each atom in the molecule gives rise to a resonance line



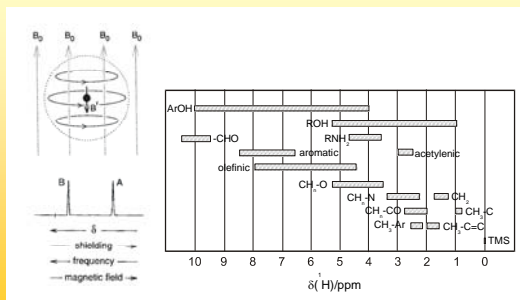
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Parameters in NMR-spectroscopy

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The chemical shift depends on the chemical environment



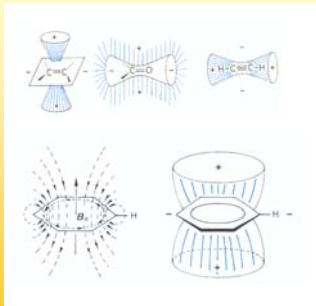
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An important factor influencing the chemical shift are anisotropy effects, that are created by small additional fields



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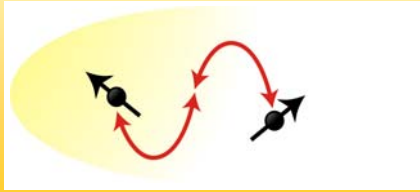
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Parameters in NMR-spectroscopy

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Scalar or J-coupling

Electrons in the bonds between the nuclei mediate an interaction, the scalar coupling



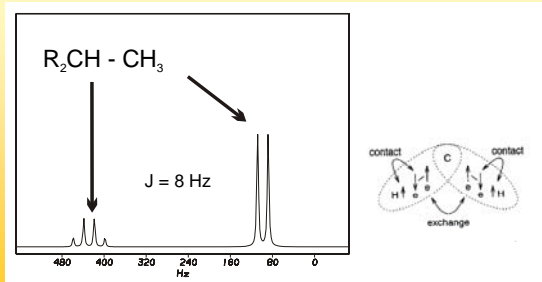
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Scalar coupling splits the signals according to the number of neighboring nuclei



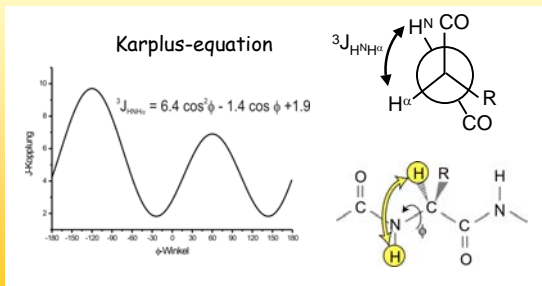
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Parameters in NMR-spectroscopy

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Scalar coupling contains structural information



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Parameters in NMR-spectroscopy

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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

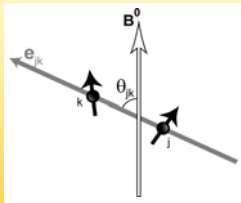


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The size of the dipolar coupling is determined by the angle between the magnetic field and the connecting line between the two nuclei

$$D \sim (3 \cos^2 \theta_{jk} - 1)$$

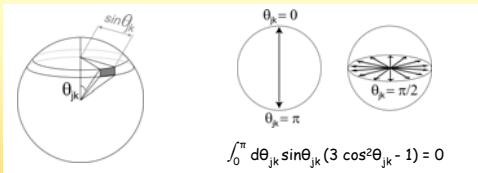


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$$\int_0^\pi d\theta_{jk} \sin\theta_{jk} (3 \cos^2\theta_{jk} - 1) = 0$$

While it is averaged to zero in solution NMR, it is present in solid state NMR and is of great importance there. In solution it is a major source of relaxation.



