Basic principles of multidimensional NMR in solution

19.03.2008

Peter Schmieder

AG Solution NMR

The program

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

General aspects of NMR-spectroscopy

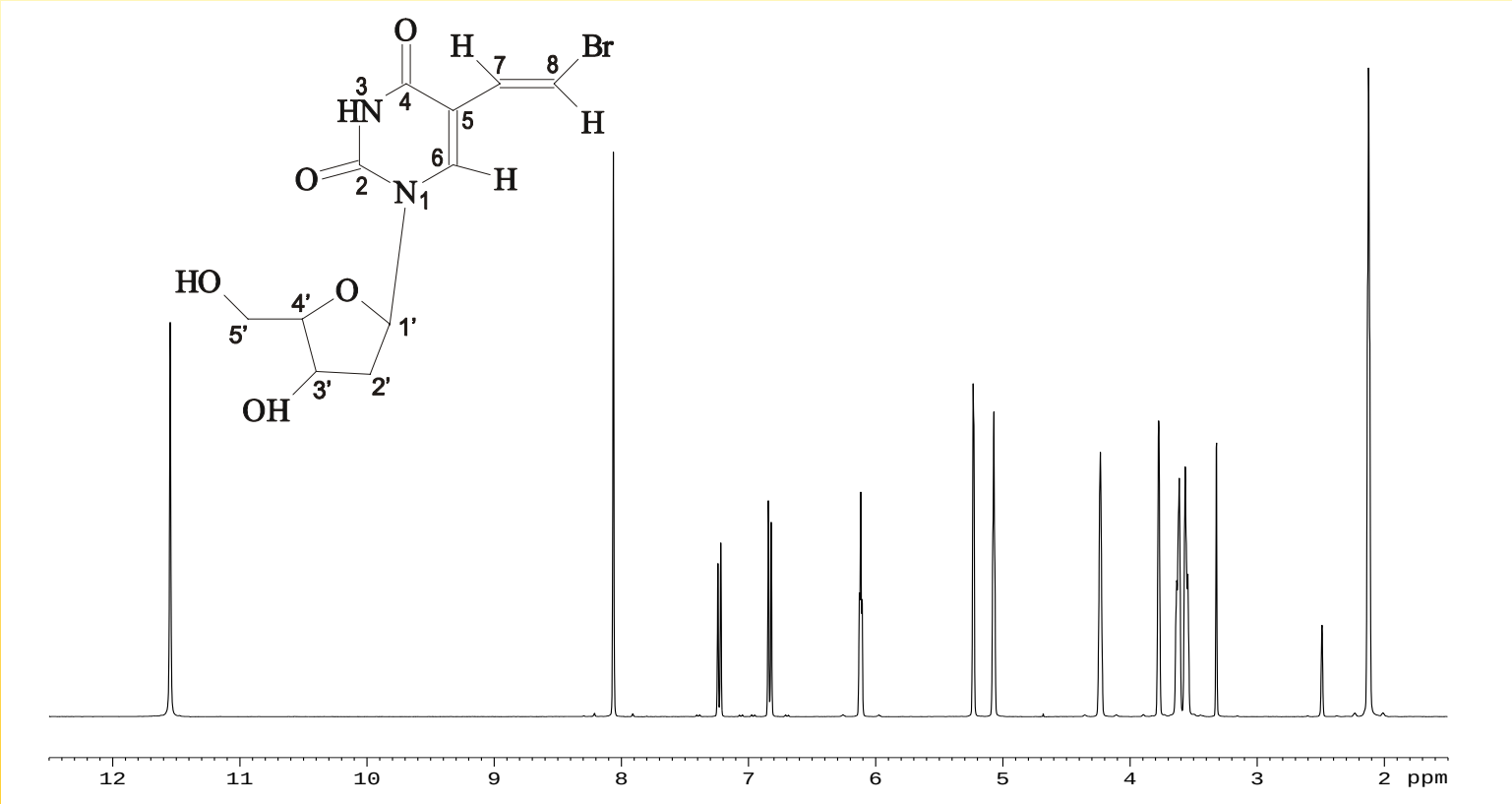
General aspects of NMR spectroscopy

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.

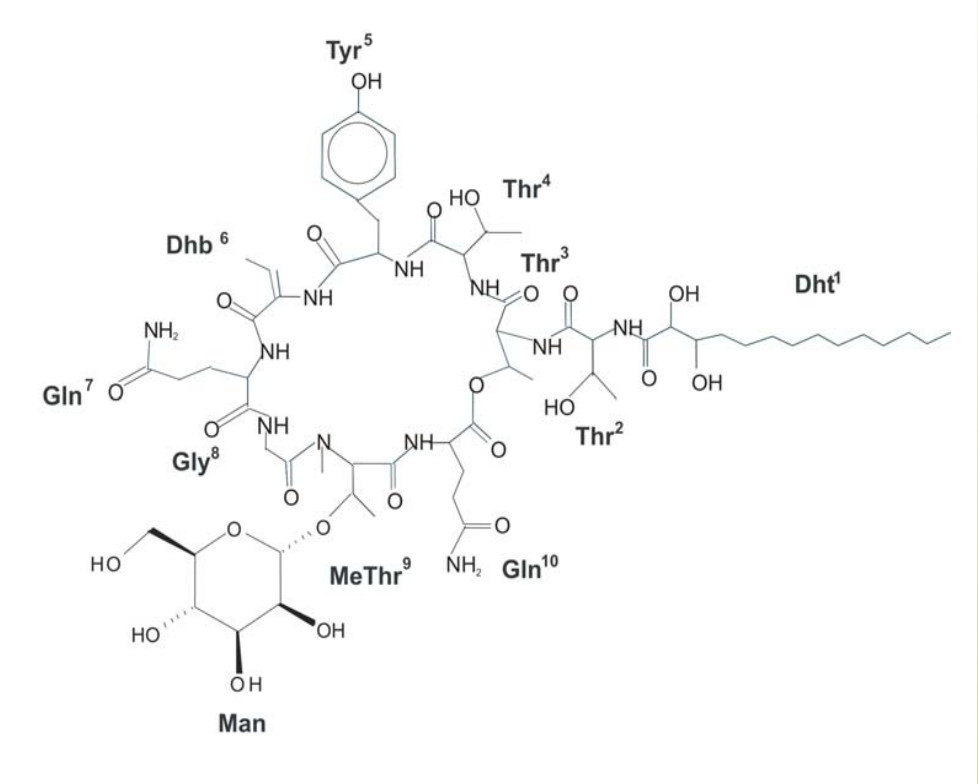
General aspects of NMR spectroscopy

Analytical method accompanying synthetic work



General aspects of NMR spectroscopy

Structure elucidation of natural compounds

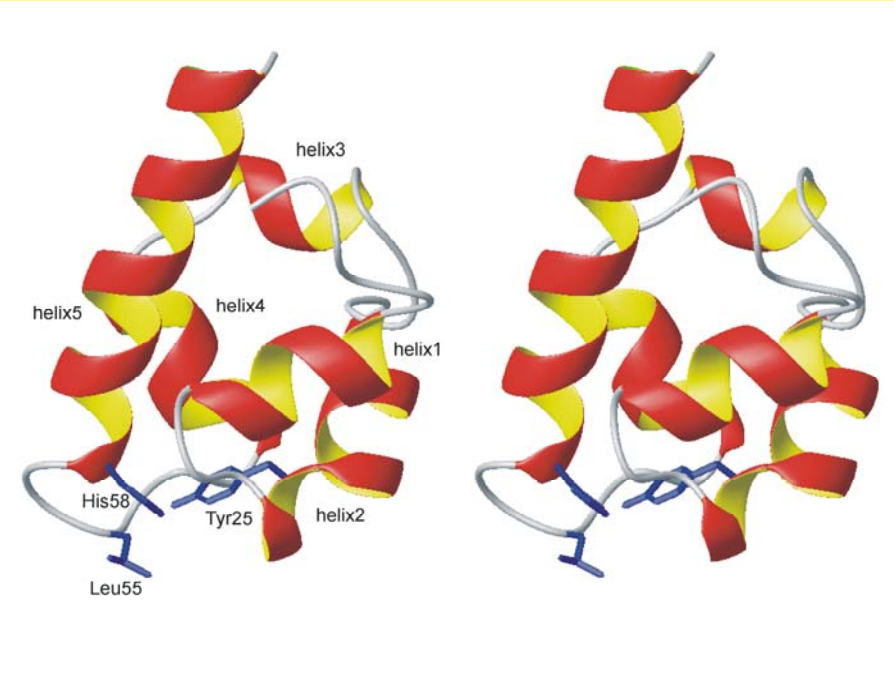


NMR is very powerful in the determination of the constitution of natural products

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

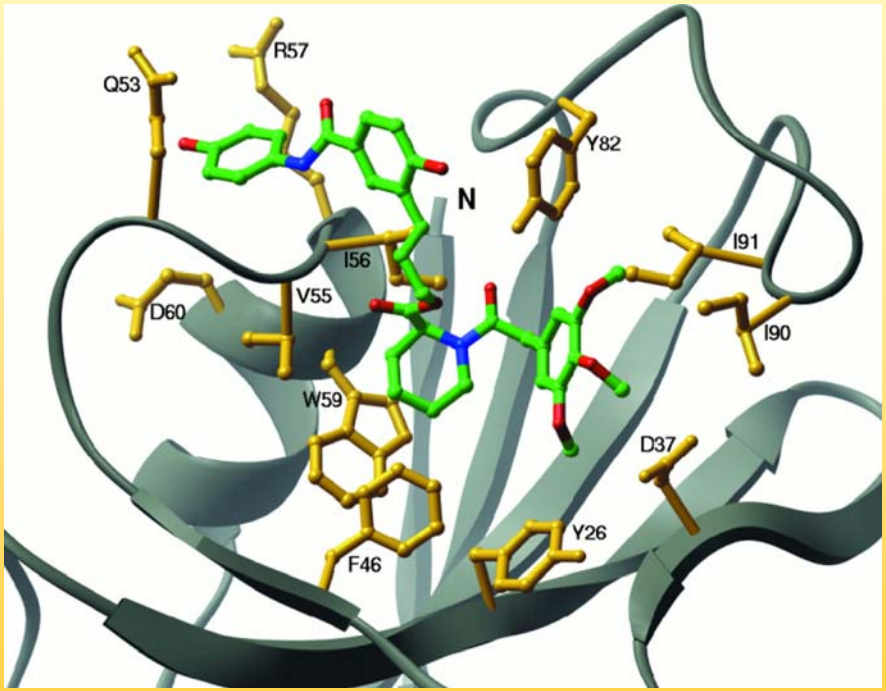
Determination of molecular interactions

NMR can be used to

detect the

interaction between

proteins and ligands

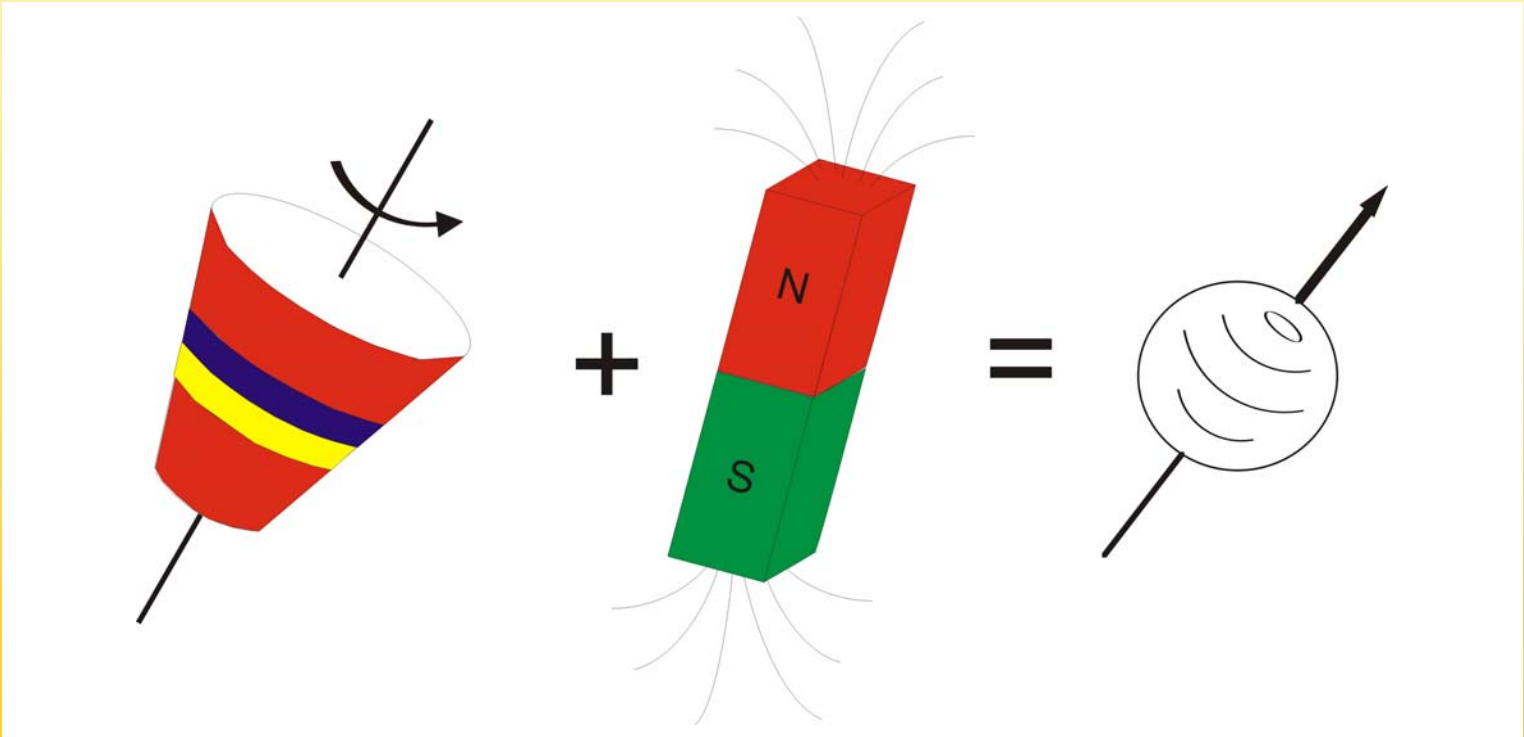


Basic principles of NMR-spectroscopy

Peter Schmieder
AG Solution NMR

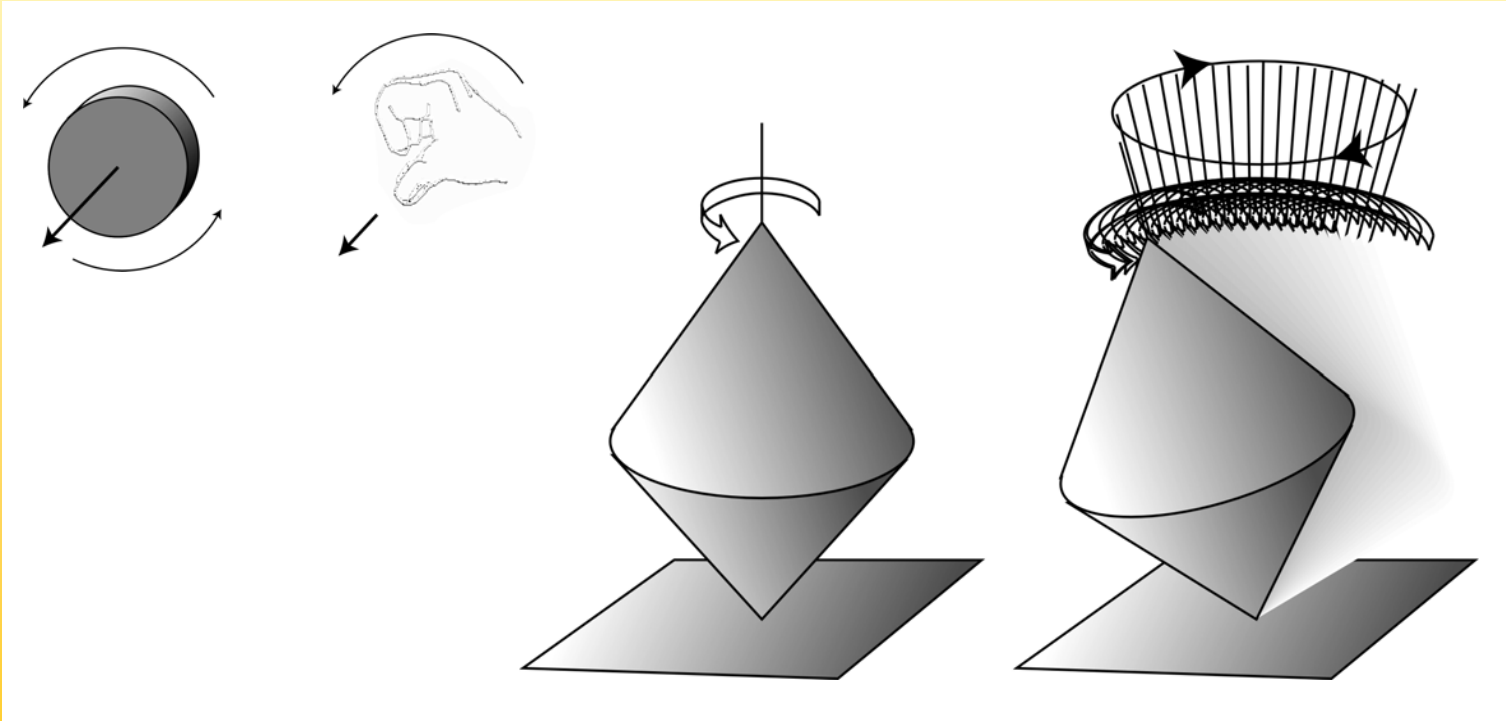
Basic principles of NMR-spectroscopy

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



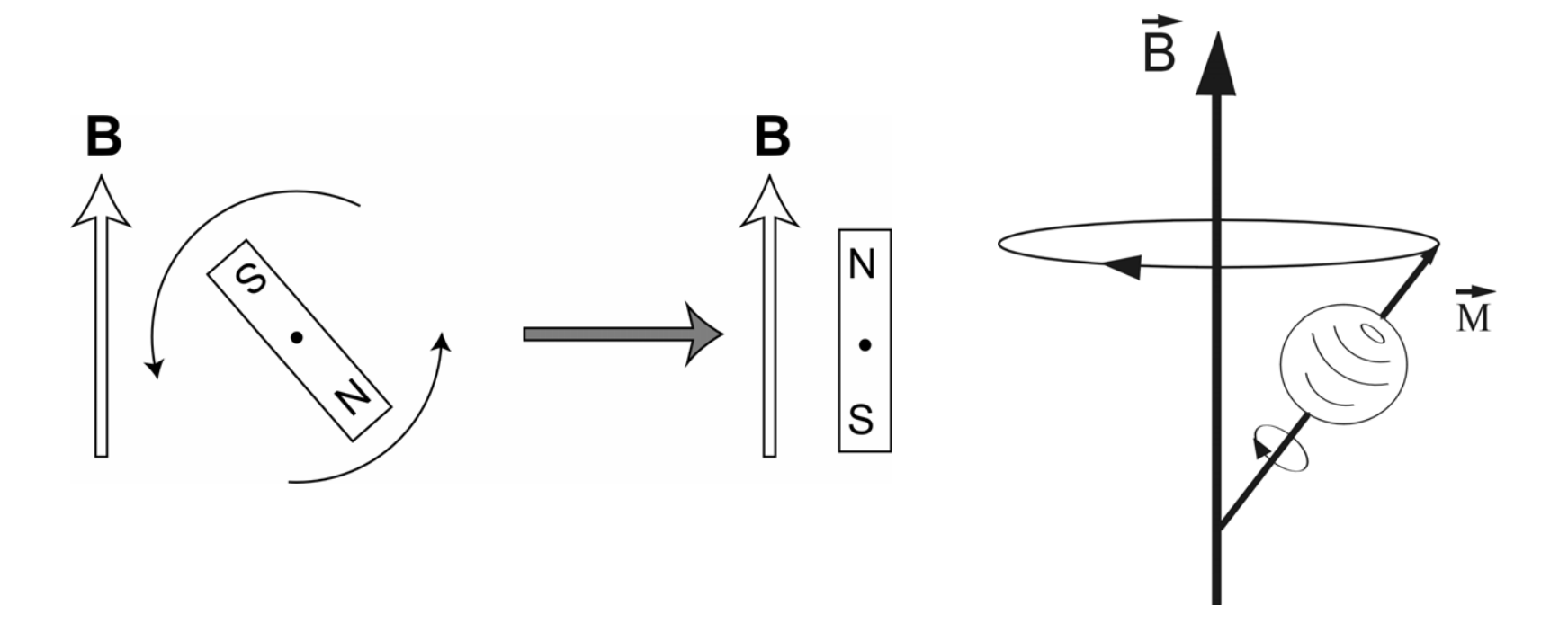
Basic principles of NMR-spectroscopy

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



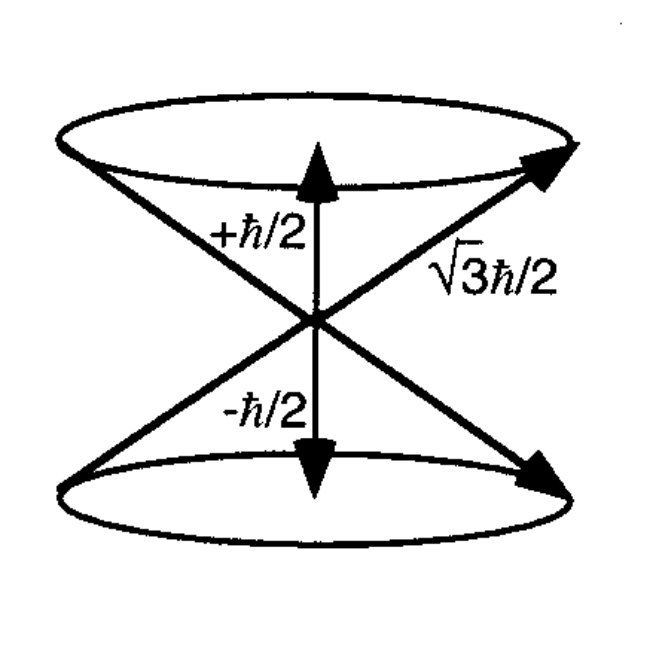
Basic principles of NMR-spectroscopy

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

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.