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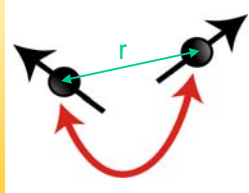
Parameters in NMR-spectroscopy

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

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

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



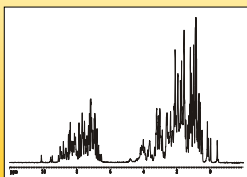
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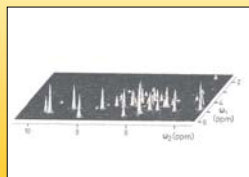
Multidimensional NMR-spectroscopy

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1D-NMR:
2 axis
intensity vs. frequency



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



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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



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Multidimensional NMR-spectroscopy

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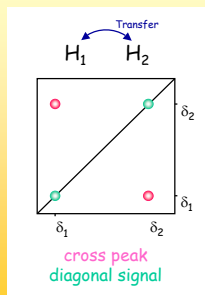
homonuclear spectra

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



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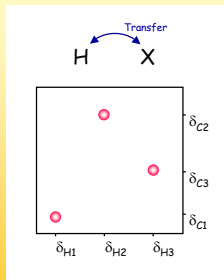
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Multidimensional NMR-spectroscopy

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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.



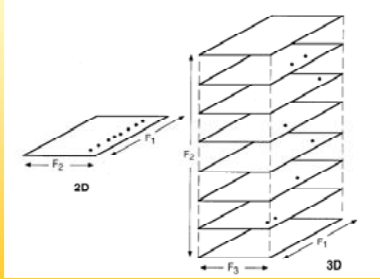
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3D-NMR



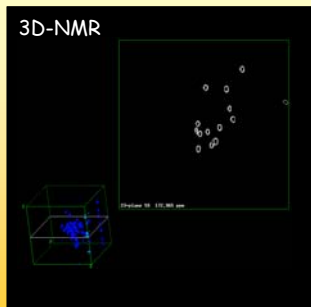
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3D-NMR



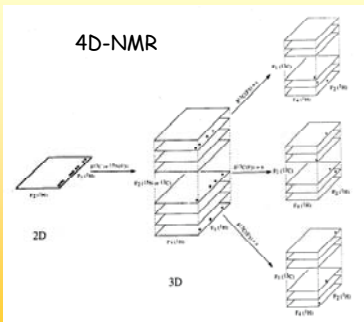
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4D-NMR



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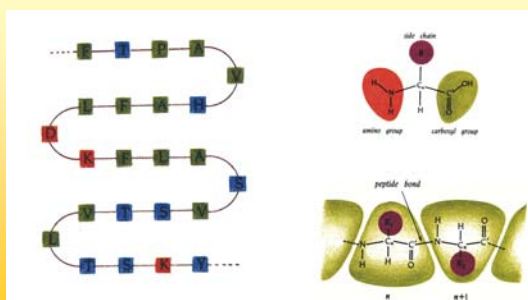
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Protein structures



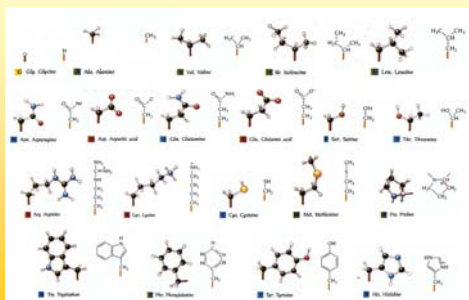
Protein structures

Primary structure



Protein structures

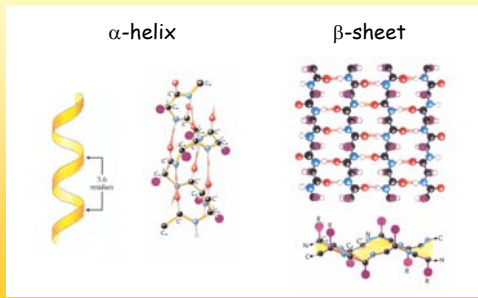
20 proteinogenic amino acids



Protein structures

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secondary structure



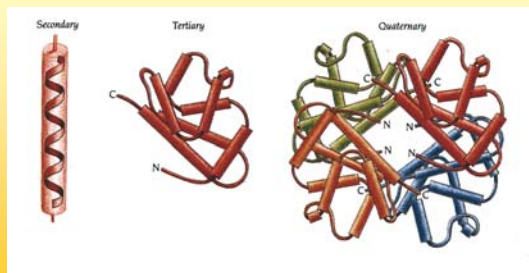
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Protein structures

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Levels of structural organization



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NMR-spectroscopy of proteins

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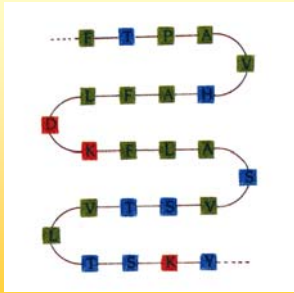


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NMR-spectroscopy of proteins