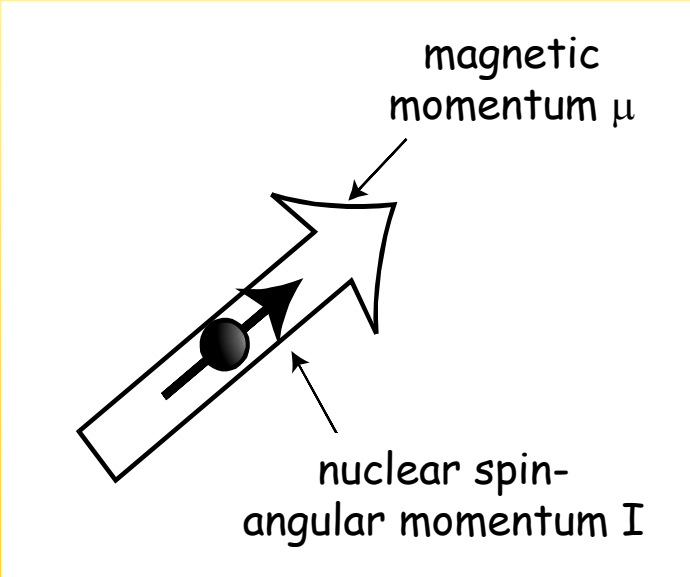
Symbolic representation of the two states of the spin

Basic principles of NMR-spectroscopy

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

$$\mu = \gamma \mathbf{I}$$

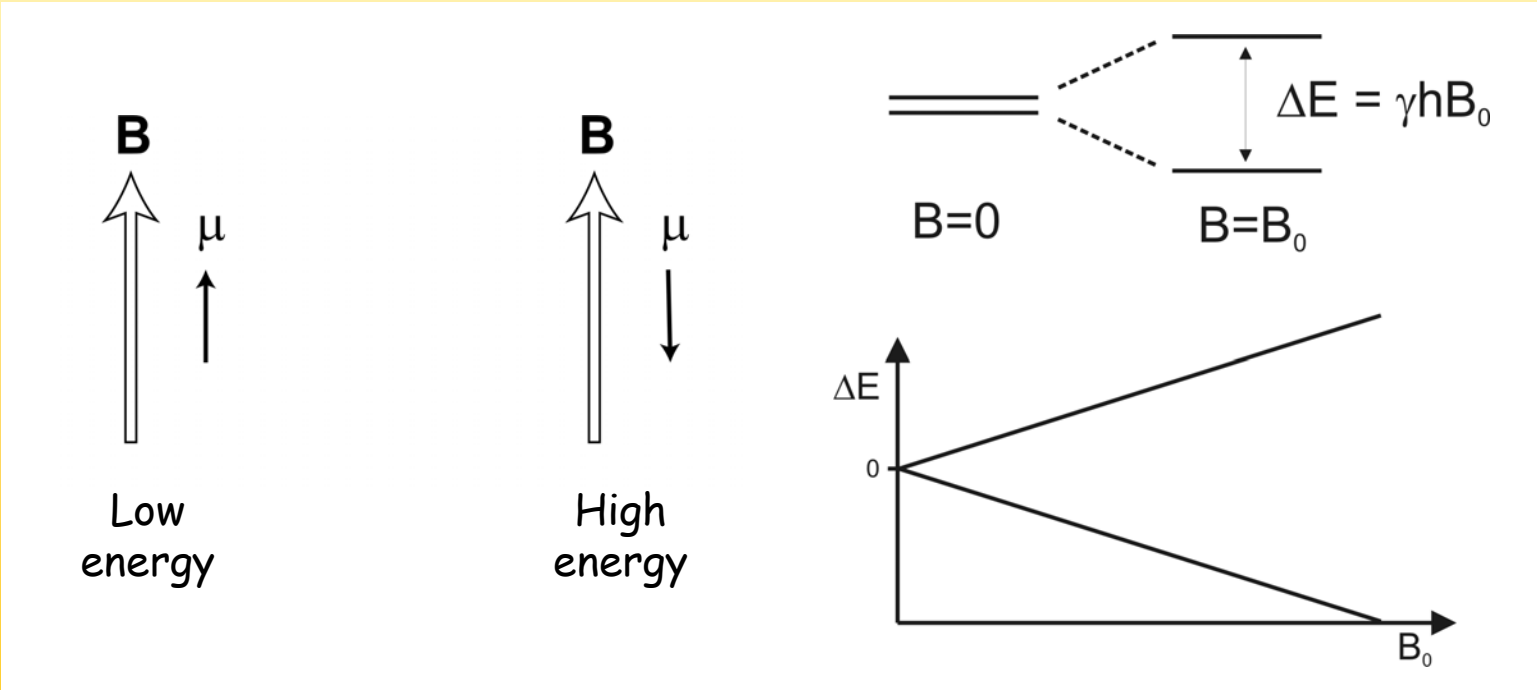
The bigger the gyromagnetic ratio the bigger the magnetic moment and hence the detectable effect



Basic principles of NMR-spectroscopy

The energy of the two possible states is not equal:

$$E = -\mu \mathbf{B} \qquad \Delta E = \hbar \gamma B_0$$



Basic principles of NMR-spectroscopy

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

$$\Delta E = h\,\nu_0$$

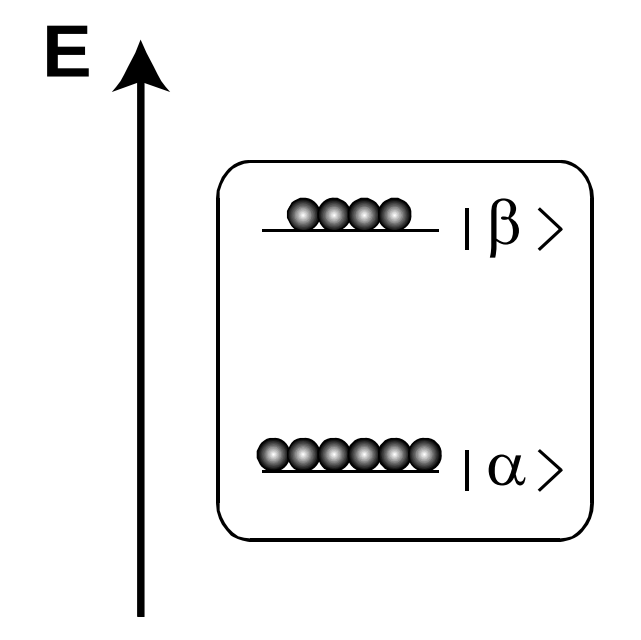
$$\nu_0 = \gamma\, B_0 / 2\pi$$

$$\omega_0 = 2\pi\,\nu_0 = \gamma\, B_0$$

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

Basic principles of NMR-spectroscopy

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



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

Basic principles of NMR-spectroscopy

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 !)

Basic principles of NMR-spectroscopy

Magnetic properties of relevant nuclei

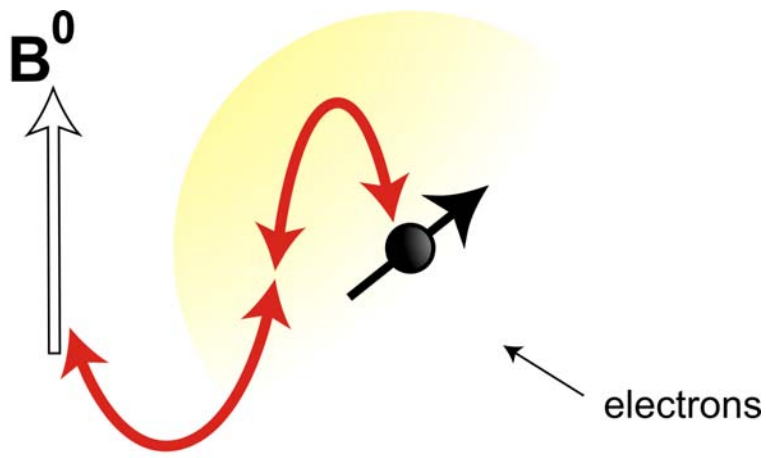
Isotop	Spin	Natürliche Häufigkeit	gyromagnetisches Verhältnis g	NMR-Frequenz bei 2.35 T
1H	1/2	99.98	26.7522	100.000
2H	1	0.015	4.1066	15.351
3H	1/2	0	28.5350	106.663
7Li	3/2	92.58	10.3976	38.863
11B	3/2	80.42	8.5847	32.084
12C	0	98.89		
13C	1/2	1.11	6.7283	25.144
14N	1	99.63	1.9338	7.224
15N	1/2	0.37	-2.7126	10.133
17O	5/2	0.037	-3.6280	13.557
19F	1/2	100.0	25.1815	94.077
23Na	3/2	100.0	7.0704	26.451
25Mg	5/2	10.13	-1.6389	6.1195
31P	1/2	100.0	10.8394	40.481
35Cl	3/2	75.53	2.6242	9.798
39K	3/2	93.1	1.2499	4.667
43Ca	7/2	0.145	-1.8028	6.728
51V	7/2	99.76	0.052	26.289
57Fe	1/2	2.19	0.8687	3.231
75As	3/2	100.0	4.5961	17.126
77Se	1/2	7.58	5.1214	19.067
113Cd	1/2	12.26	-5.9609	22.182

Parameters in NMR-spectroscopy

Parameters in NMR-spectroscopy

Chemical shift

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



$$B_{eff} = (1 - \sigma)\, B_0$$

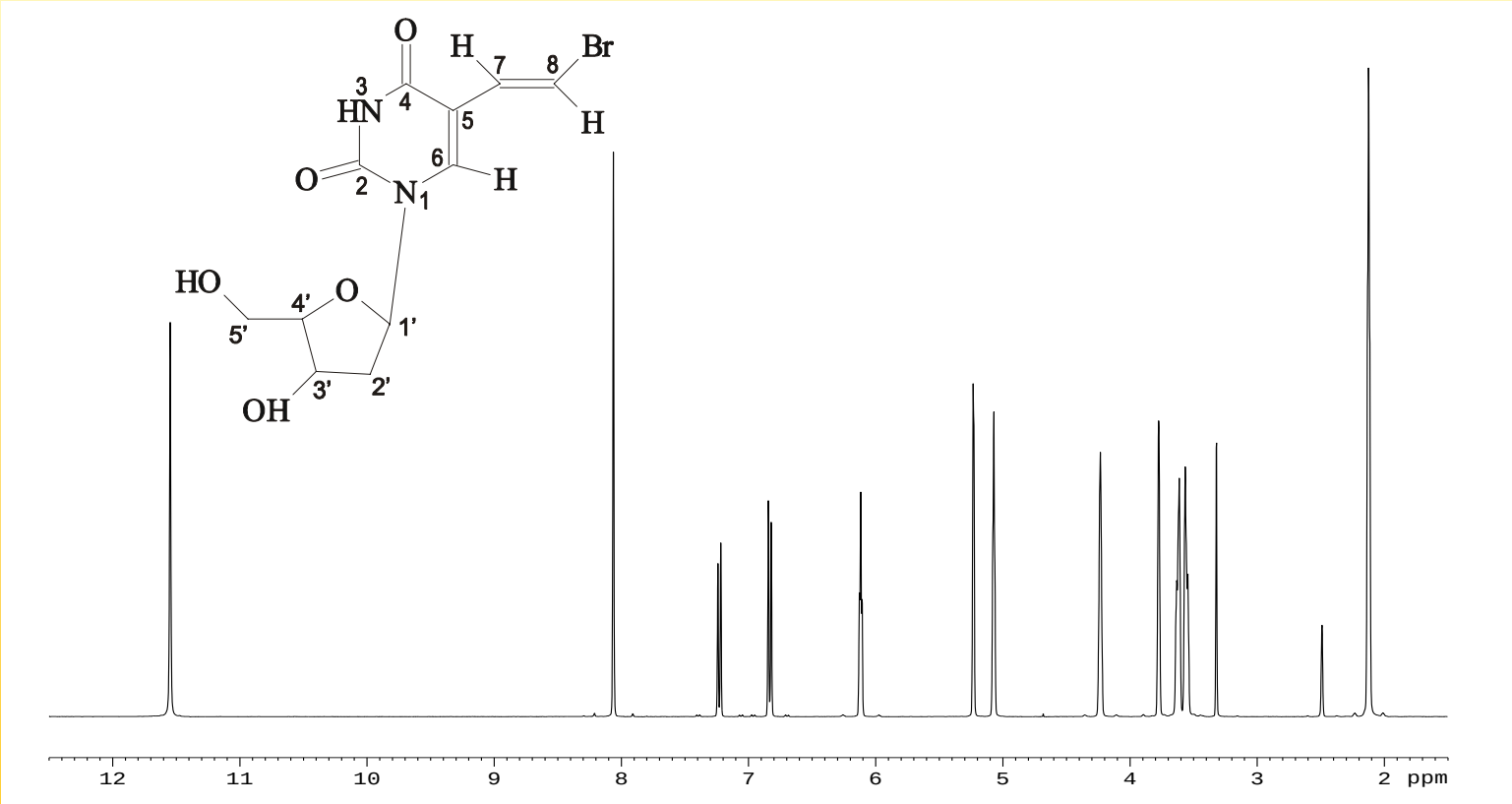
$$\omega = \gamma\, (1 - \sigma)\, B_0$$

$$\delta = (\omega - \omega_{ref}) / \omega_0 \times 10^6$$

$$= (\sigma_{ref} - \sigma) \times 10^6$$

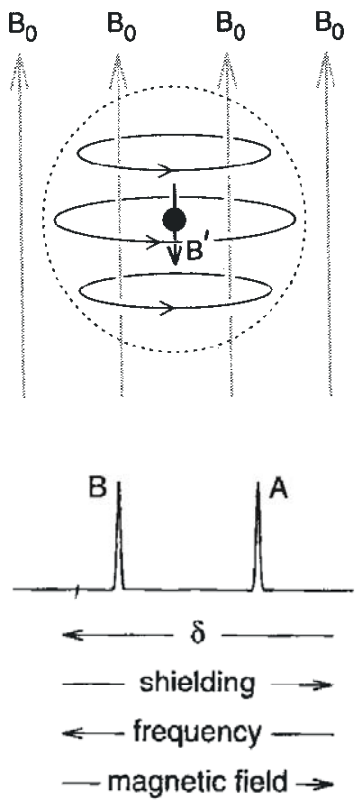
Parameters in NMR-spectroscopy

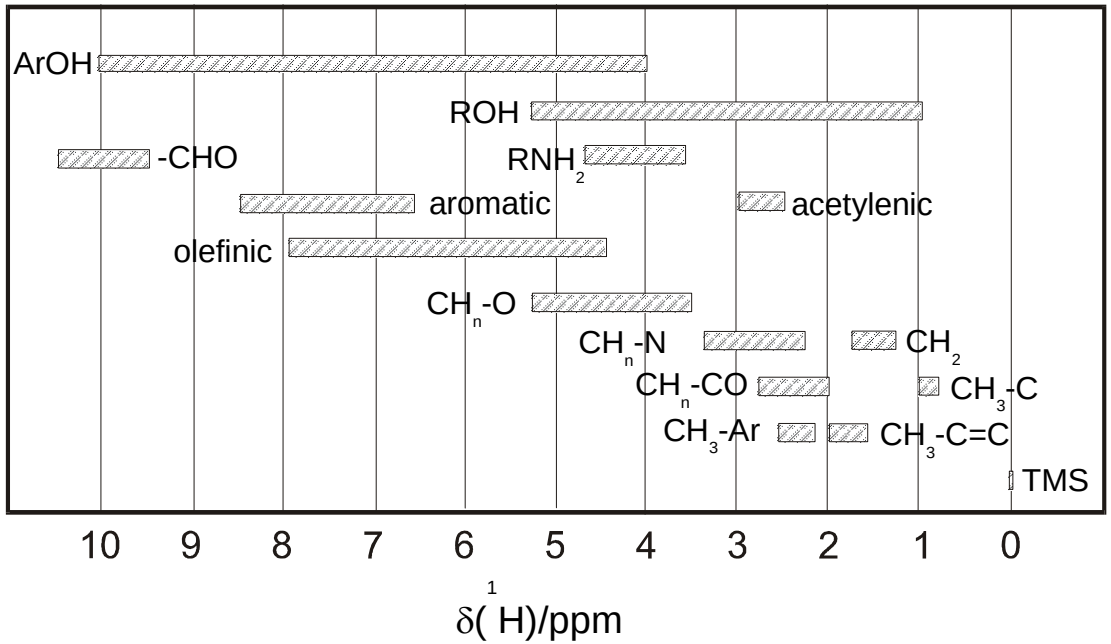
Each atom in the molecule gives rise to a resonance line



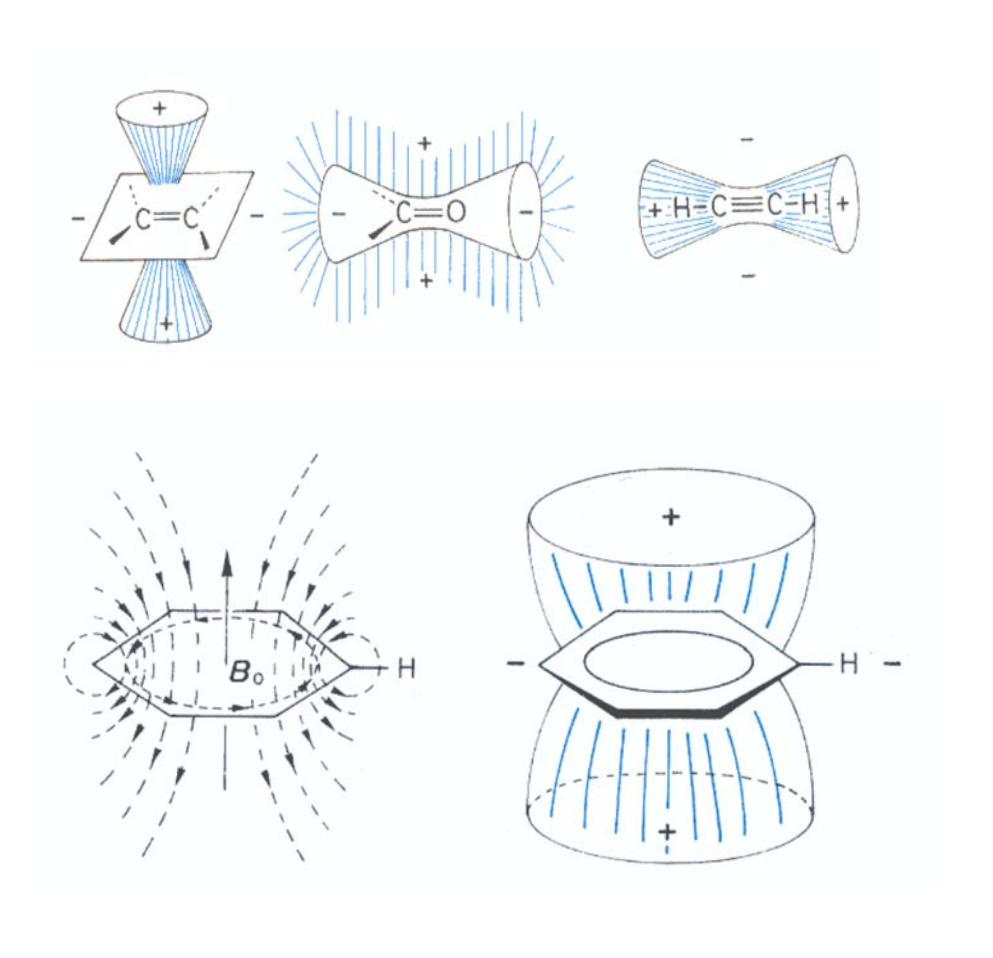
Parameters in NMR-spectroscopy

The chemical shift depends on the chemical environment





Parameters in NMR-spectroscopy

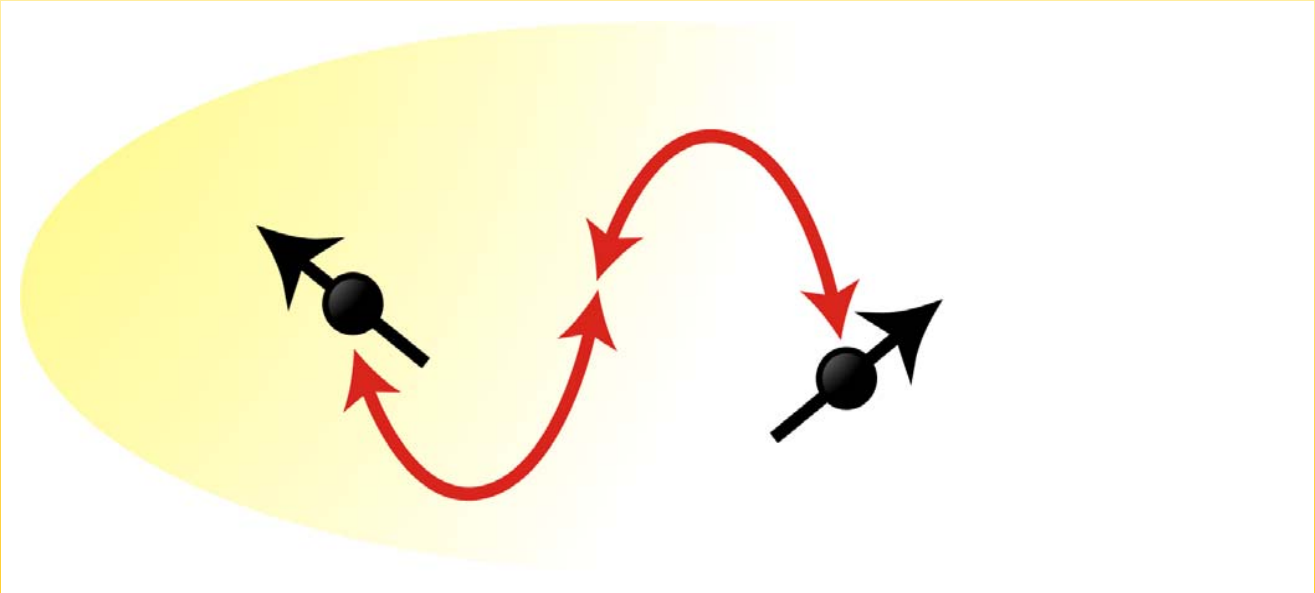


An important factor influencing the chemical shift are anisotropy effects, that are created by small additional fields