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

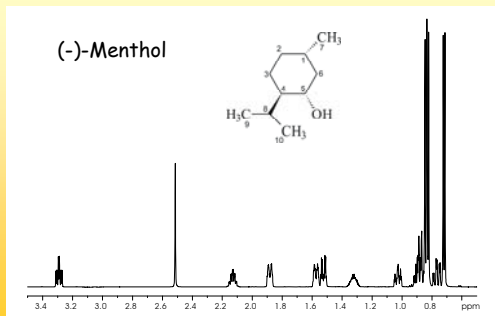


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NMR-spectroscopy of proteins

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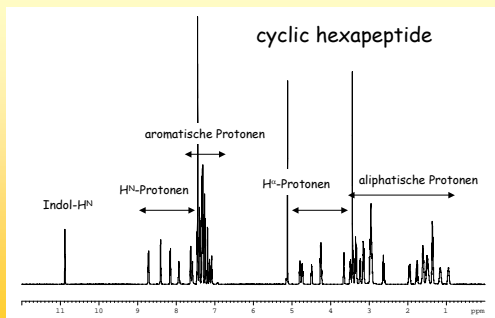


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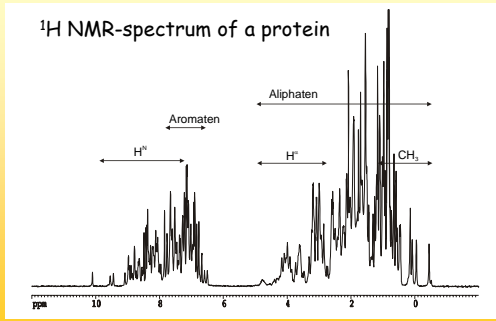


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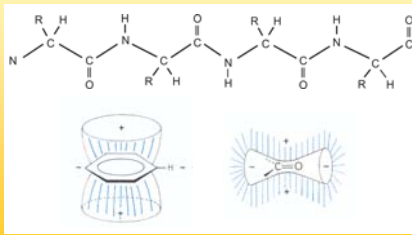
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Differences in chemical shifts can be produced by structure and the accompanying anisotropy effect



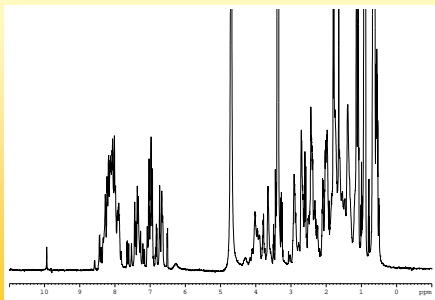
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NMR-spectroscopy of proteins

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¹H NMR-spectrum of an unfolded protein

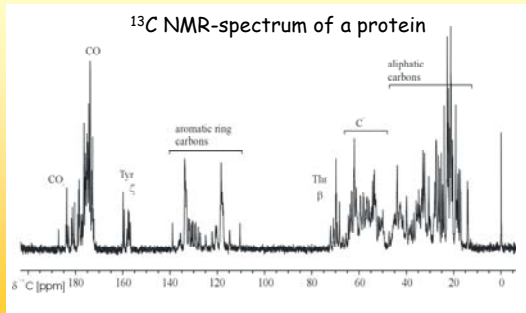


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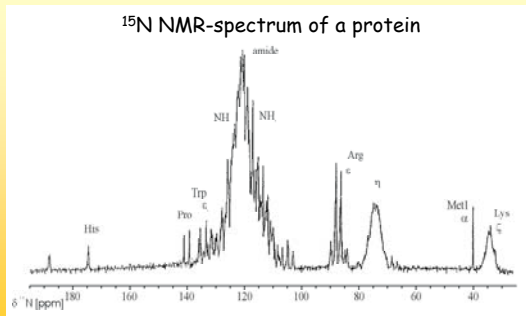


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Sequence specific assignment

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Sequence specific assignment

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The solution of the assignment problem is the **sequence-specific assignment**

Two strategies exist:

In case of small proteins or peptides where usually only unlabeled material is available the strategy is based on homonuclear spectra (**COSY, TOCSY, NOESY**)

In case of larger proteins labeling with ^{13}C and ^{15}N is necessary and heteronuclear triple resonance experiments (**CBCA(CO)NNH, CBCANNH**) are recorded



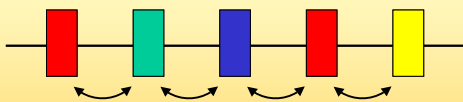
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Sequence specific assignment