Parameters in NMR-spectroscopy

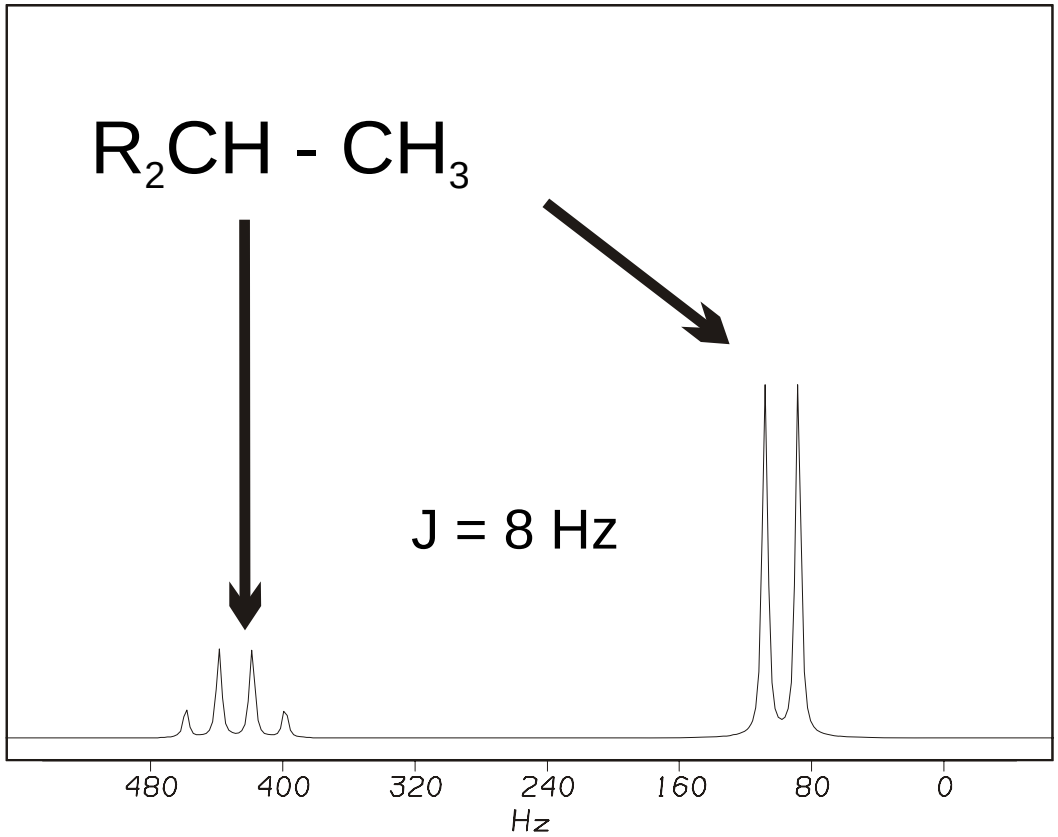
Scalar or J-coupling

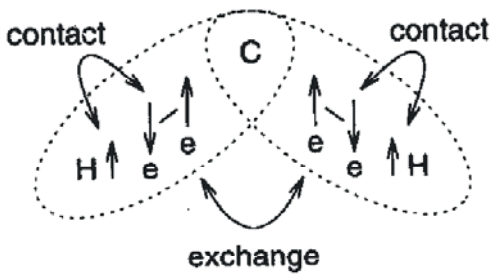
Electrons in the bonds between the nuclei mediate an interaction, the scalar coupling



Parameters in NMR-spectroscopy

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

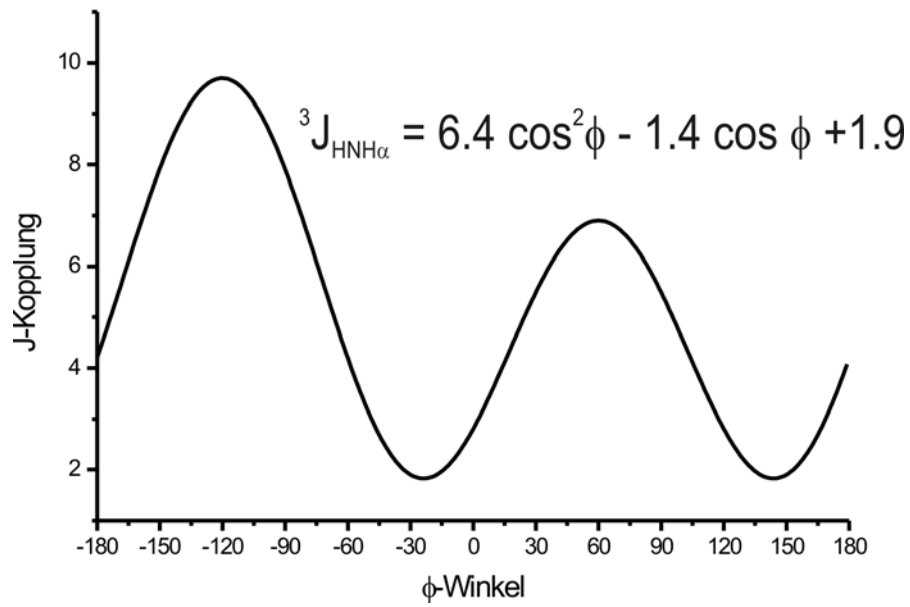


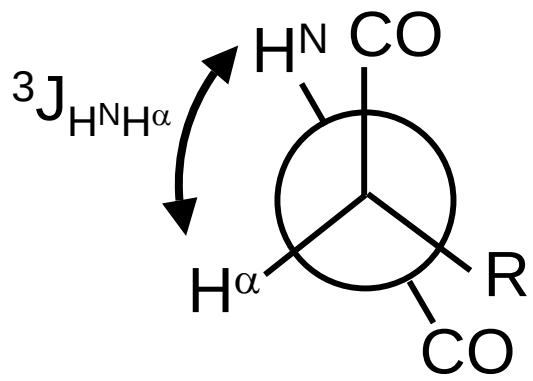


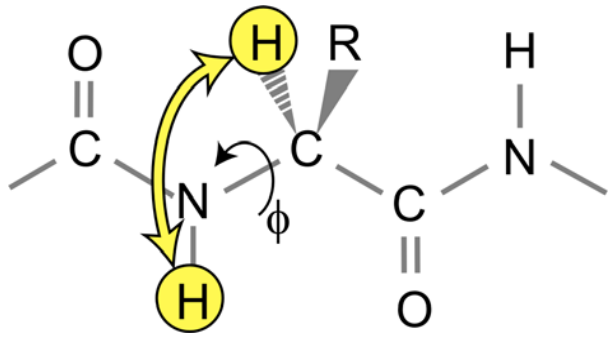
Parameters in NMR-spectroscopy

Scalar coupling contains structural information

Karplus-equation



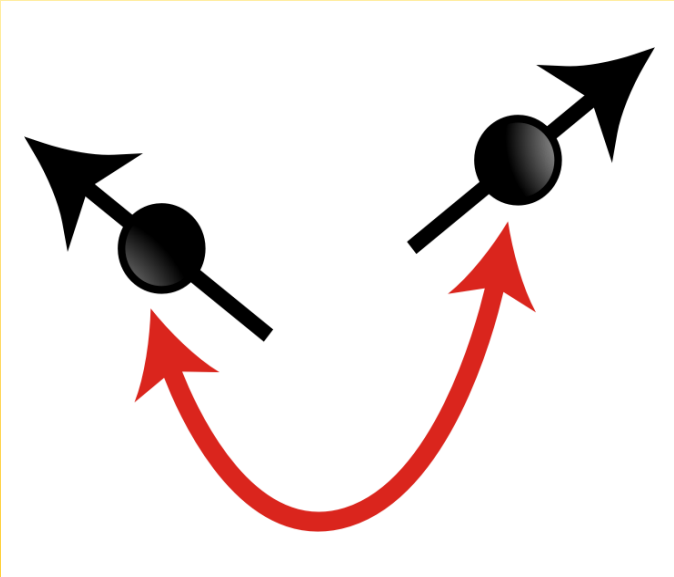




Parameters in NMR-spectroscopy

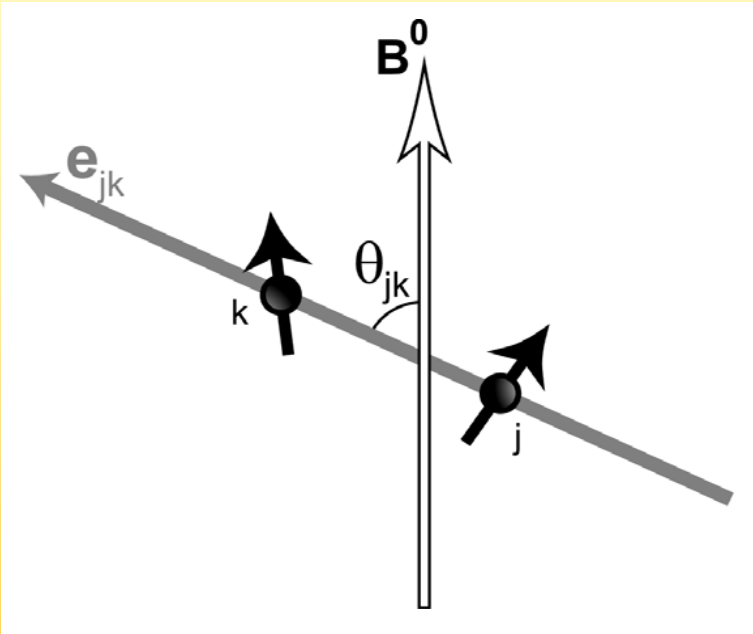
Dipolar coupling

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

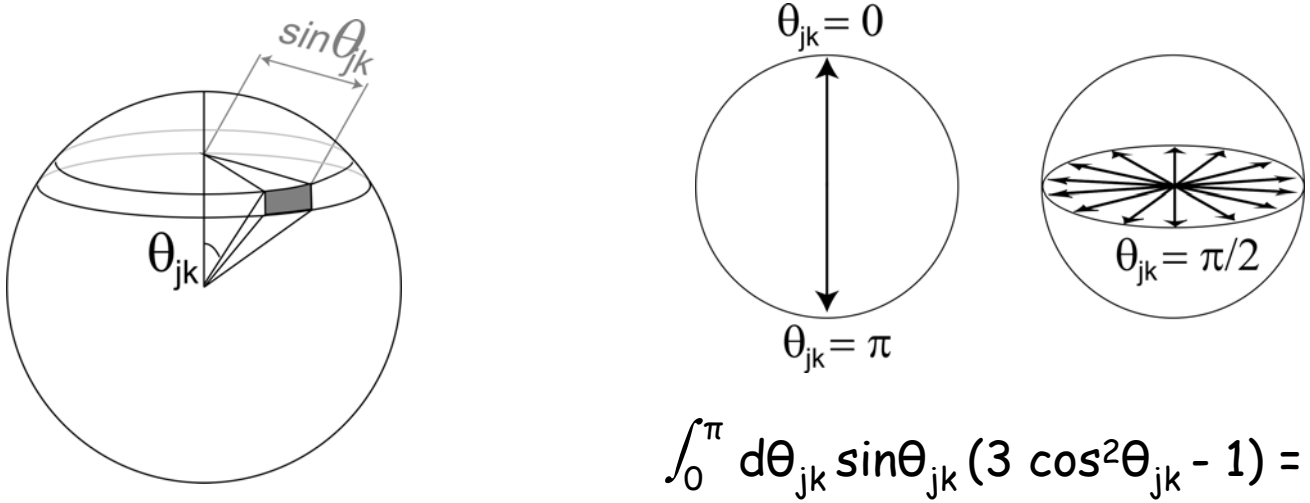
Parameters in NMR-spectroscopy



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

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

Parameters in NMR-spectroscopy



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

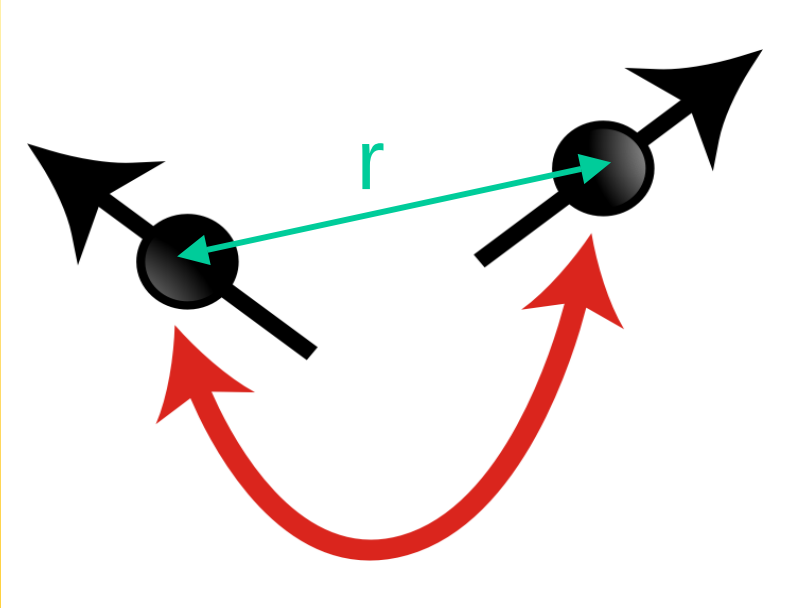
While it is averaged to zero in solution NMR, it is present in solid state NMR and is of great importance there. In solution it is a major source of relaxation.

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

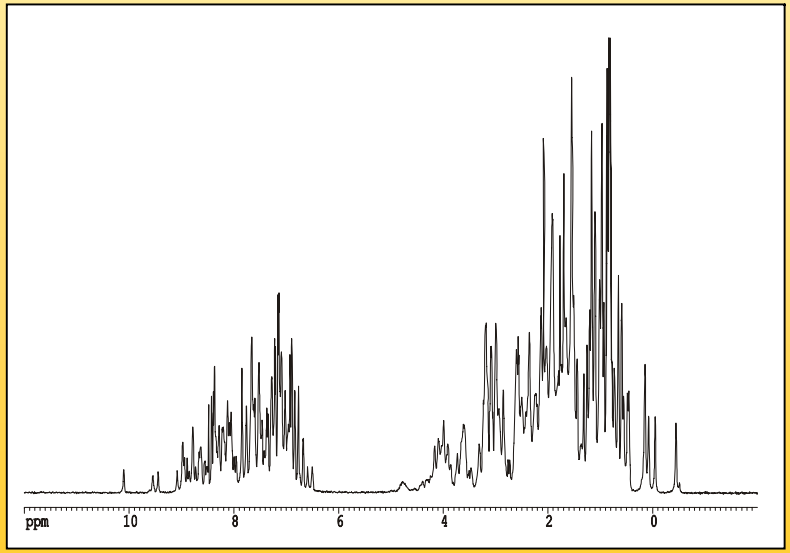


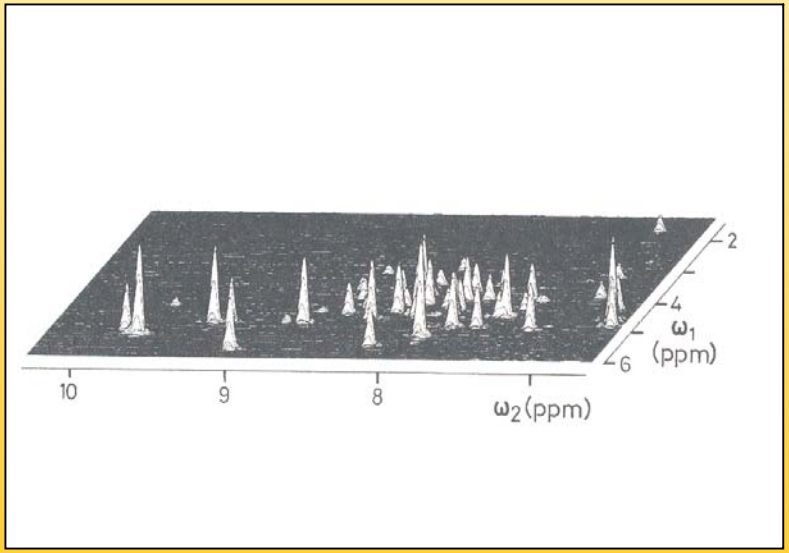
Multidimensional NMR-spectroscopy

Multidimensional NMR-spectroscopy

1D-NMR:
2 axis
intensity vs. frequency

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





Multidimensional NMR-spectroscopy

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

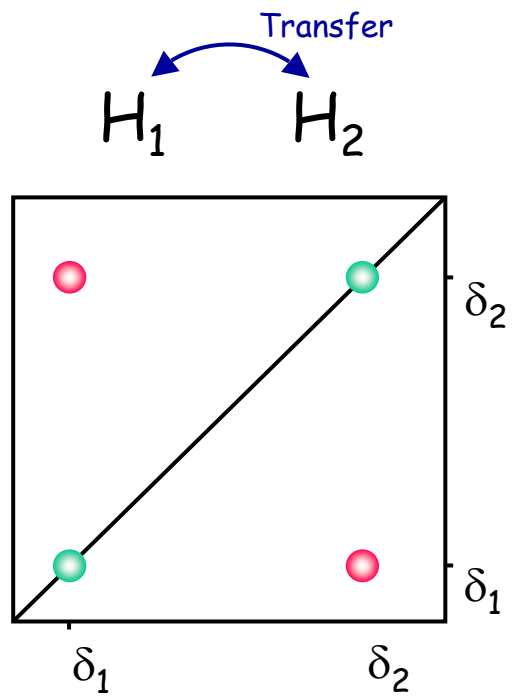
homonuclear spectra

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

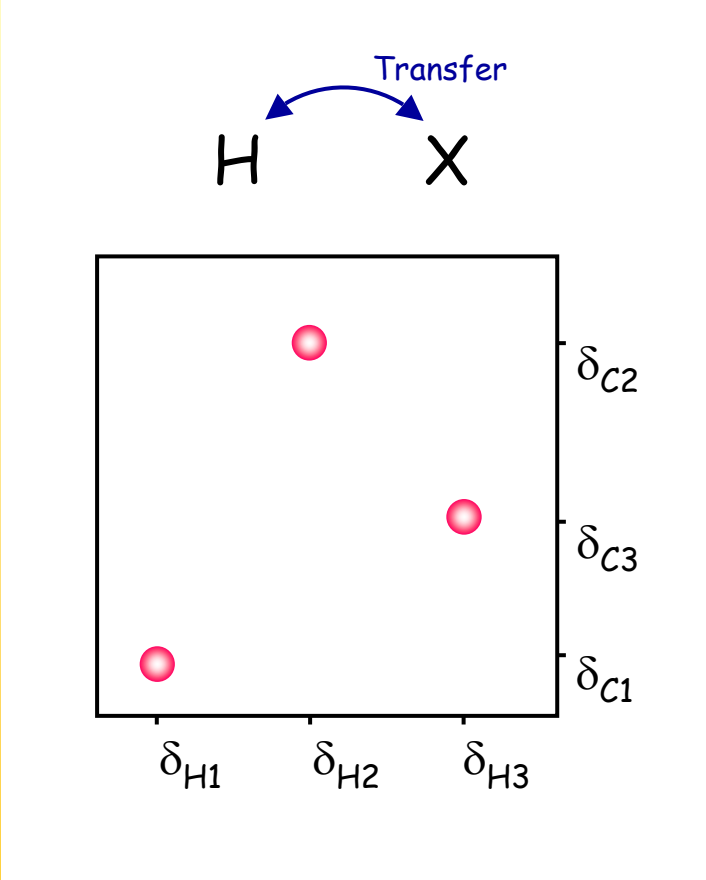


cross peak
diagonal signal

Multidimensional NMR-spectroscopy

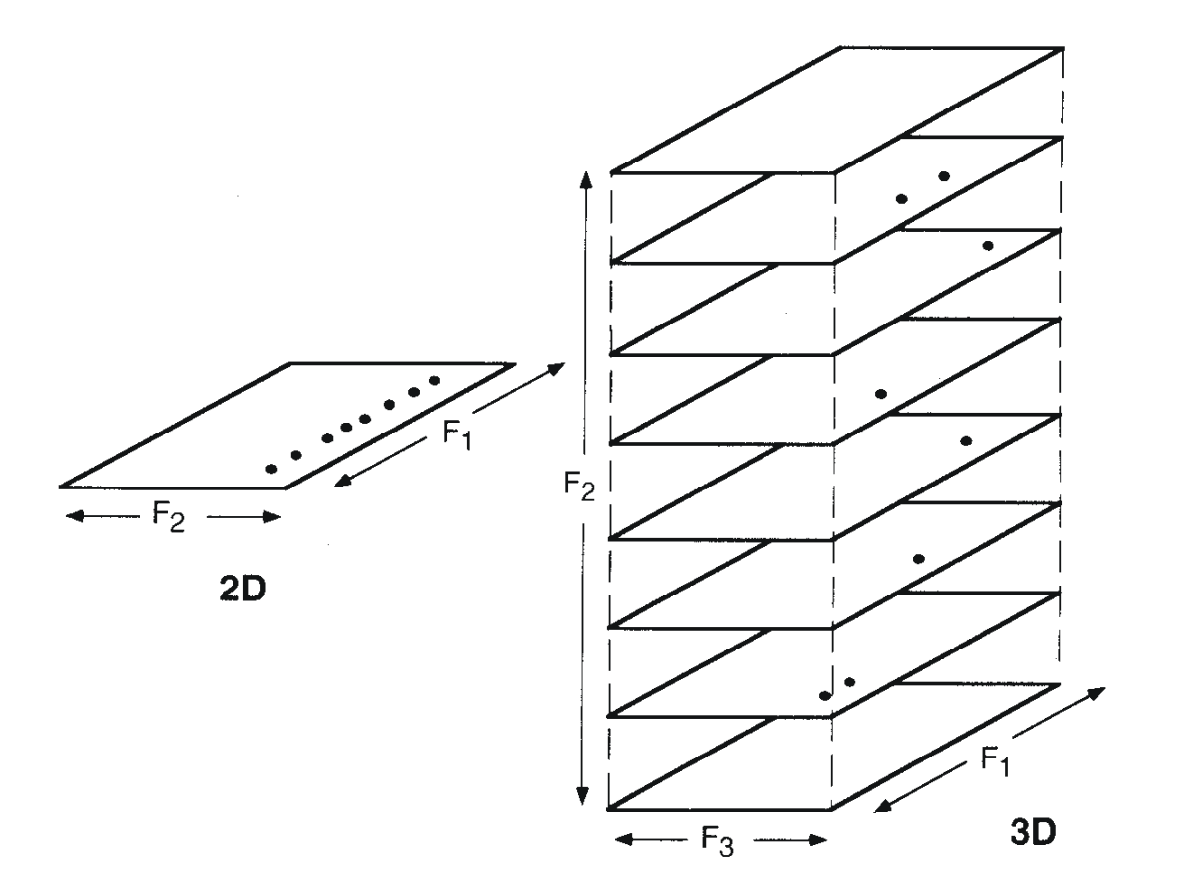
heteronuclear spectra

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.