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Sequence-specific assignment



1. Which amino acid type is present (which color)
2. Which amino acid is next to which (neighborhood)
3. Comparison of subsequences with that of the protein



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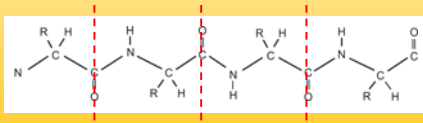
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Sequence specific assignment

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Assignment using homonuclear spectra:

Each amino acid represents a separate set of signals, a spin system, since amino acids are separated by the carbonyl carbon that does not have a proton attached. Homonuclear spectra that utilize scalar couplings (**COSY, TOCSY**) are used to establish the amino acid type



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Sequence specific assignment

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The neighborhood of the amino acids are then detected by through space interactions, i.e. in NOESY spectra.

Inter- and intra-residue signals are separated by comparison between the scalar-coupling spectra that can only show intra-residual peaks and the NOESY

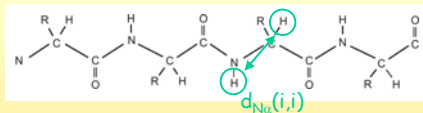


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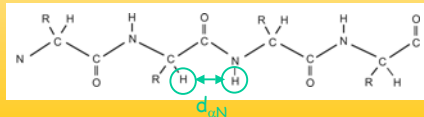
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The distance from the H^N to the H^α of the same amino acids, $d_{N\alpha}(i,i)$, is always short enough to yield an NOE. The same is true for the distance from the H^N to the H^α of the amino acid (i-1), $d_{\alpha N}$



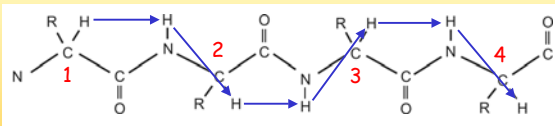
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A neighborhood of amino acids is thus established



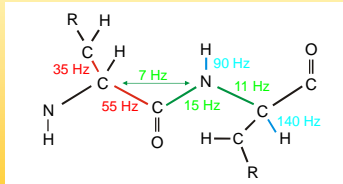
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Sequence specific assignment

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Triple resonance experiments use the couplings between ^1H , ^{13}C and ^{15}N



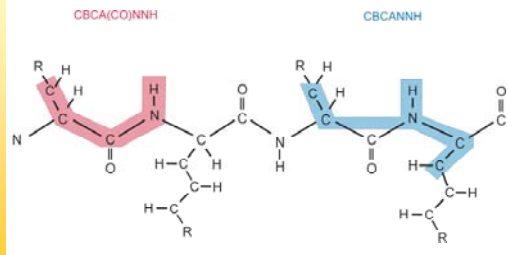
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Mainchain assignment using triple resonance experiments



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A structure determination using NMR-spectroscopy



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A protein structure determination

The diagram illustrates a cyclical process for protein structure determination. It starts with **Bioinformatics** (represented by a colorful bar chart), leading to **Protein expression** (represented by a gel electrophoresis image). This is followed by **Data acquisition** (represented by NMR spectra), **Resonance assignment** (represented by a chemical structure with peaks), **Structurally relevant information** (represented by a chemical structure with highlighted atoms), and **Structure calculation** (represented by a 3D protein model). An arrow points from the final step back to the first, completing the cycle.

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A protein structure determination

Bioinformatics (1)

This diagram shows a detailed view of protein bioinformatics. It features a central protein backbone with various domains and motifs highlighted in different colors (red, blue, yellow, green). Labels include 'Protein domain', 'Protein motif', 'Protein interaction', and 'Protein structure'. A legend at the bottom identifies symbols for different types of domains and motifs.

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A protein structure determination

Bioinformatics (2)

This slide displays a sequence alignment or database search result. It shows multiple protein sequences aligned horizontally, with specific residues highlighted in green and yellow. The sequences are labeled with accession numbers and protein names. A legend at the bottom explains the symbols used in the alignment.