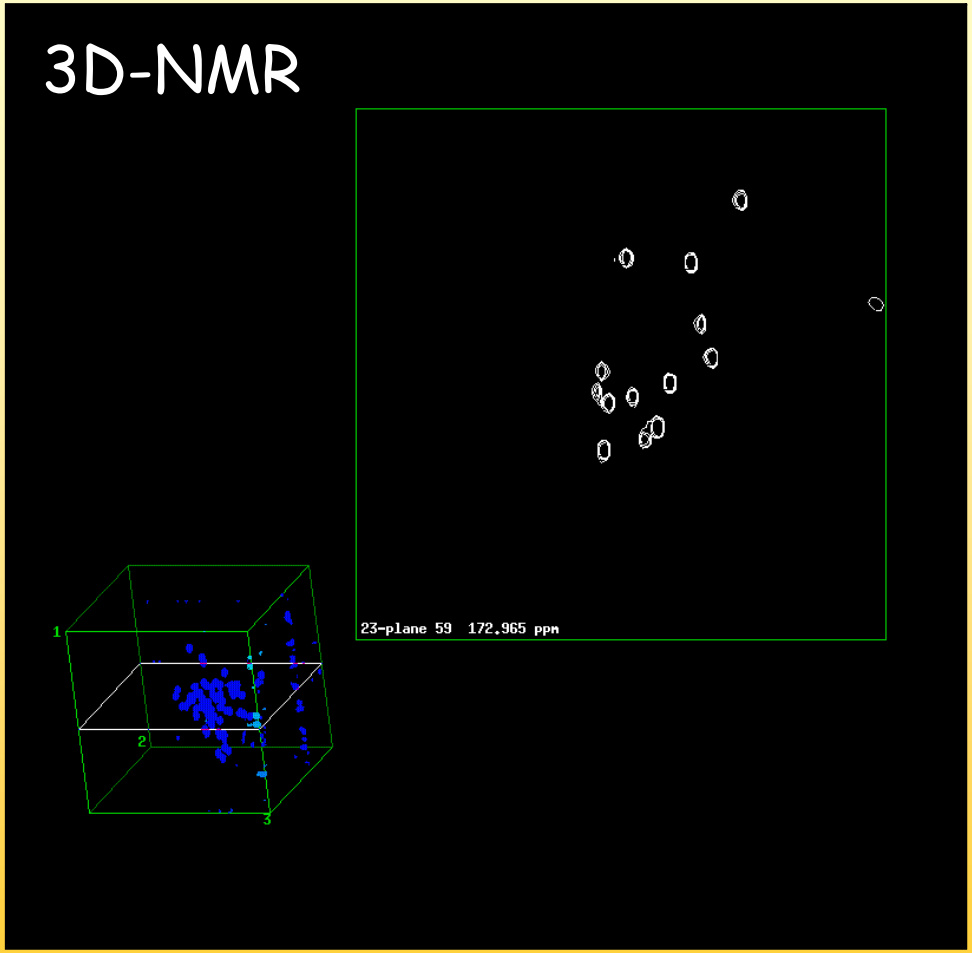


Multidimensional NMR-spectroscopy

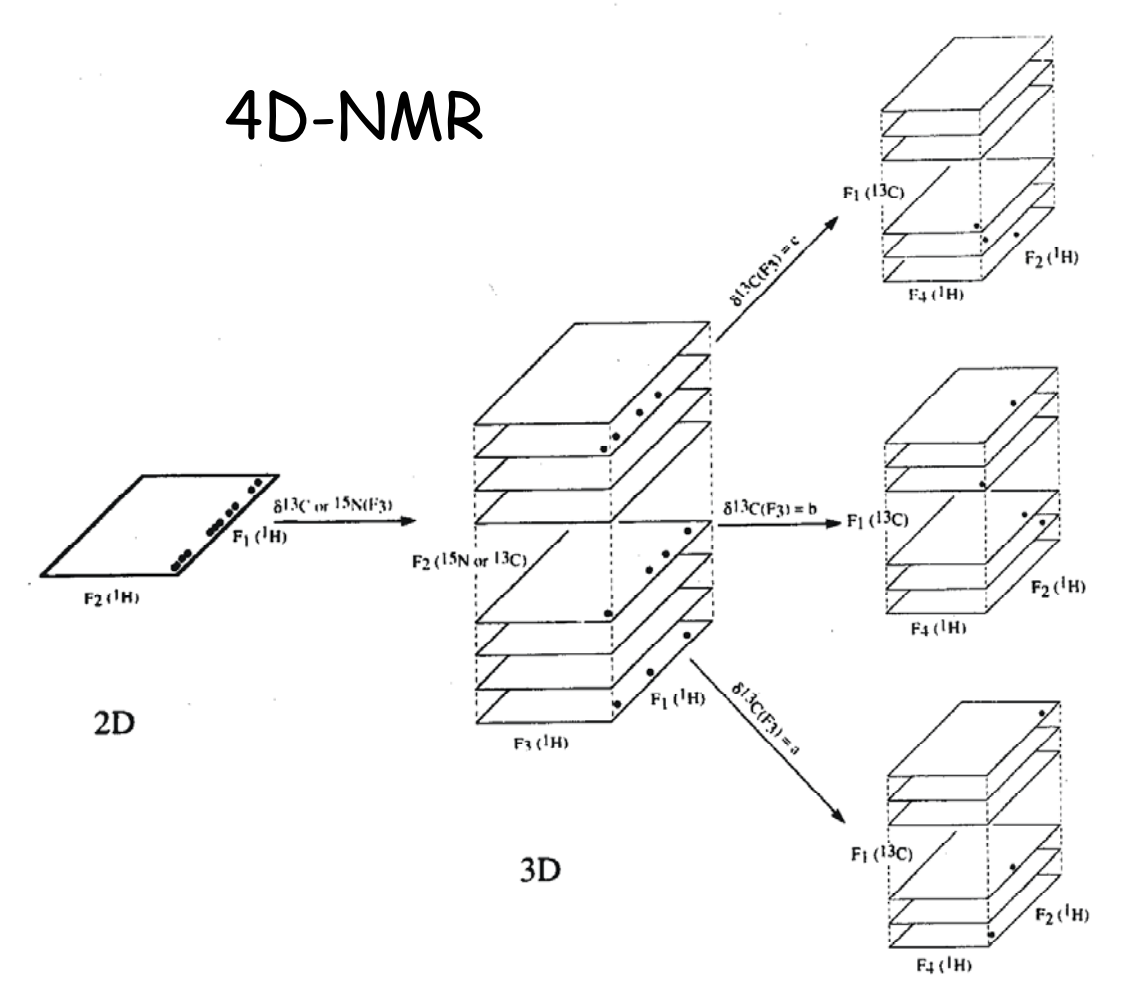
3D-NMR



Multidimensional NMR-spectroscopy



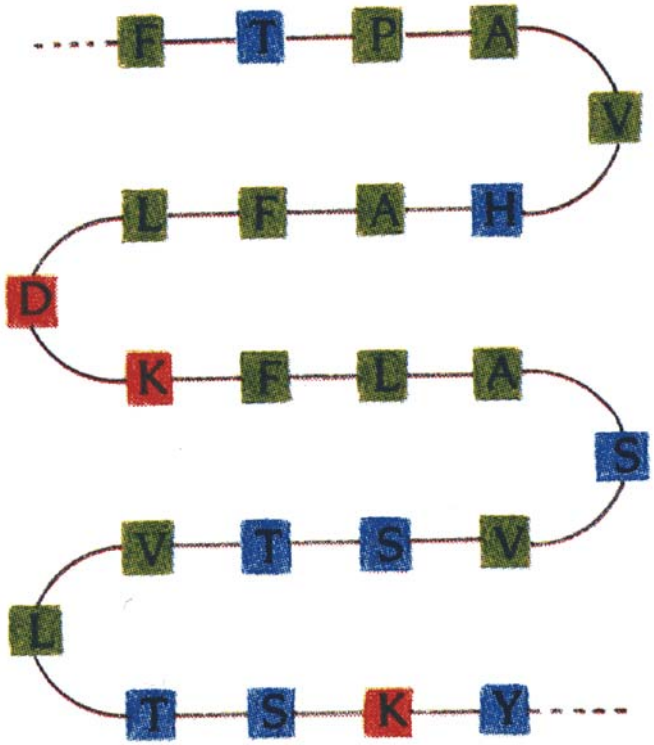
Multidimensional NMR-spectroscopy

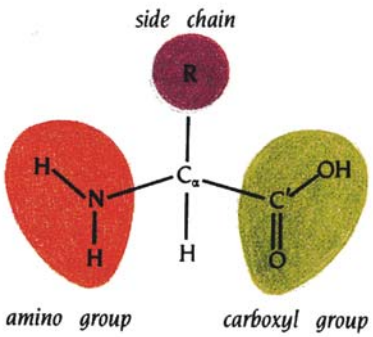


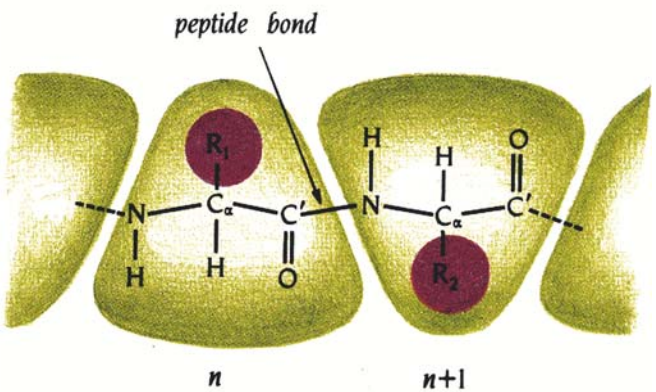
Protein structures

Protein structures

Primary structure

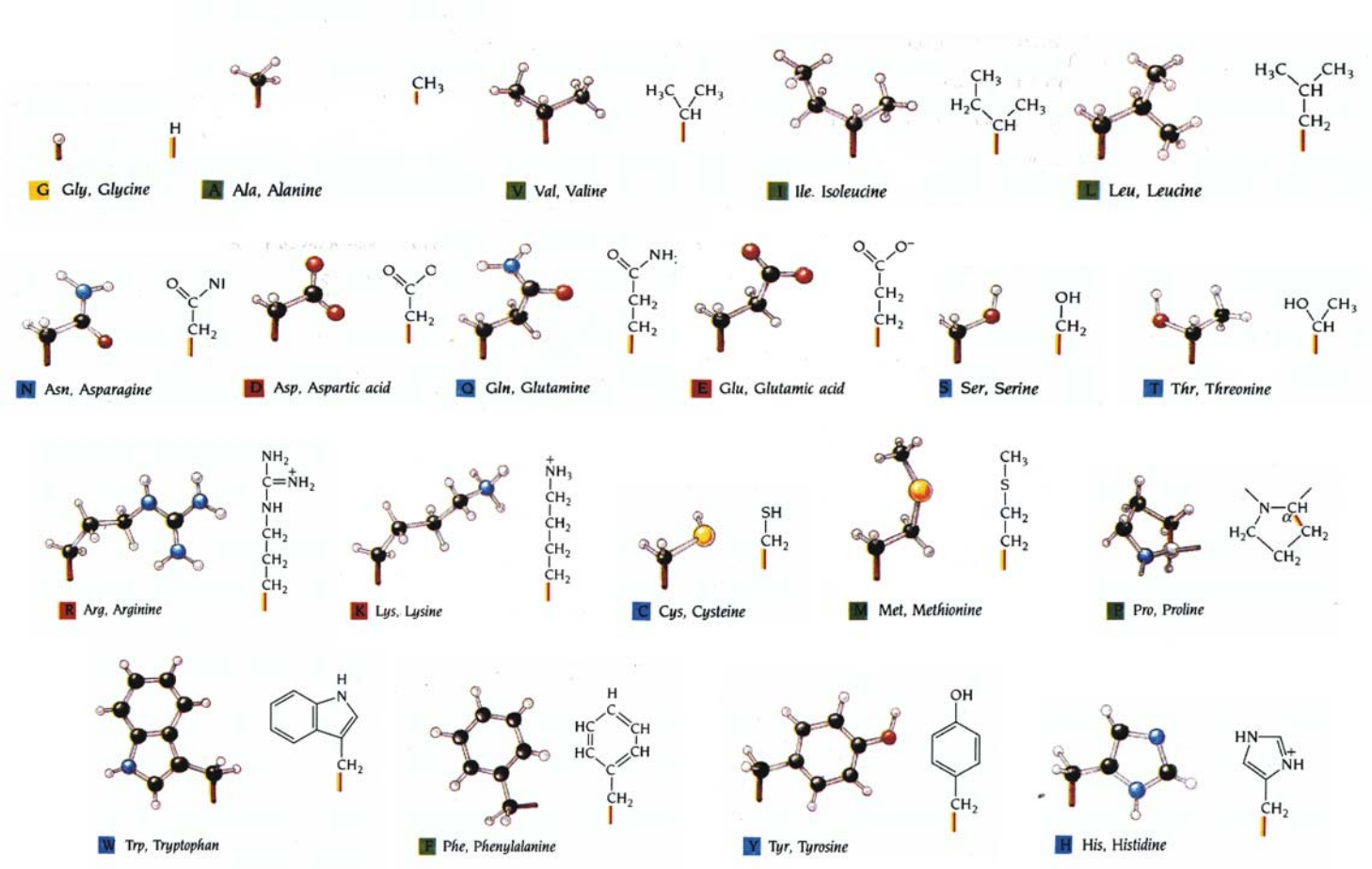






Protein structures

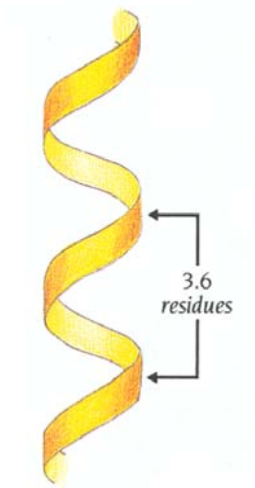
20 proteinogenic amino acids

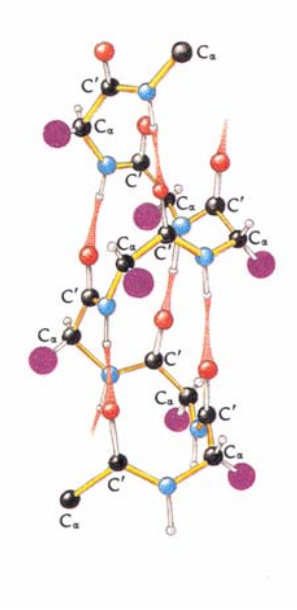


Protein structures

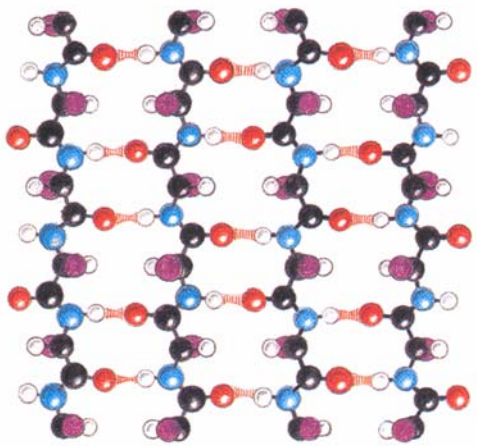
secondary structure

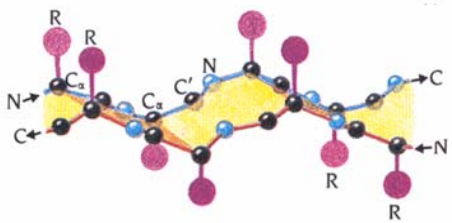
α-helix





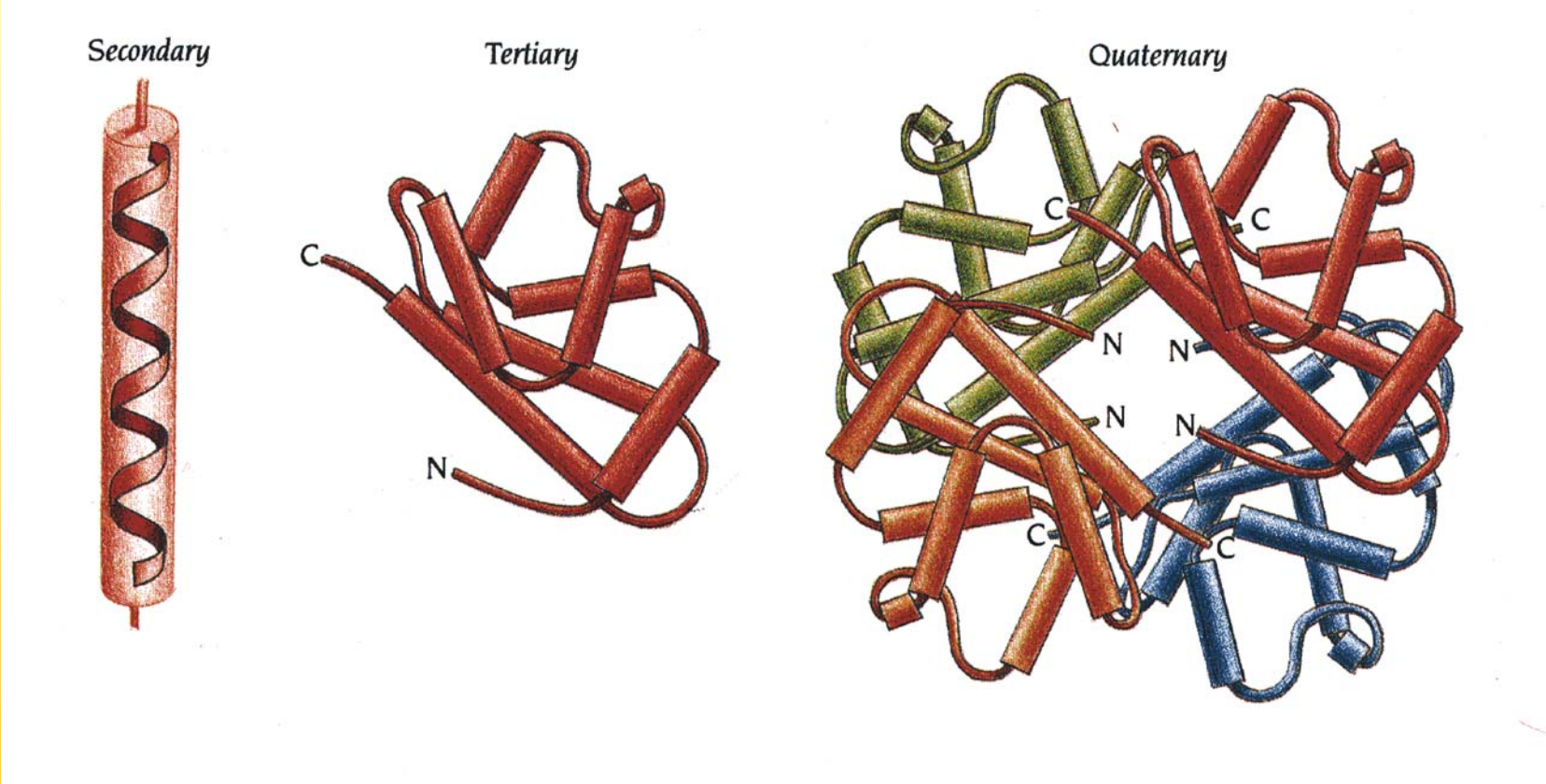
β-sheet





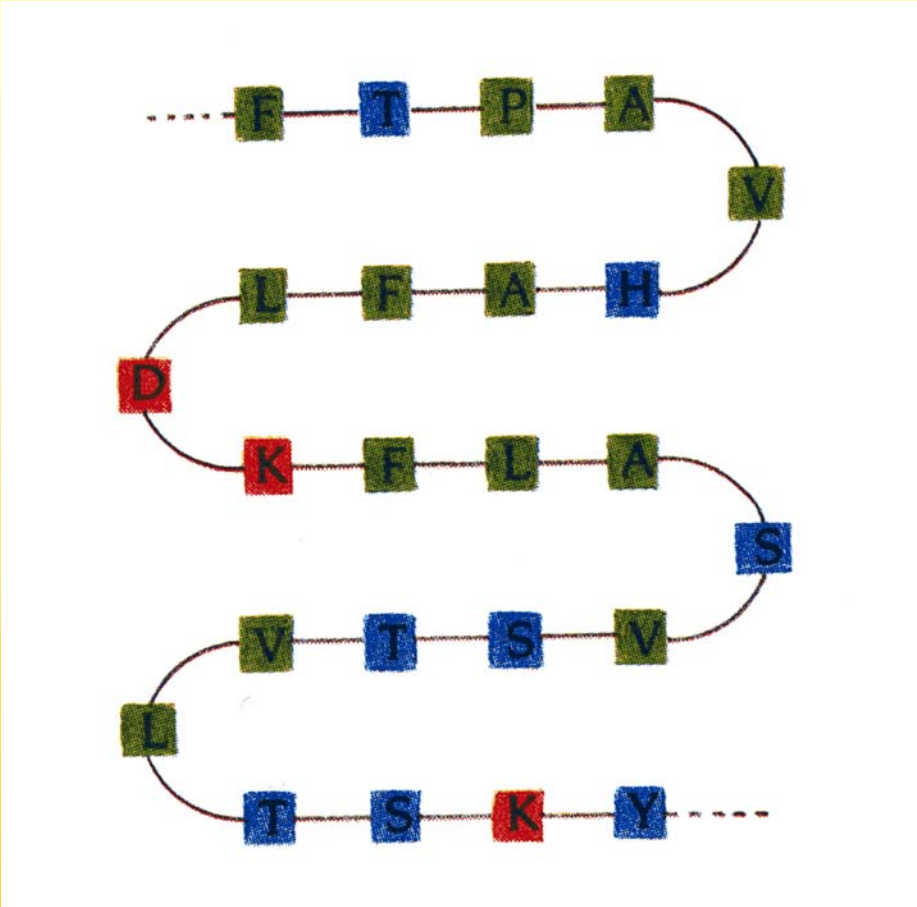
Protein structures

Levels of structural organization



NMR-spectroscopy of proteins

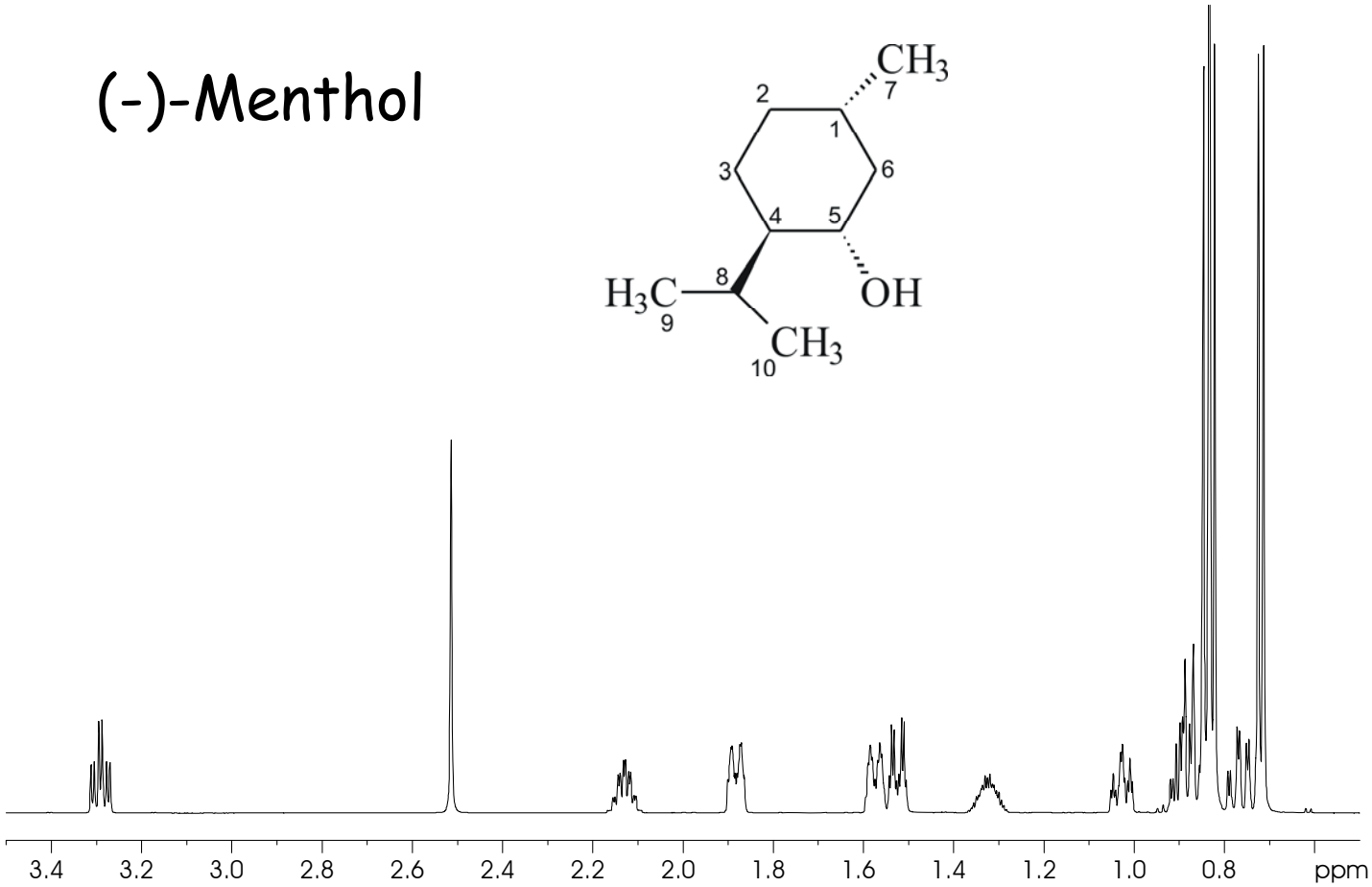
NMR-spectroscopy of proteins



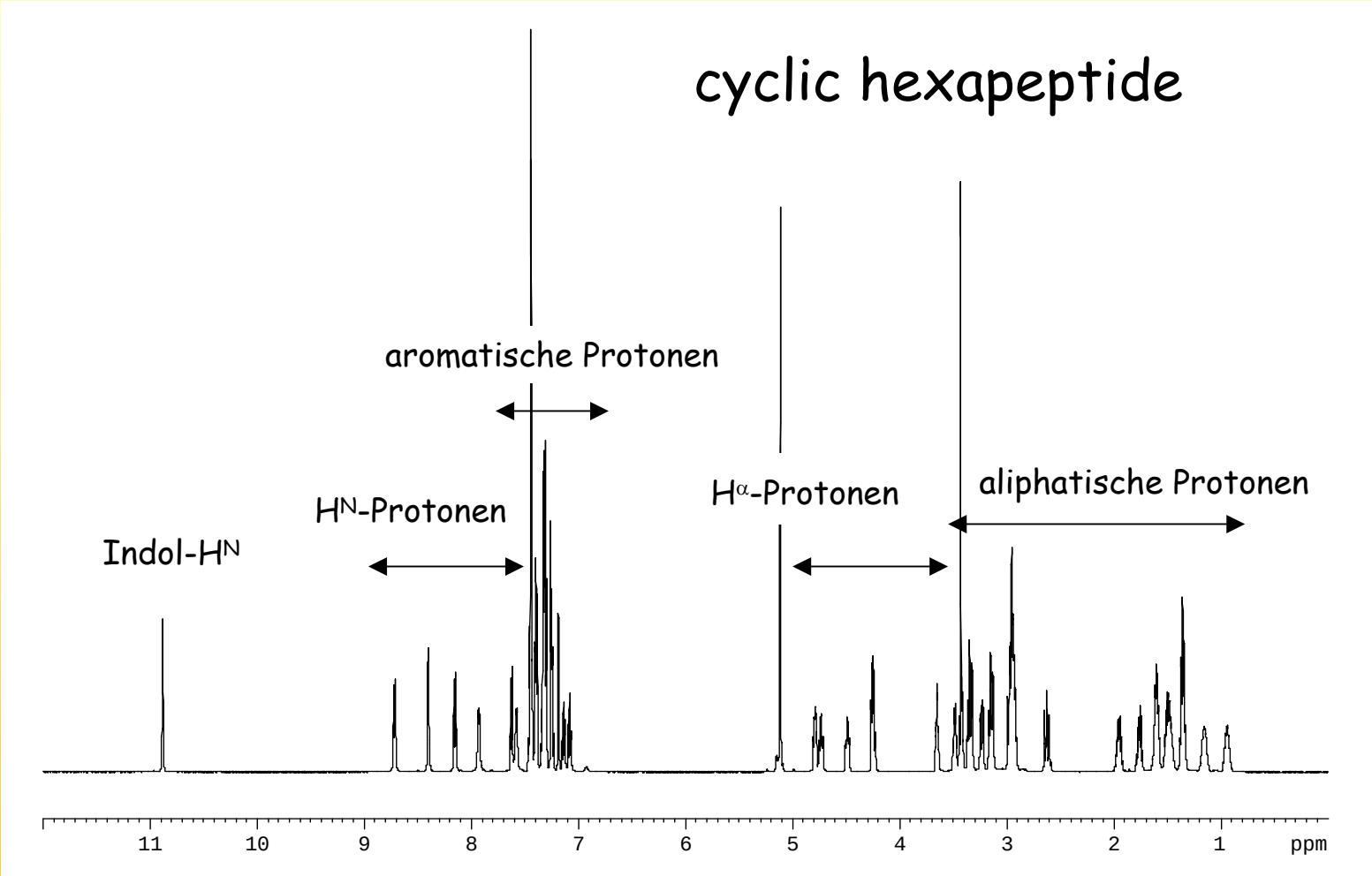
The major problem of protein NMR results from the fact that proteins are polymers, i.e. the repetition of almost identical subunits

NMR-spectroscopy of proteins

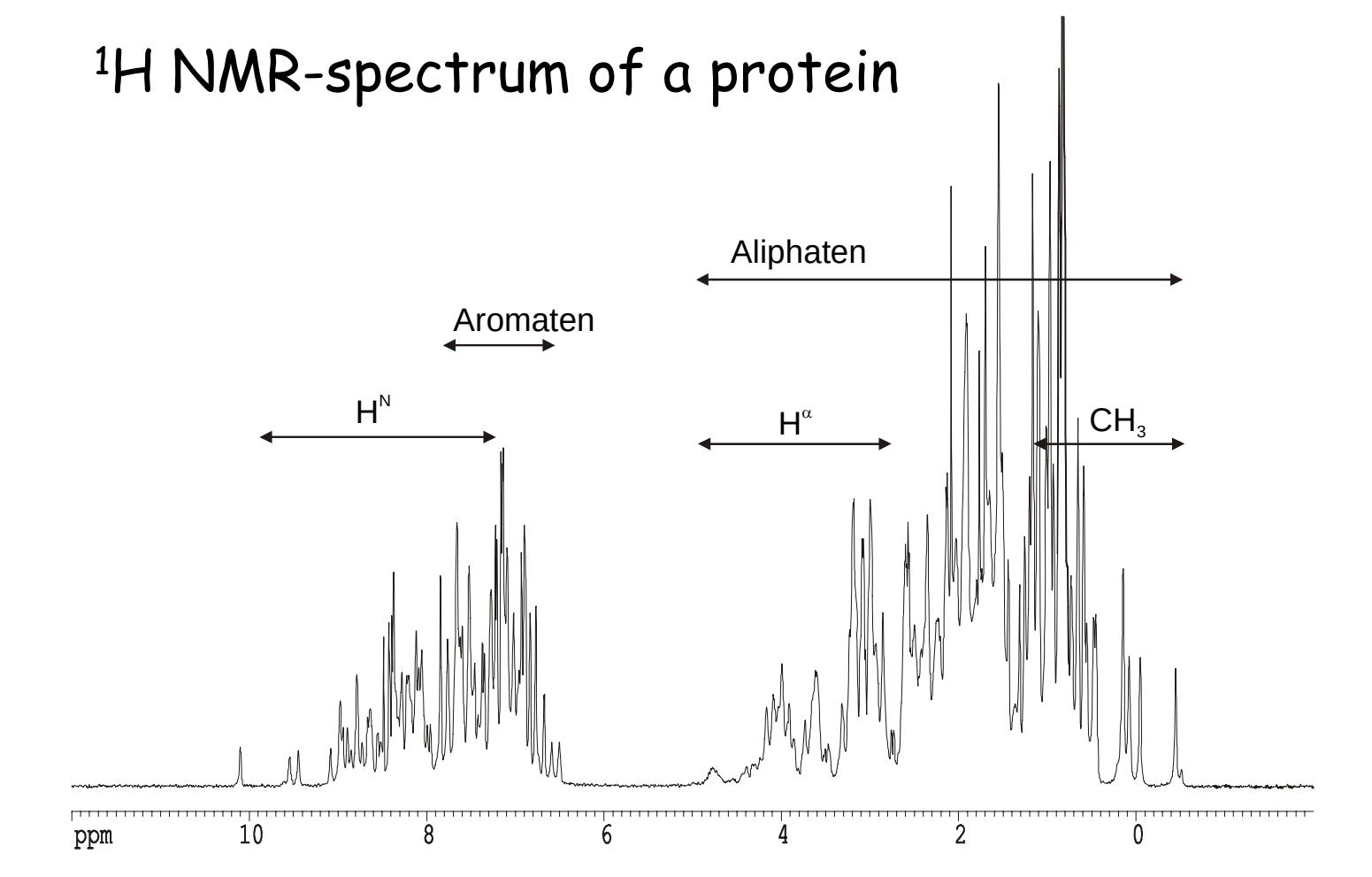
(-)-Menthol



NMR-spectroscopy of proteins

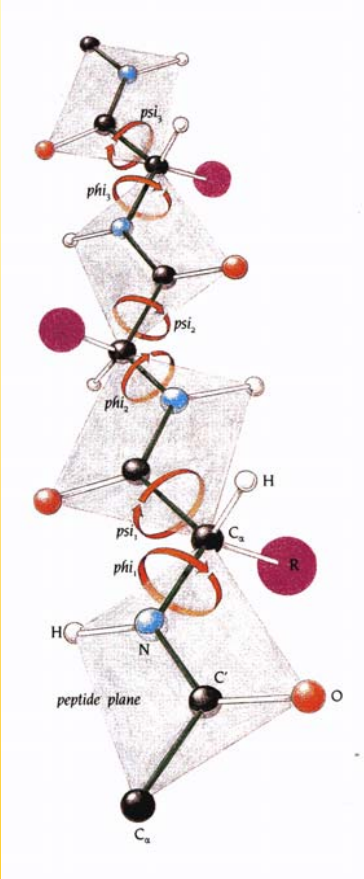


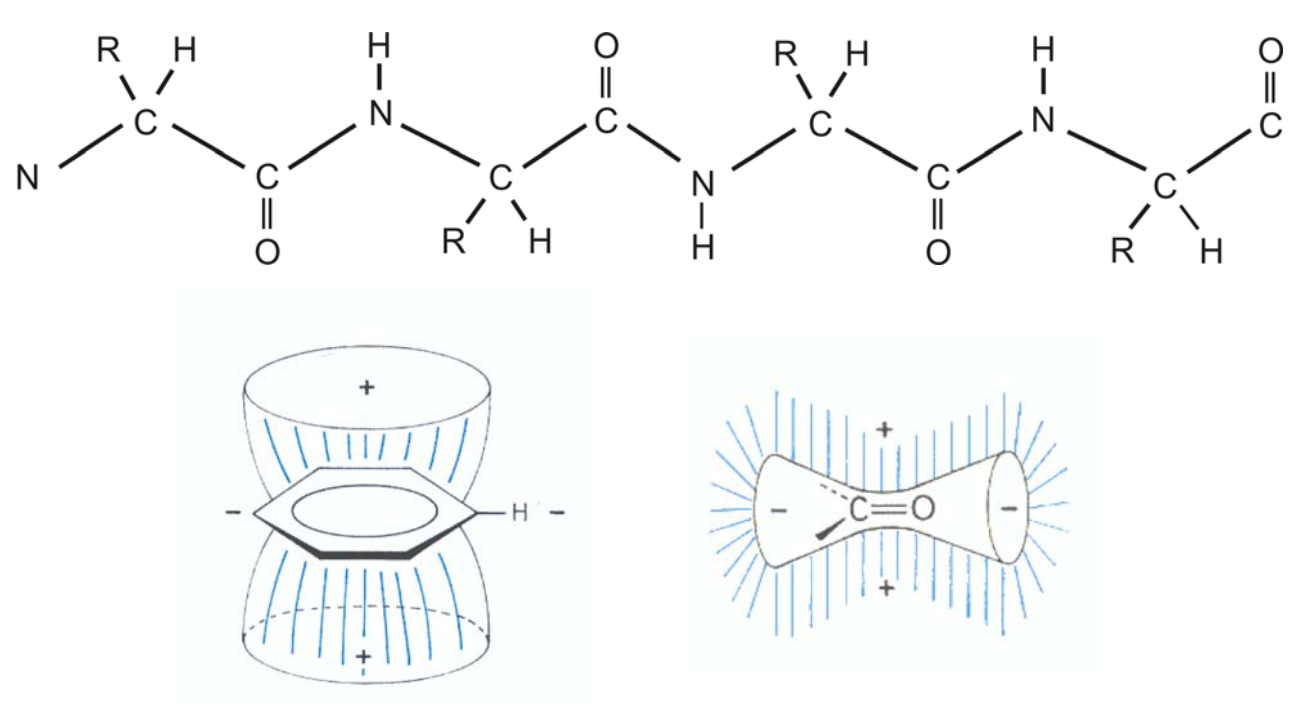
NMR-spectroscopy of proteins



NMR-spectroscopy of proteins

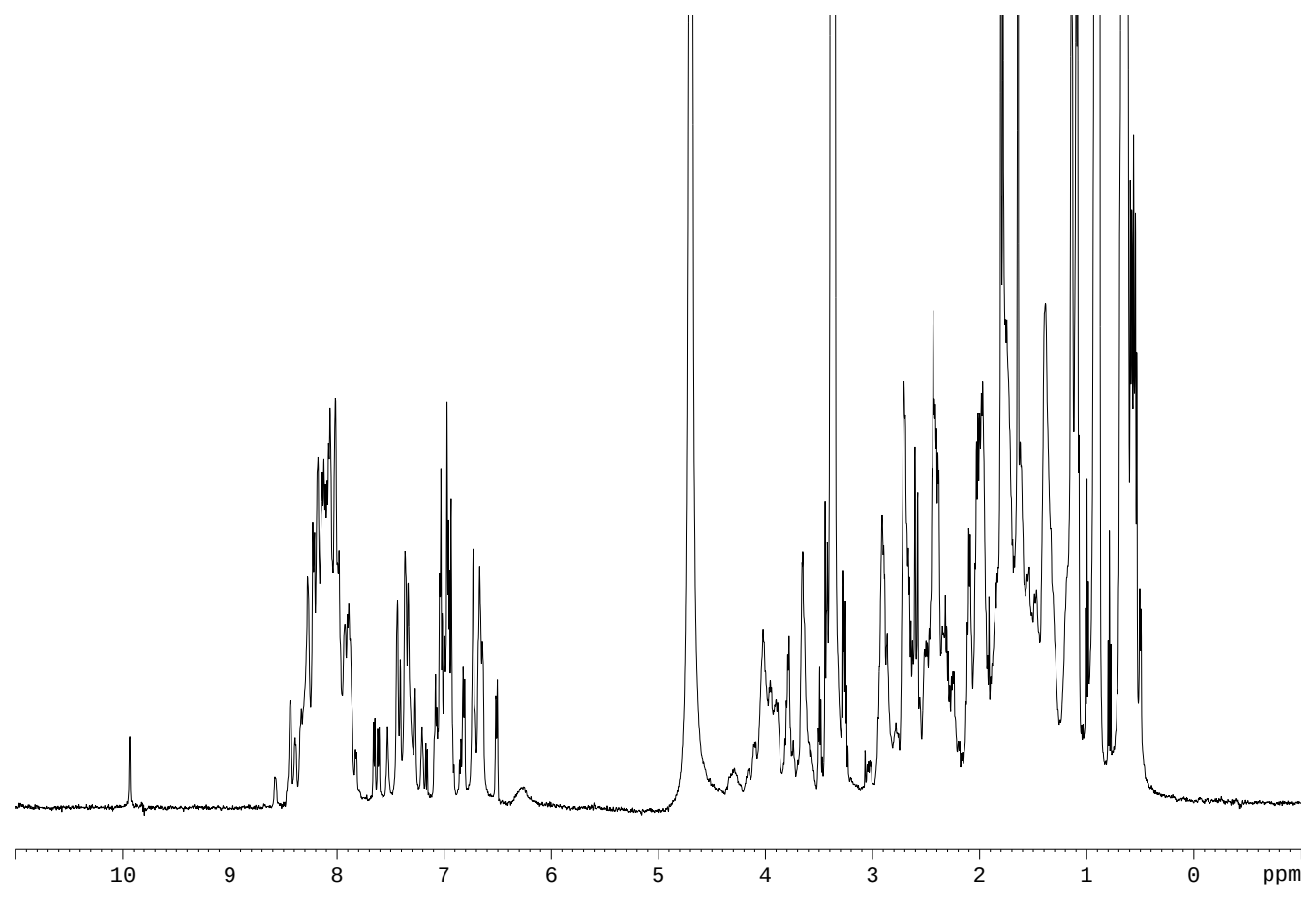
Differences in chemical shifts can be produced by structure and the accompanying anisotropy effect