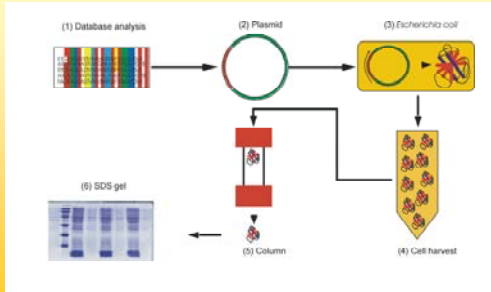
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Protein expression and purification



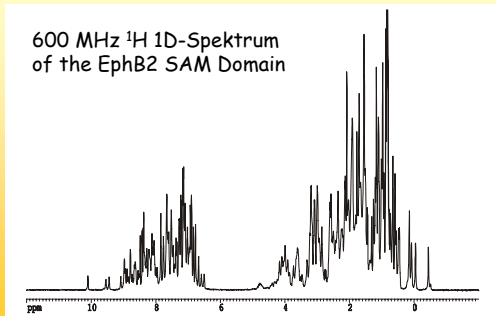
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A protein structure determination

68/93

600 MHz ¹H 1D-Spektrum
of the EphB2 SAM Domain



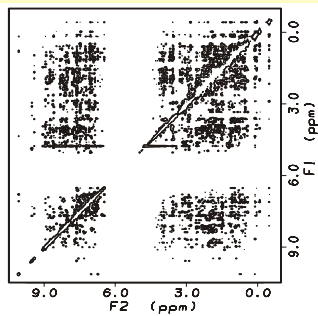
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2D-NOESY
(100 msec)
of the EphB2
SAM Domain



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With increasing size of the protein the interpretation of homonuclear spectra alone becomes increasingly difficult.

With the introduction of nitrogen and carbon labels this problem can be ameliorated because of the better resolution in the heteronuclear spectra and the option to record well resolved 3D spectra.

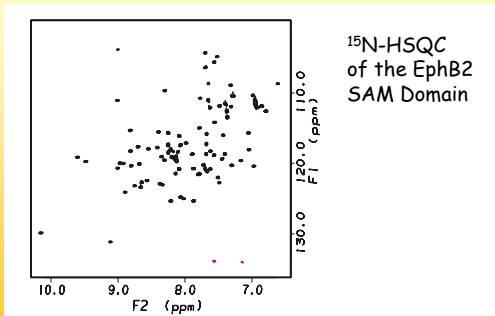


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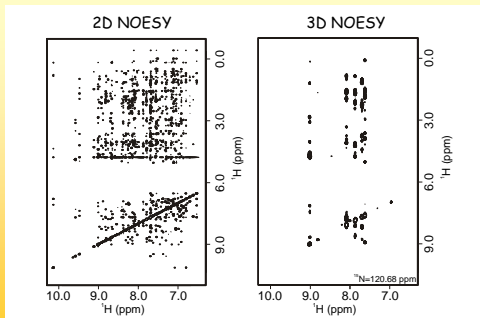


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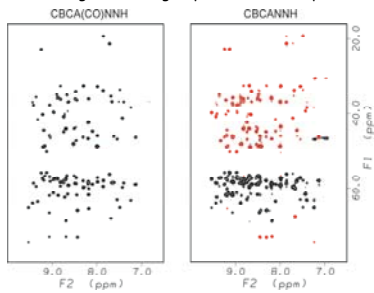
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Mainchain assignment using triple resonance experiments



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List of relevant experiments

^{15}N -HSQC, ^{13}C -HSQC

^{15}N -NOESY-HSQC, ^{13}C -NOESY-HSQC

CBCA(CO)NNH, CBCANNH

HNCO, HN(CA)CO

HNCA, HN(CO)CA

(H)C(CO)NNH, H(CCO)NNH

^{15}N -relaxation time experiments



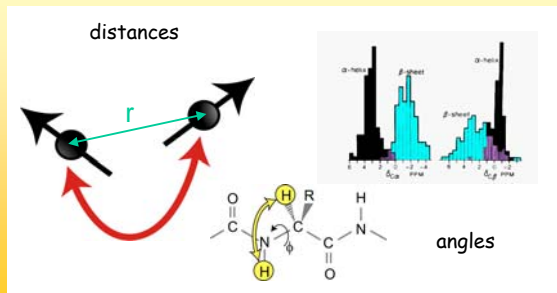
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Structurally relevant information



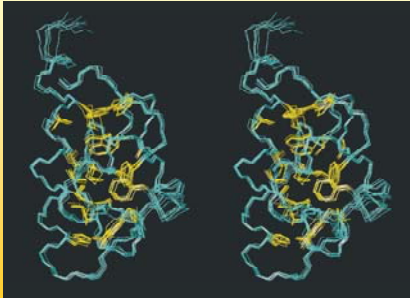
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As a result a 3D structure can be calculated