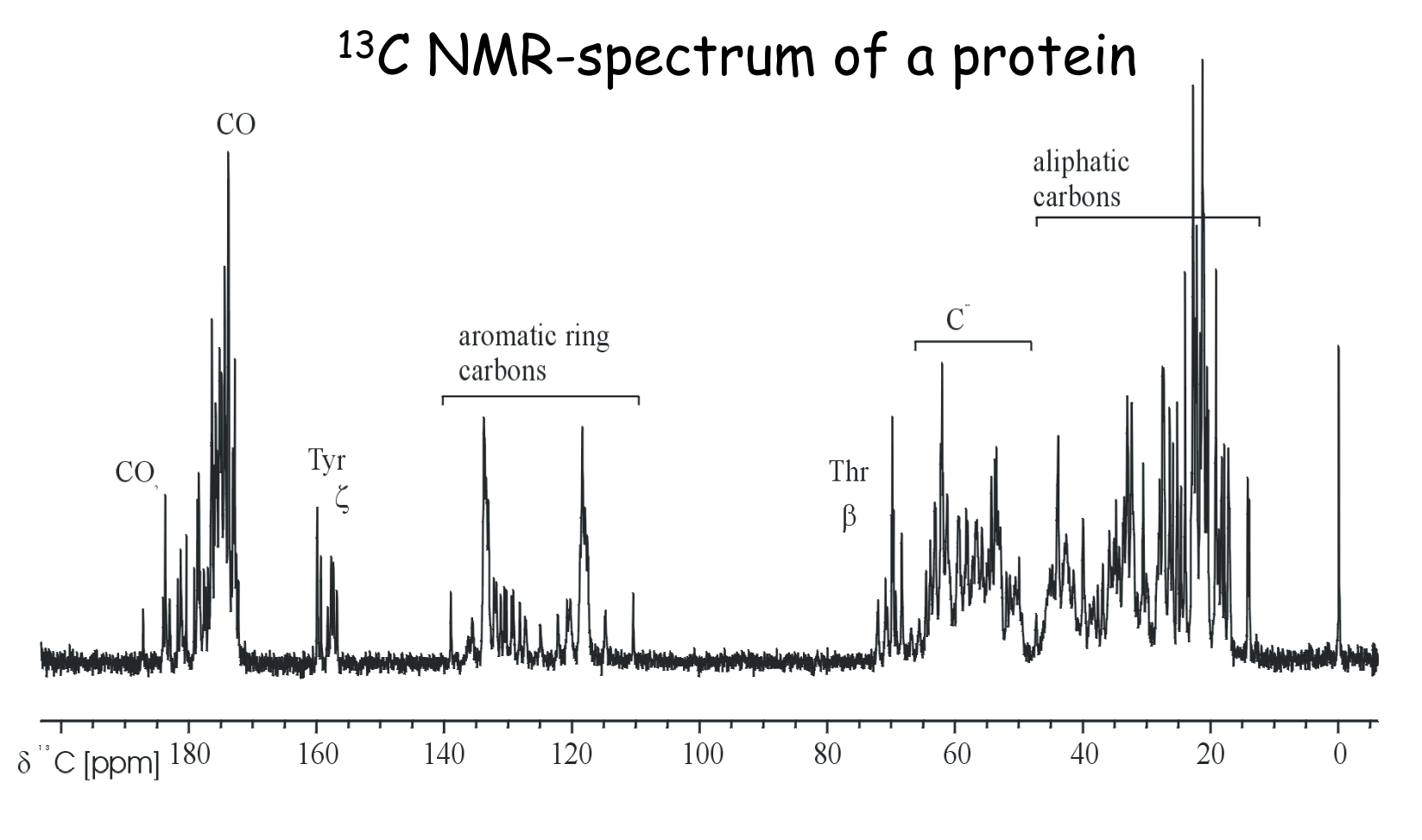


NMR-spectroscopy of proteins

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

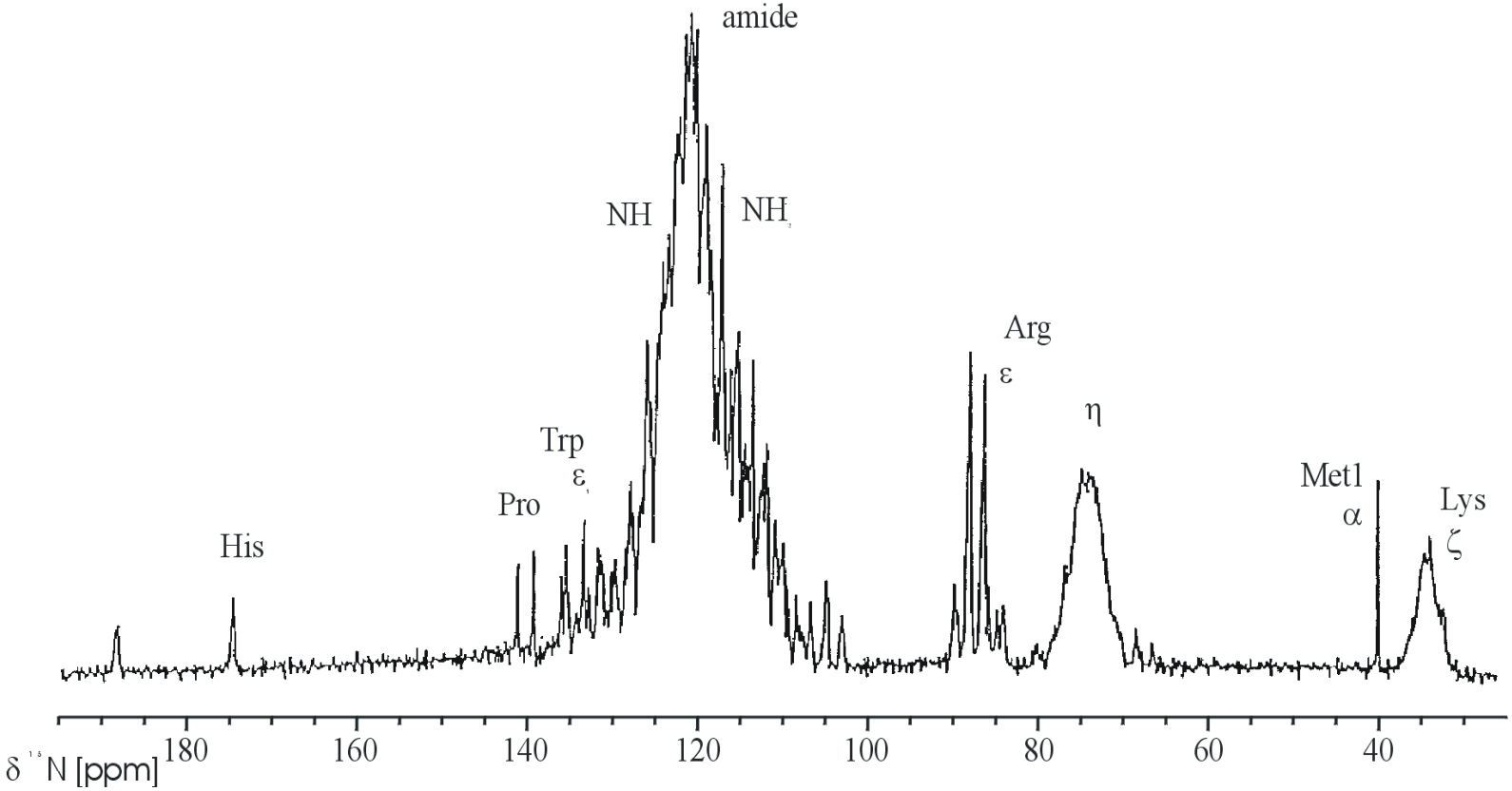


NMR-spectroscopy of proteins



NMR-spectroscopy of proteins

^{15}N NMR-spectrum of a protein



Sequence specific assignment

Sequence specific assignment

The solution of the assignment problem is the

sequence-specific assignment

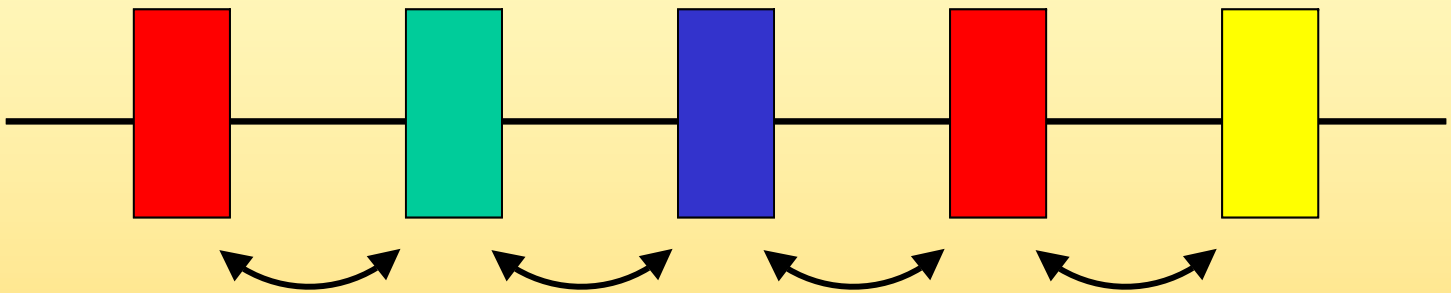
Two strategies exist:

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

Sequence specific assignment

Sequence-specific assignment

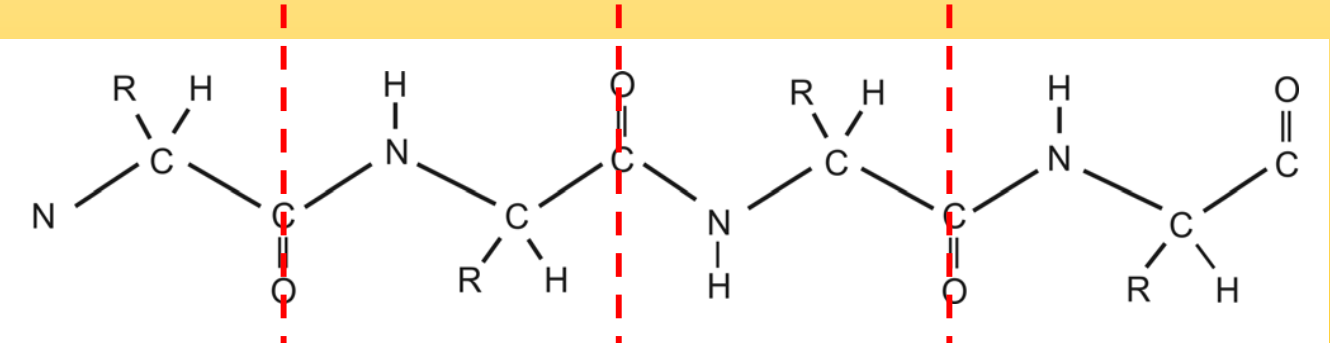


1. Which amino acid type is present (which color)
2. Which amino acid is next to which (neighborhood)
3. Comparison of subsequences with that of the protein

Sequence specific assignment

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

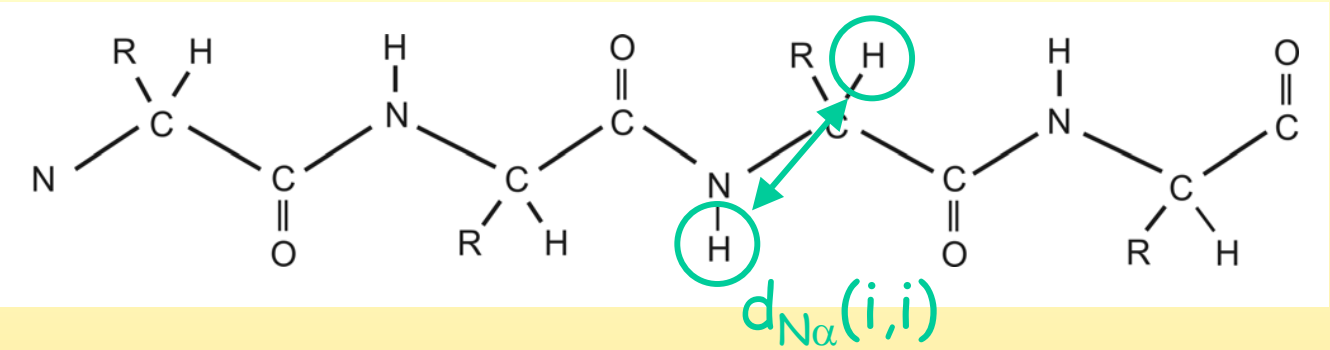


Sequence specific assignment

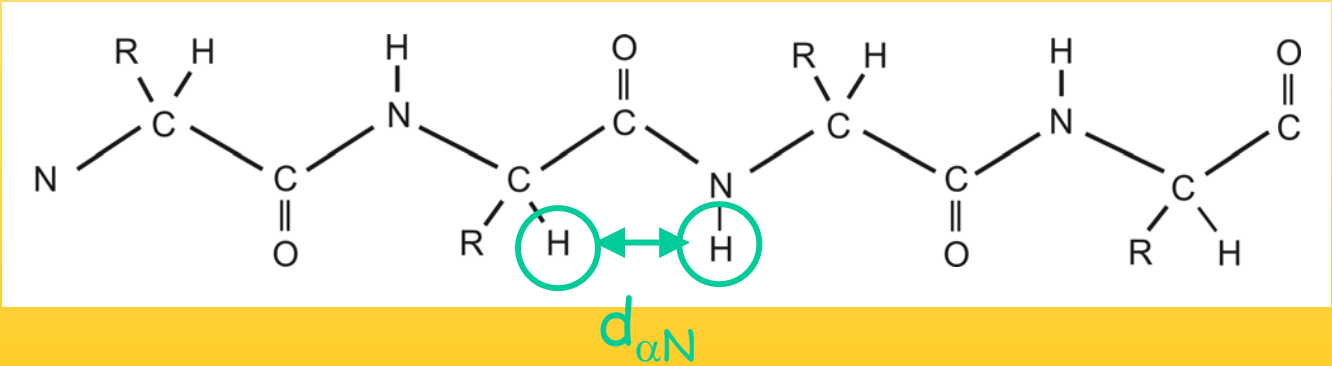
The neighborhood of the amino acids are then detected by through space interactions, i.e. in NOESY spectra.

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

Sequence specific assignment

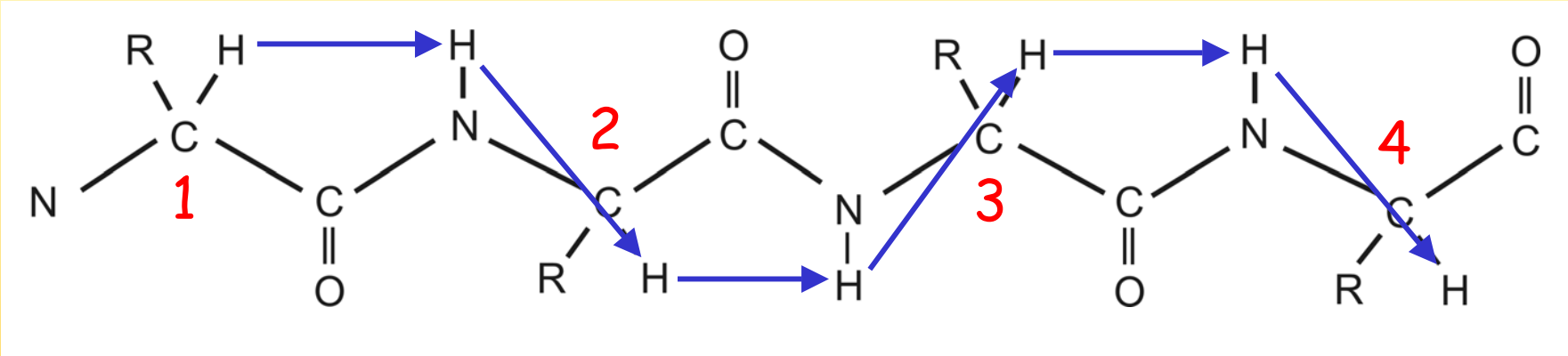


The distance from the H^N to the H^α of the same amino acids, $d_{N\alpha}(i,i)$, is always short enough to yield an NOE. The same is true for the distance from the H^N to the H^α of the amino acid (i-1), $d_{\alpha N}$



Sequence specific assignment

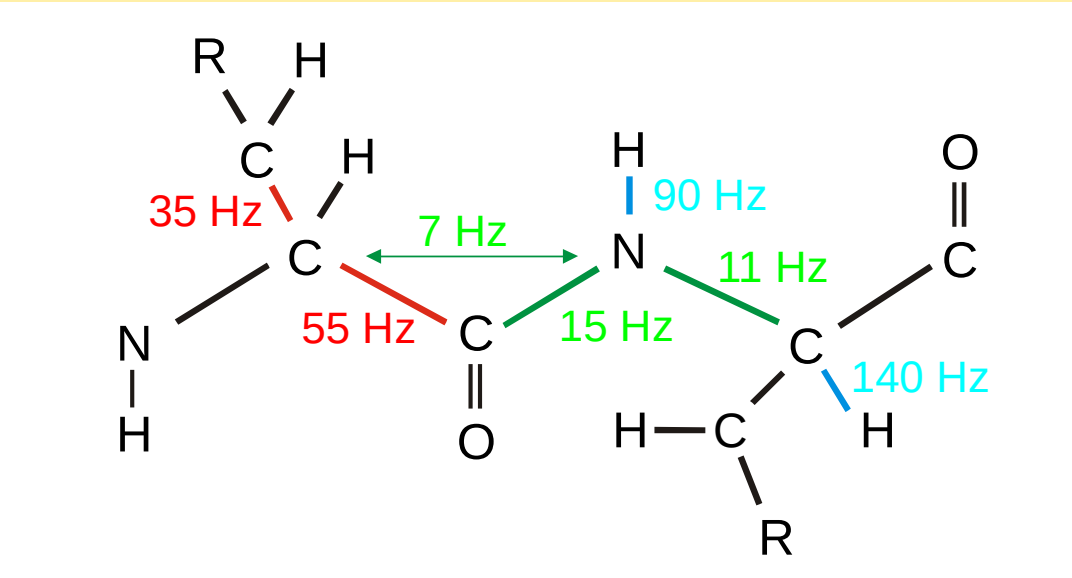
A neighborhood of amino acids is thus established



$1 \xrightarrow{d_{\alpha N}} 2 \xrightarrow{d_{N\alpha}(i,i)} 2 \xrightarrow{d_{\alpha N}} 3 \xrightarrow{d_{N\alpha}(i,i)} 3 \xrightarrow{d_{\alpha N}} 4 \xrightarrow{d_{N\alpha}(i,i)} 4$

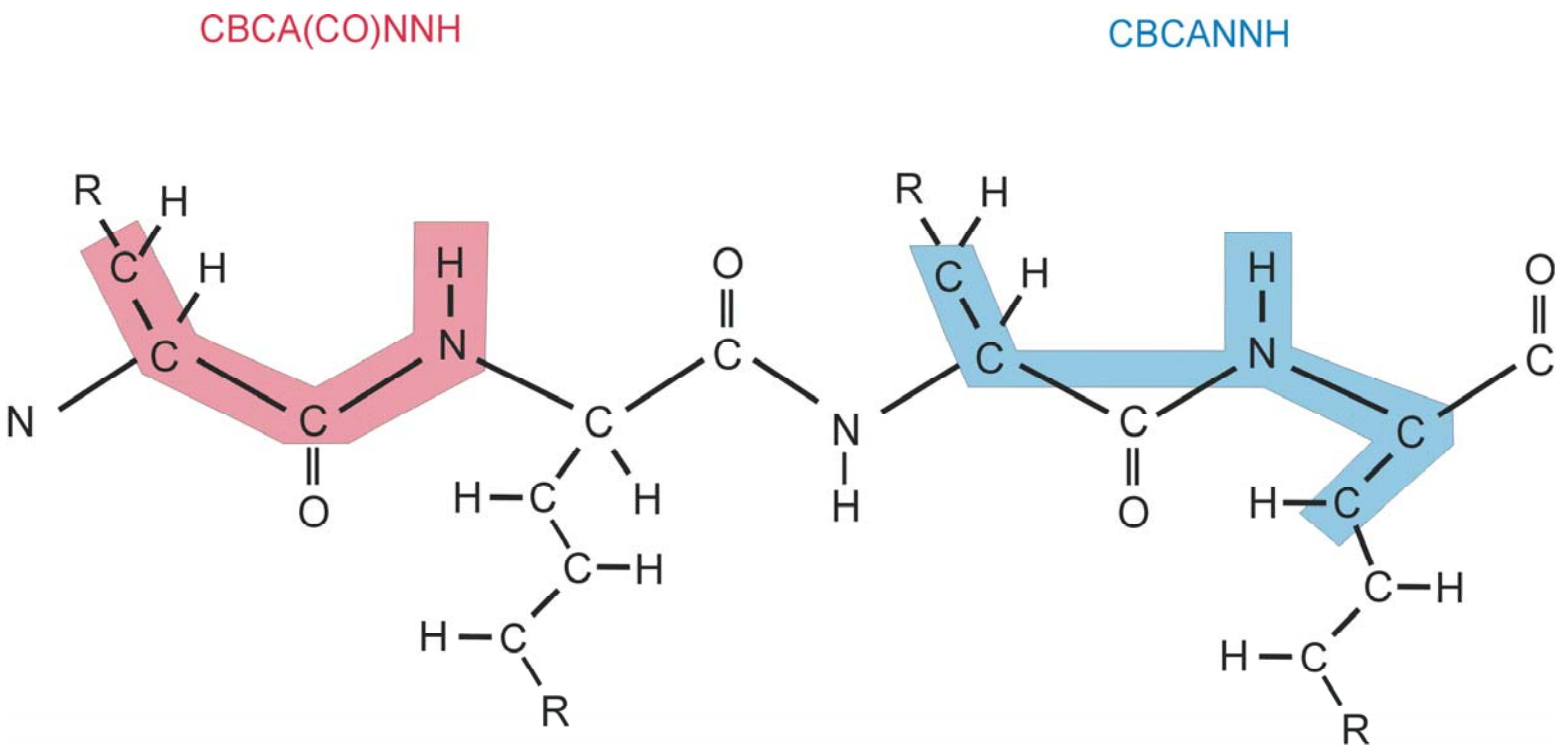
Sequence specific assignment

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



Sequence specific assignment

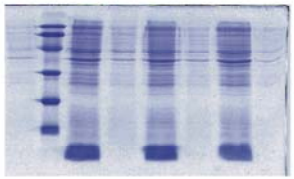
Mainchain assignment using tripel resonance experiments



A structure determination using NMR-spectroscopy

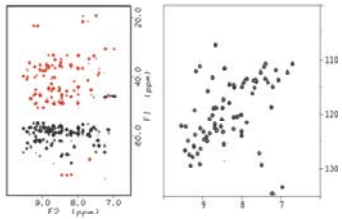
A protein structure determination

Bioinformatics

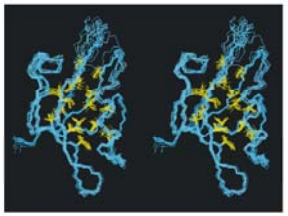


Protein expression

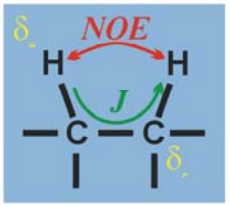
Data acquisition



Resonance assignment



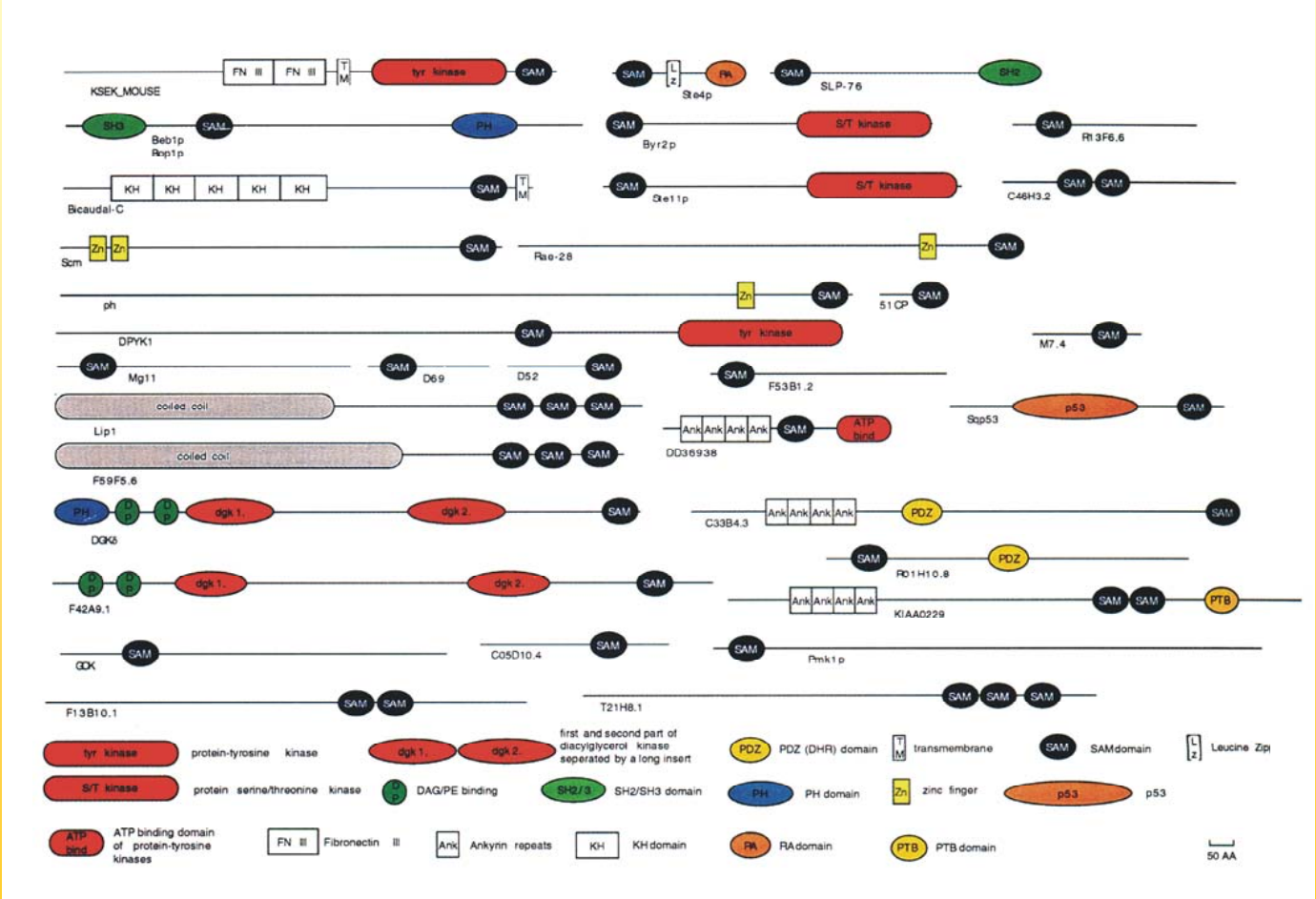
Structure calculation



Structurally relevant information

A protein structure determination

Bioinformatics (1)

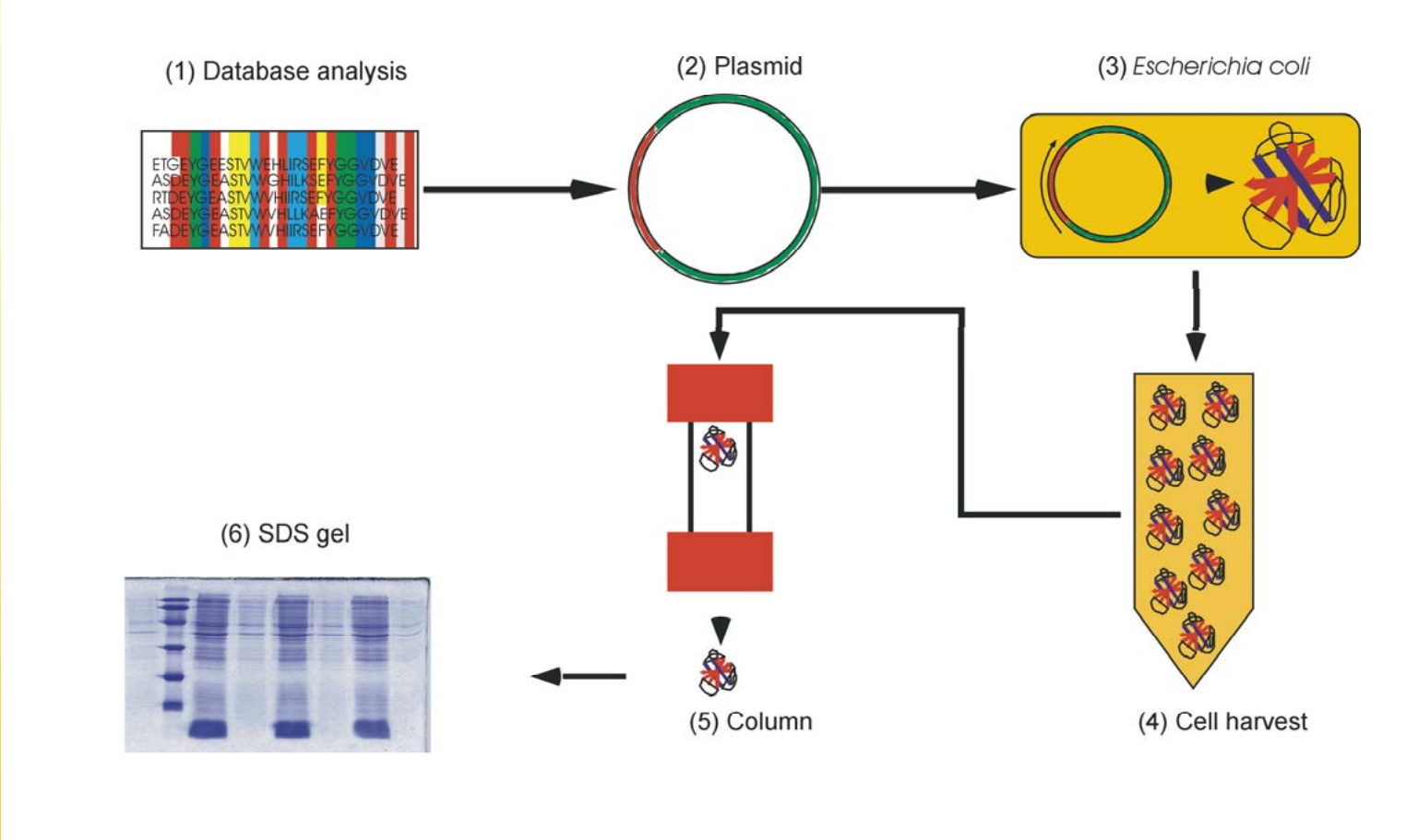


A protein structure determination

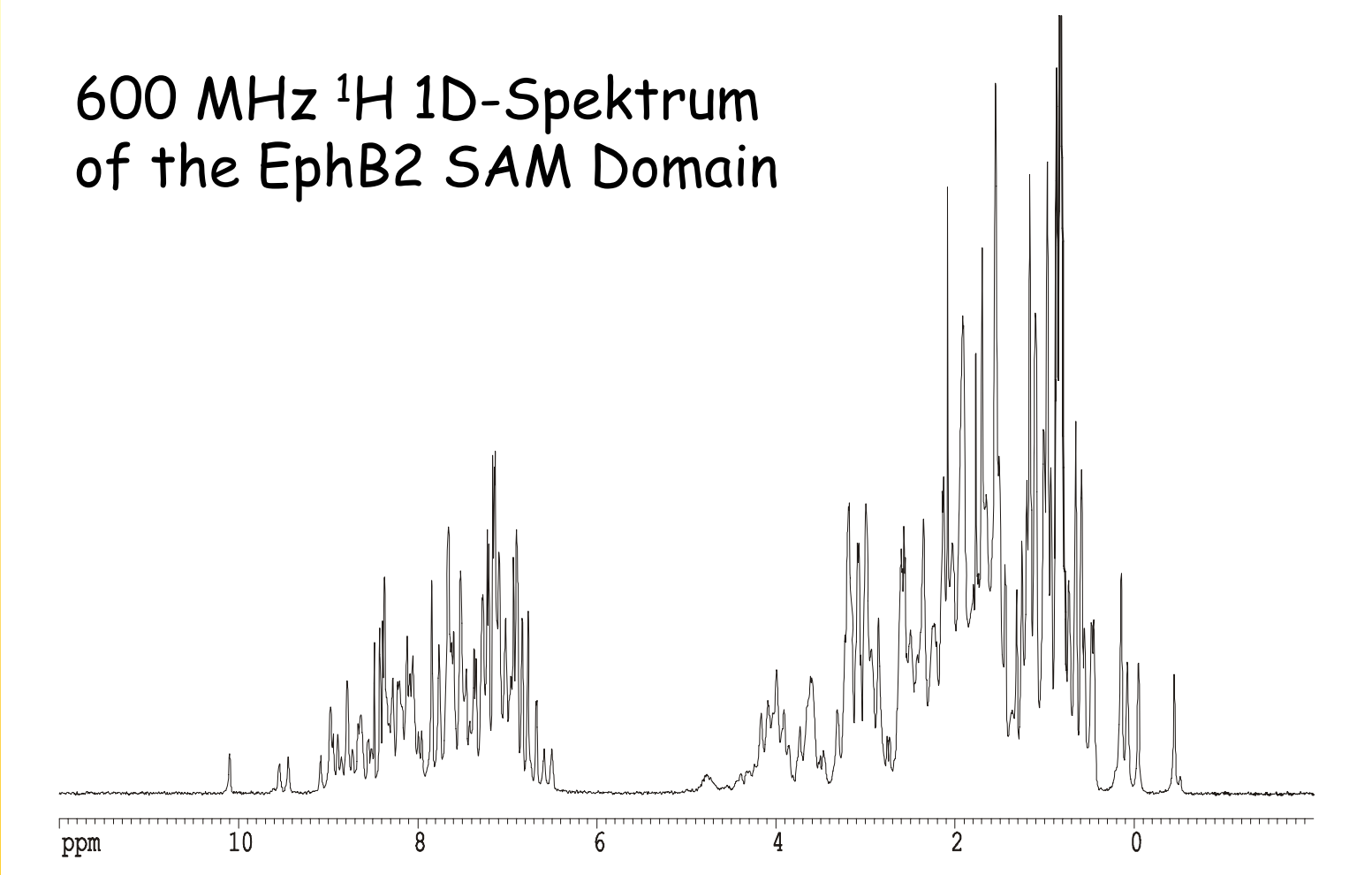
Bioinformatics (2)

A protein structure determination

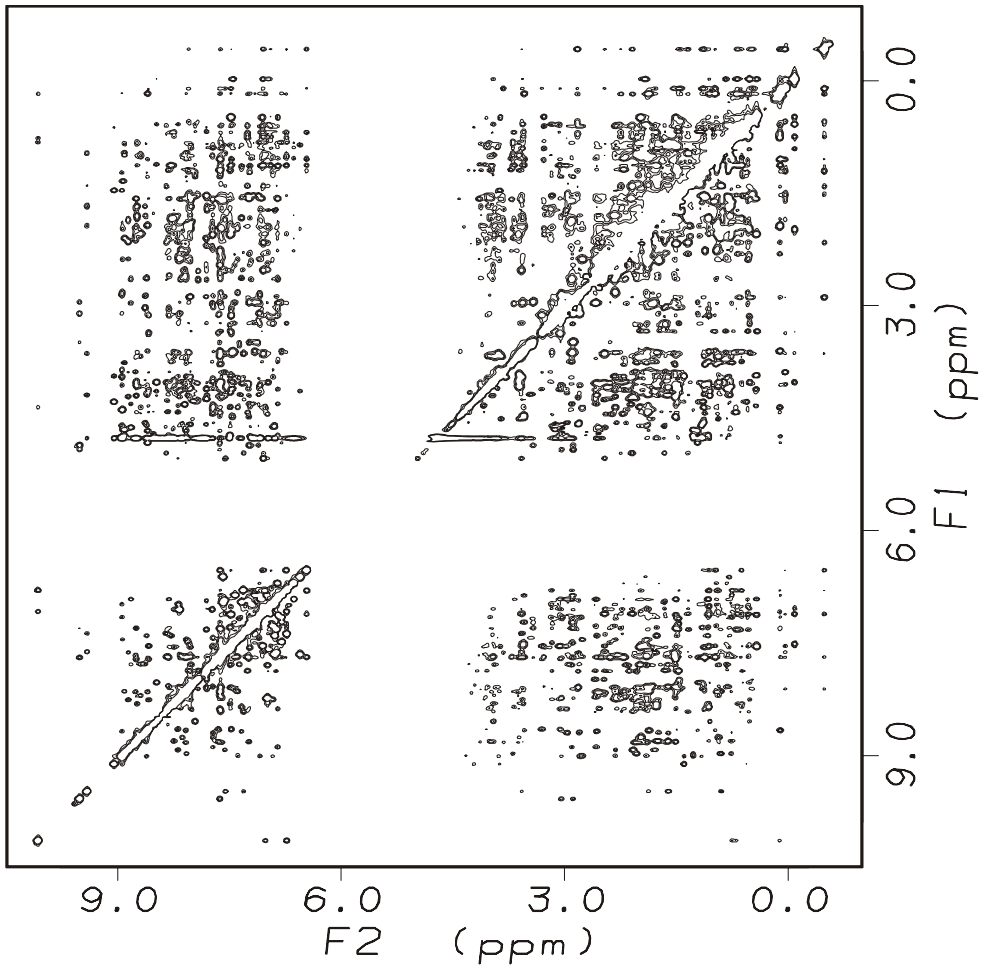
Protein expression and purification



A protein structure determination



A protein structure determination



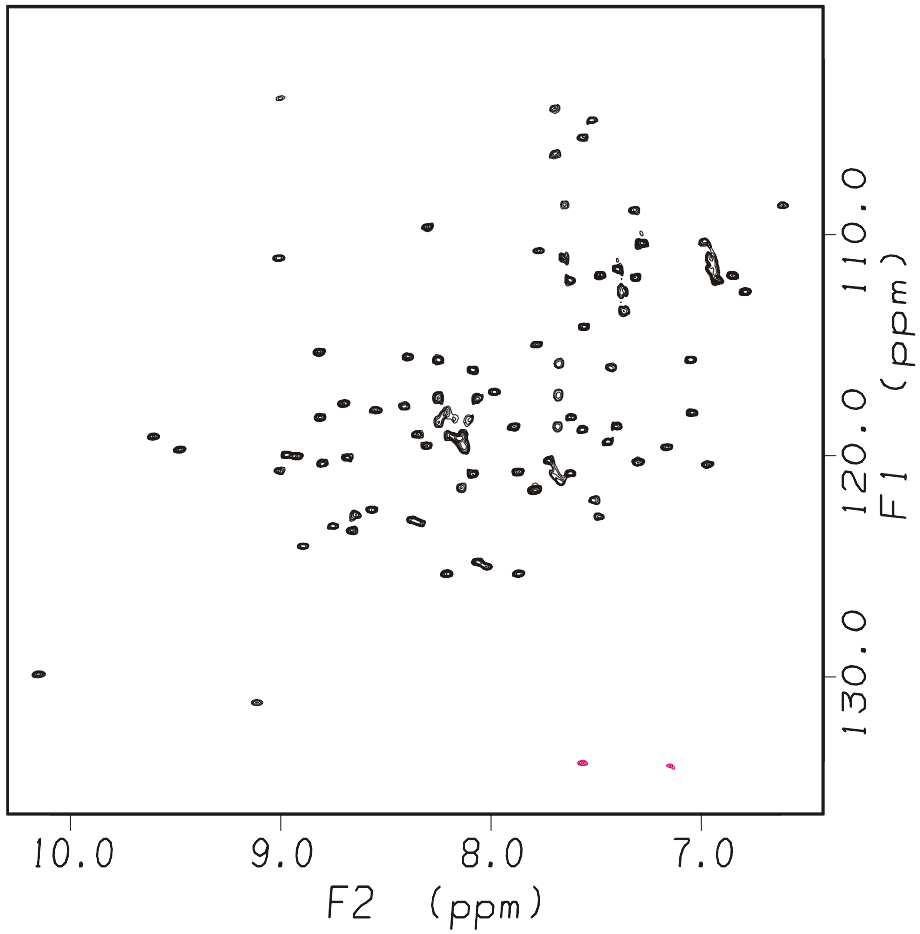
2D-NOESY
(100 msec)
of the EphB2
SAM Domain

A protein structure determination

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

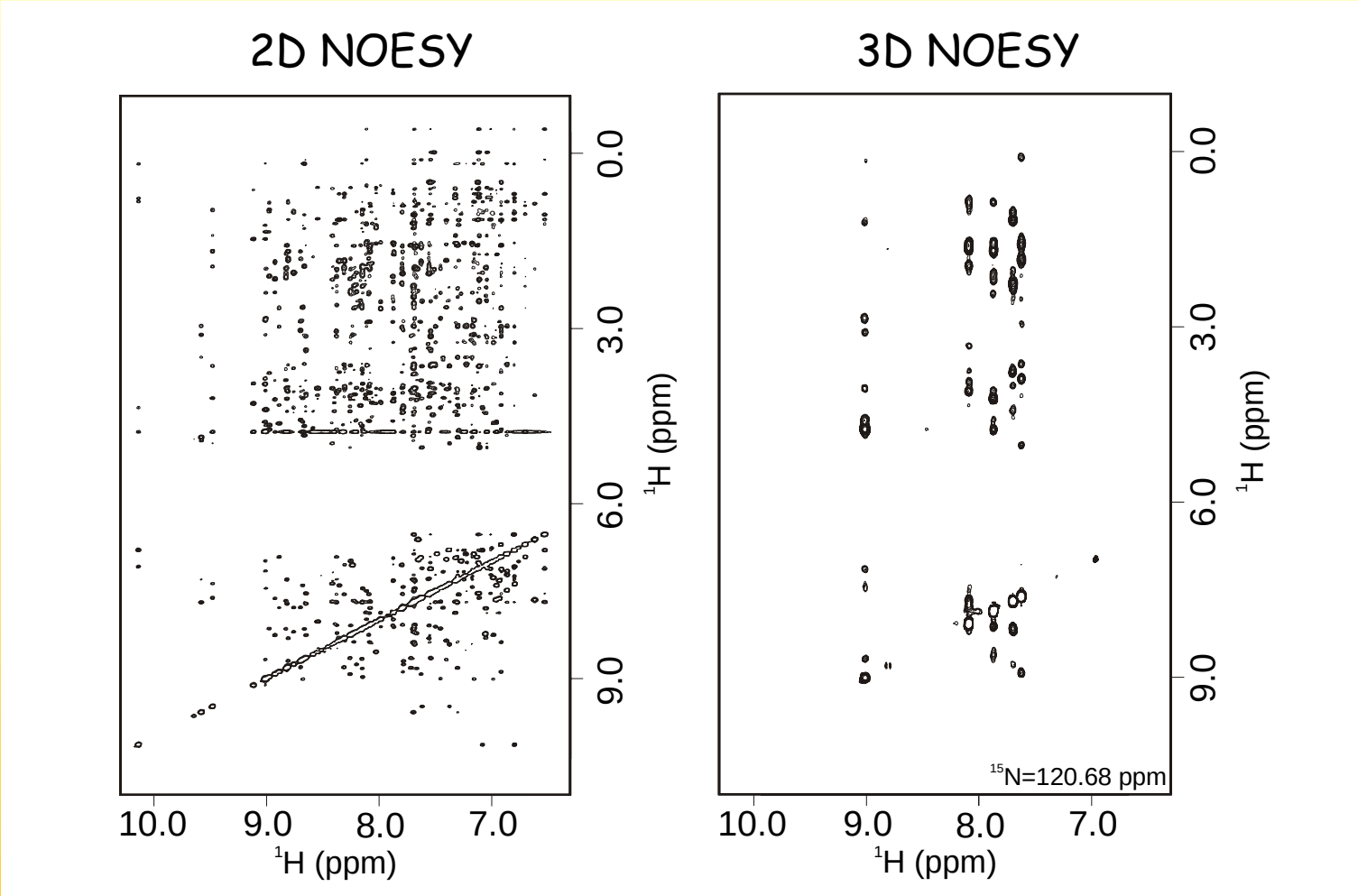
With the introduction of nitrogen and carbon labels this problem can be ameliorated because of the better resolution in the heteronuclear spectra and the option to record well resolved 3D spectra.