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Ligand-screening using NMR-
spectroscopy

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Ligand Screening

An increasingly important application of NMR-
spectroscopy is the screening of compound libraries to
identify new interaction partners for a given protein and
subsequently lead structures

There are two major types of approach, the „ligand-
detecting techniques“ and the „protein detecting
techniques“

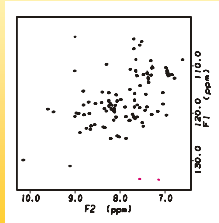
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Ligand Screening

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A technique of major importance from the class of protein-detecting techniques is called „SAR-by-NMR“

Starting point is a completely assigned two-dimensional HSQC spectra of the protein of interest

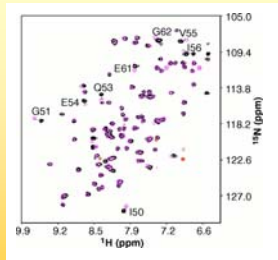


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HSQC-spectra with and without the addition of a potential ligand are compared. A shift in the spectrum with ligand relative to the one without indicates an interaction

The method can be used in a „high-throughput“ manner

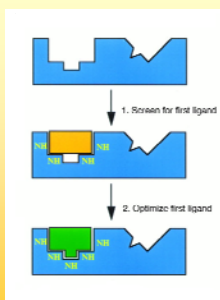


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This (hopefully) leads to the identification of a first ligand that can subsequently be optimized via synthesis and further screening

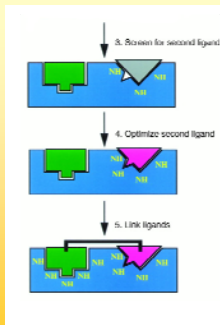


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Then a second ligand may be detected and optimized and - if possible - attached to the first to form a new, tighter binding ligand



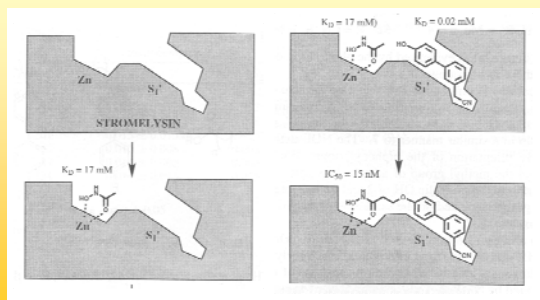
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Ligand Screening

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Application to the inhibition of stromelysin



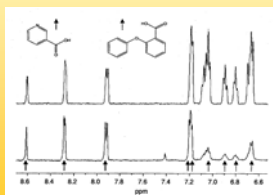
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There are numerous techniques in the class of the „ligand detecting techniques“
Two techniques of particular importance are WATERlogy and STD-NMR



The major principle is the alteration of certain properties of the ligand by the protein when bound



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Ligand Screening

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Screening can be commercially interesting....

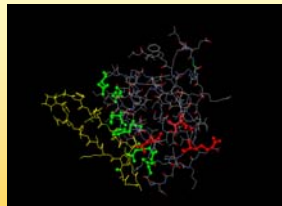
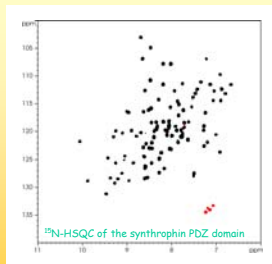


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Ligand Screening

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...but it can also be used to detect specific interactions



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Summary

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using protein-NMR-spectroscopy it is possible

to determine the structure of small to medium sized proteins

to study protein-protein or protein-ligand interactions



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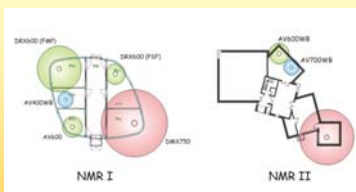
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Want to know more ?




Vorlesung L845 (TV Berlin)
Mehrdimensionale NMR-Spektroskopie
 Grundlagen und Anwendung in der Strukturauflösung
 Mittwochs 16-18 Uhr
 Hörsaal C 264
 Peter Schmieder
 Vorlesungsbeginn 26.04.04
 Info/Anmeldung unter
 www.fmp-berlin.de/schmieder/teaching/vorlesung_L845.htm



The NMR facility



That's it

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