A protein structure determination



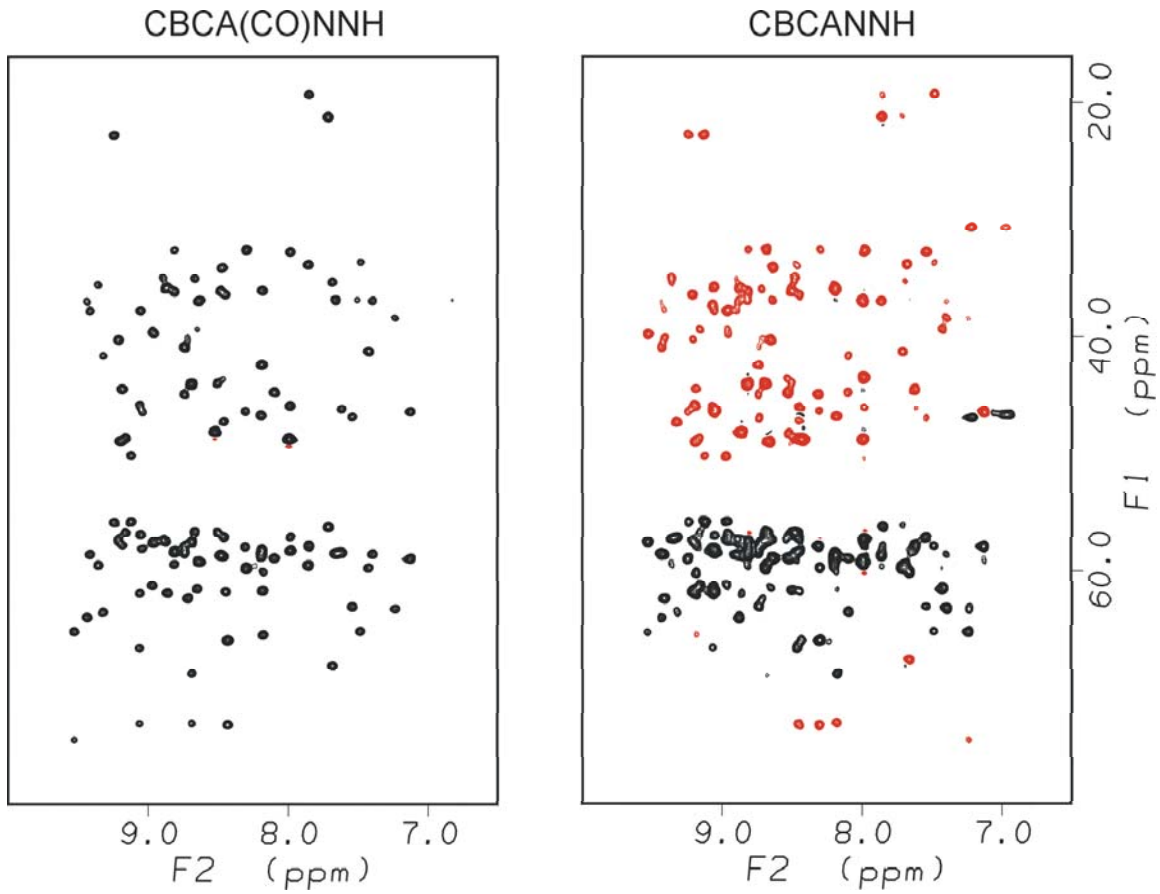
^{15}N-HSQC of the EphB2 SAM Domain

A protein structure determination



A protein structure determination

Mainchain assignment using tripel resonance experiments



A protein structure determination

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

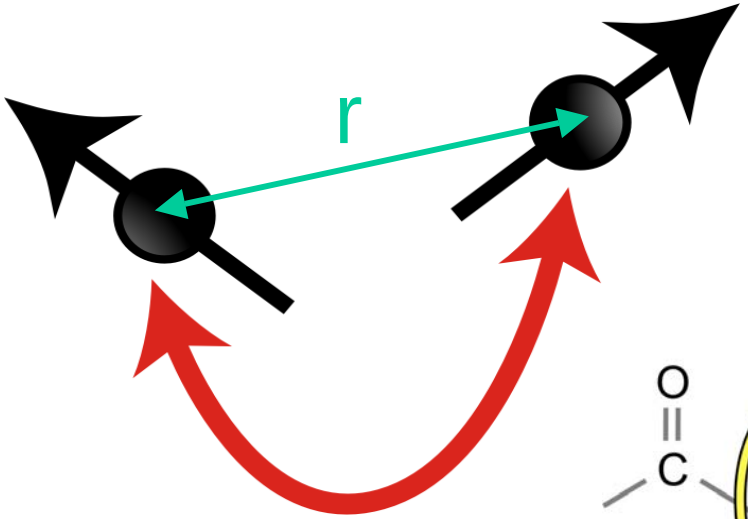
(H)C(CO)NNH, H(CCO)NNH

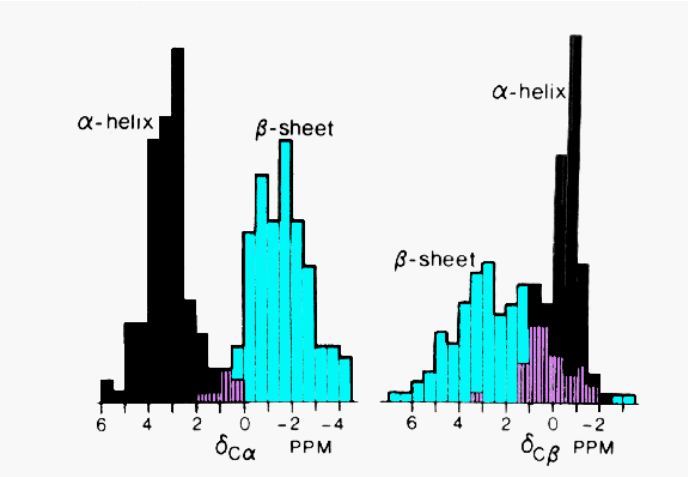
^{15}N-relaxation time experiments

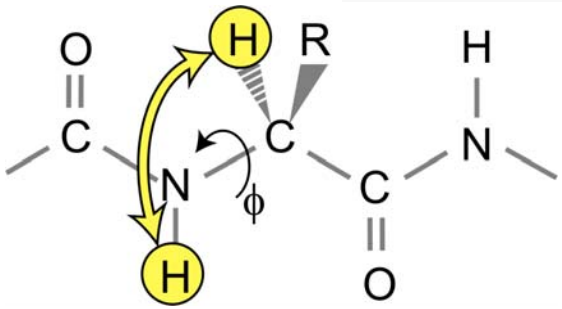
A protein structure determination

Structurally relevant information

distances



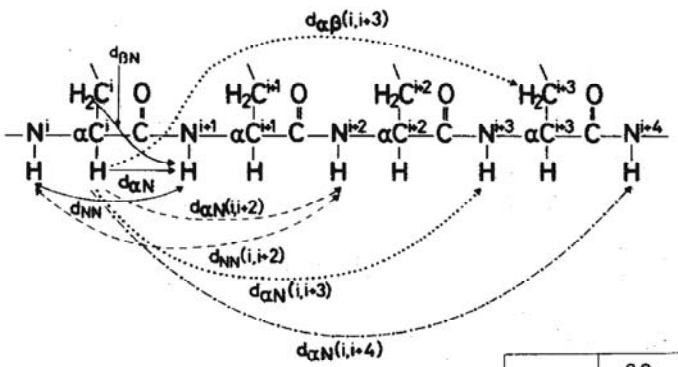


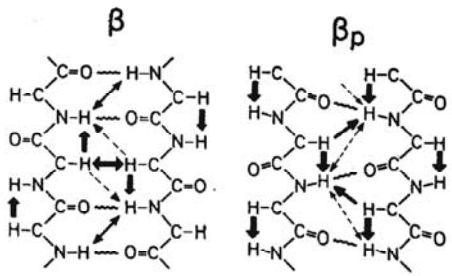


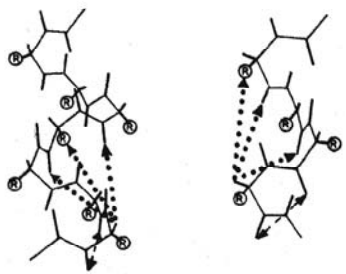
angles

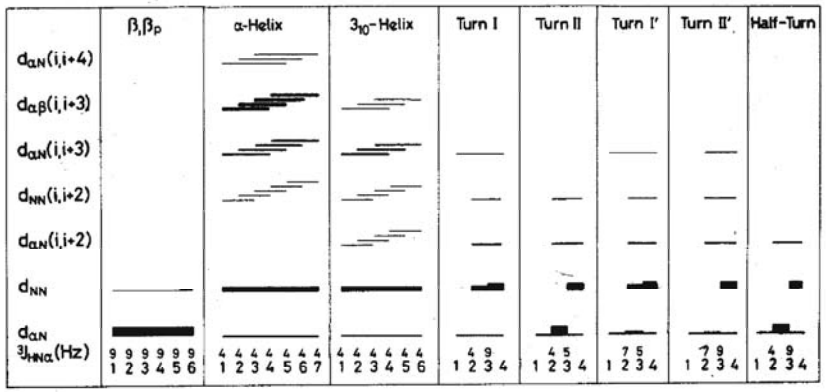
A protein structure determination

Distances give information on elements of secondary stucture



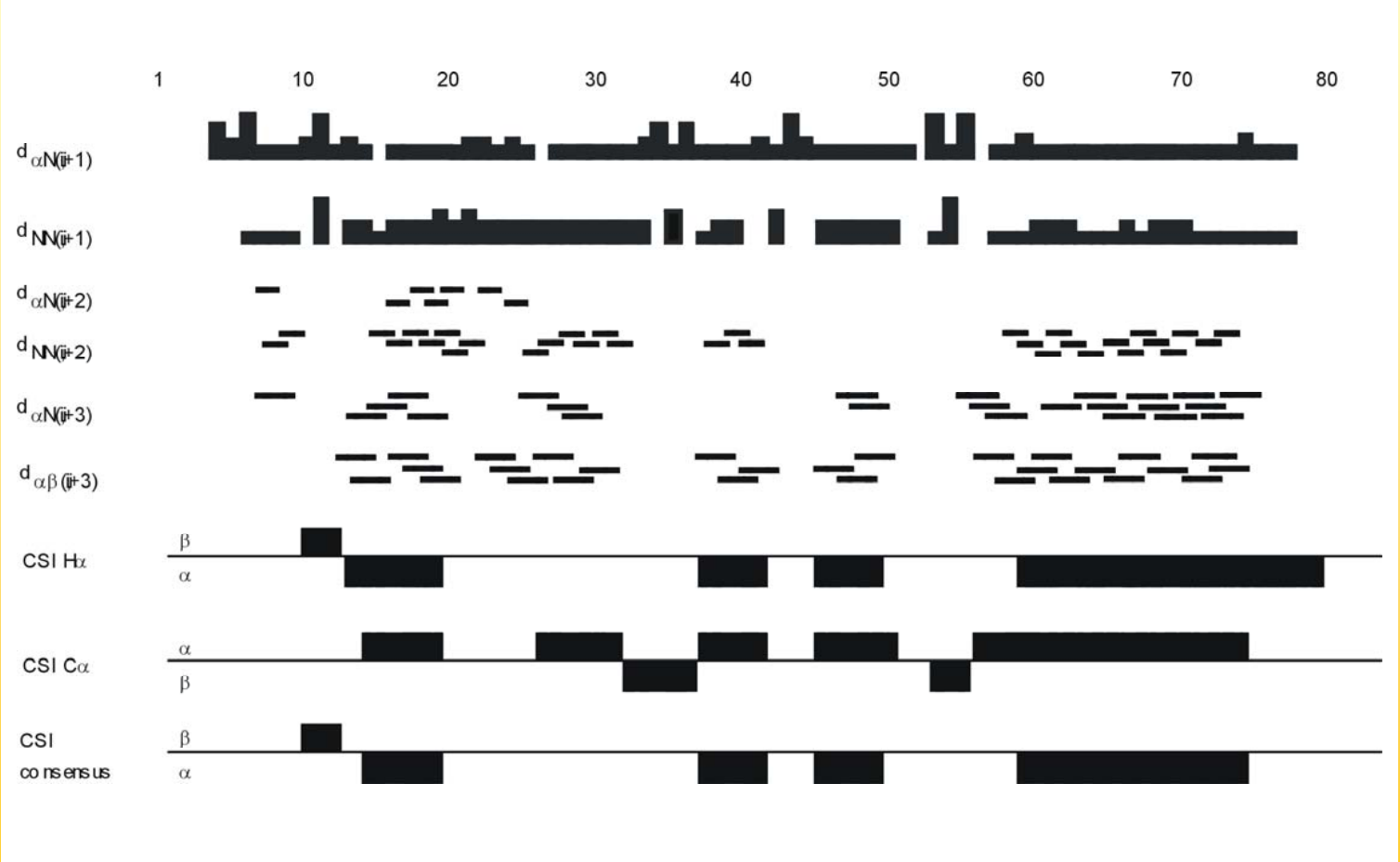






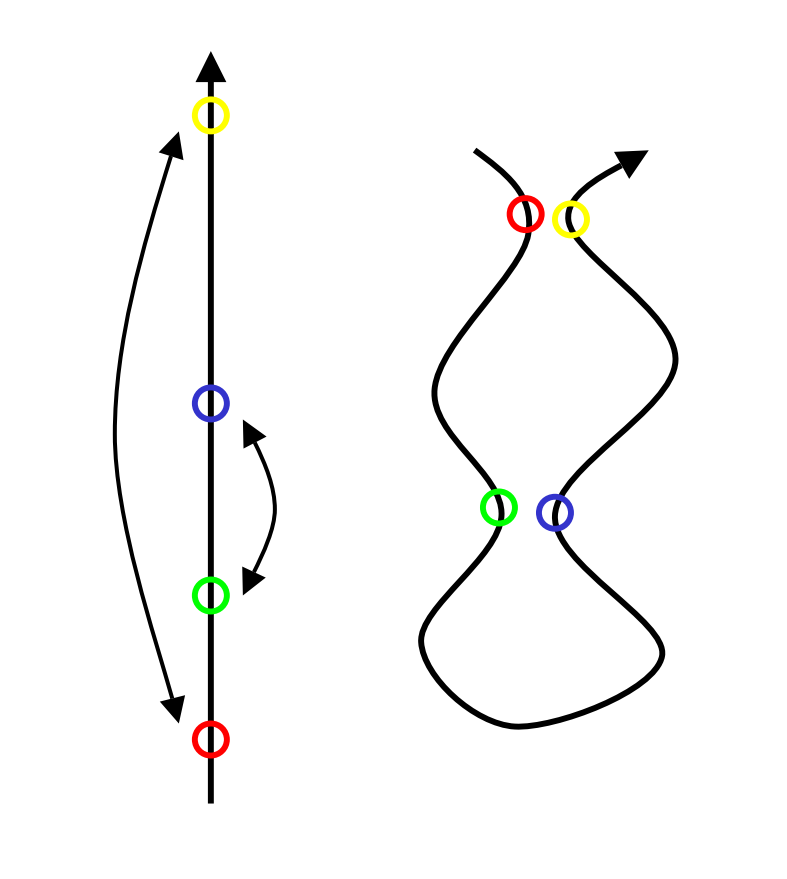
A protein structure determination

Structurally relevant information



A protein structure determination

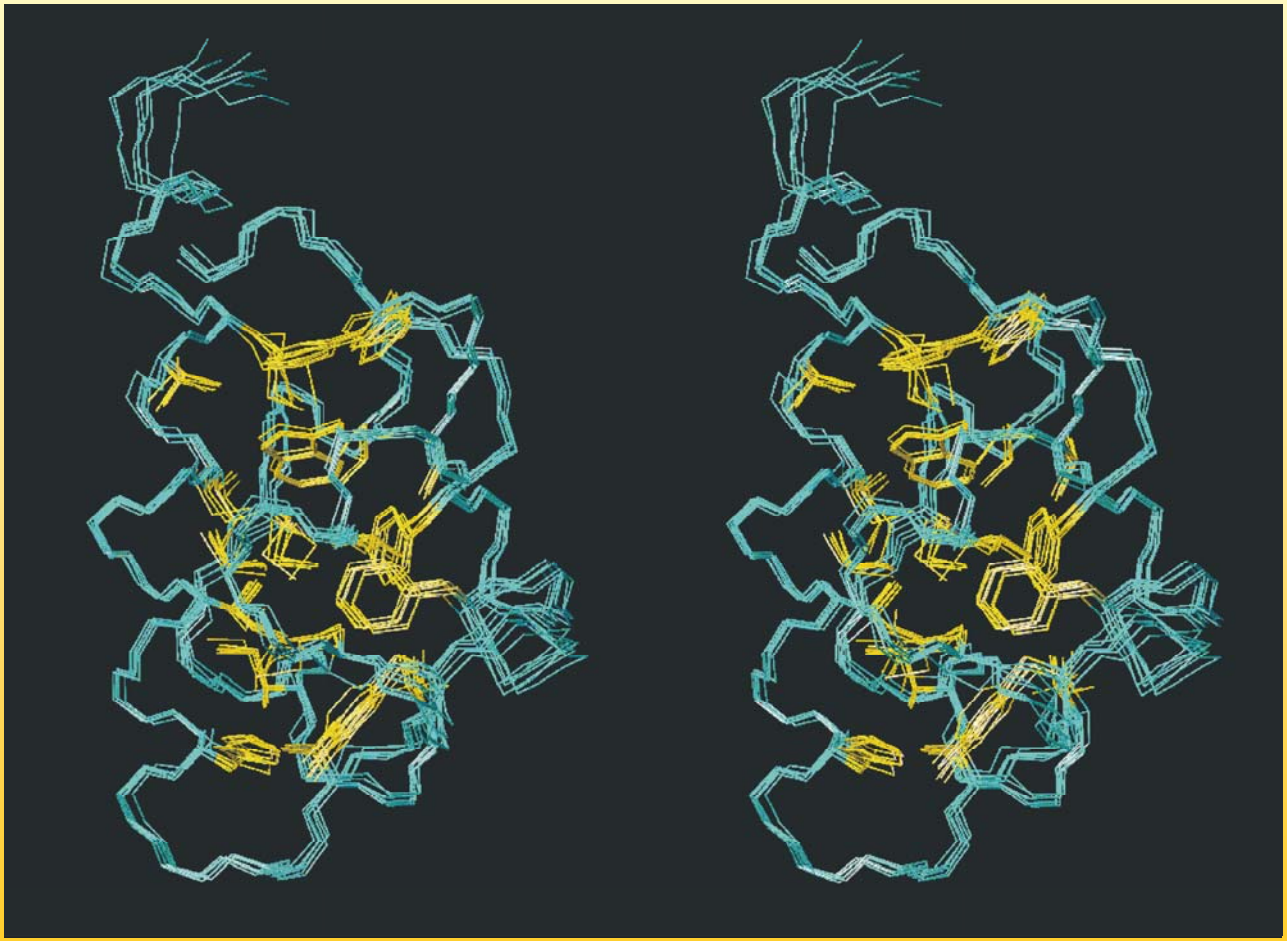
Distances determine the overall strcutre of the protein



Few distances are enough to „fold up" the protein

A protein structure determination

As a result a 3D structure can be calculated



Ligand-screening using NMR-spectroscopy

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

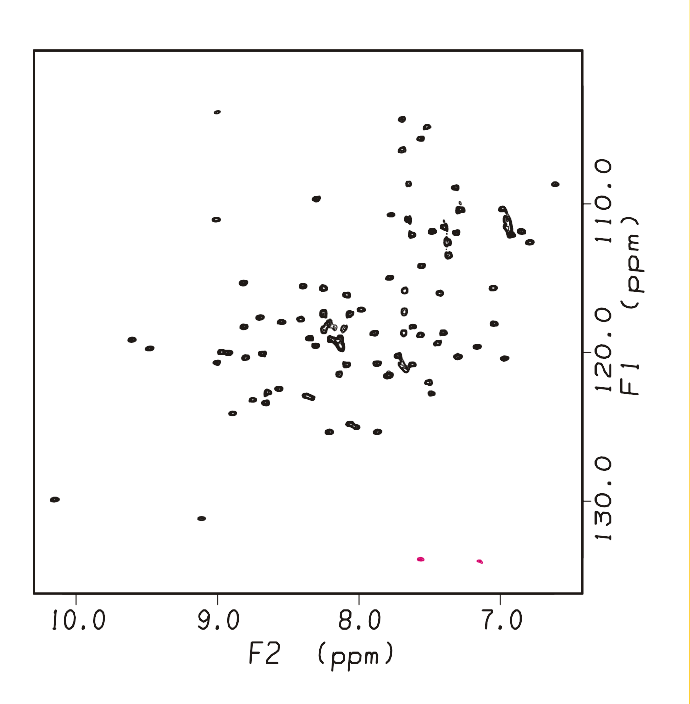
There are two major types of approach, the „ligand-detecting techniques" and the „protein detecting techniques"

Ligand Screening

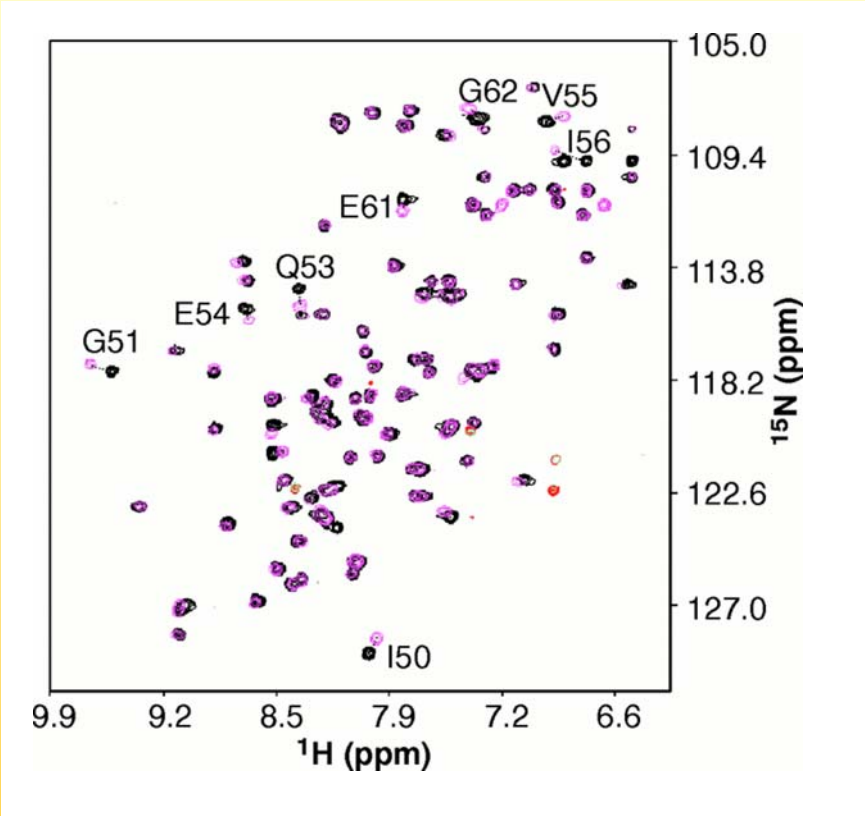
A technique of major importance from the class of protein-detecting techniques is called

„SAR-by-NMR"

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



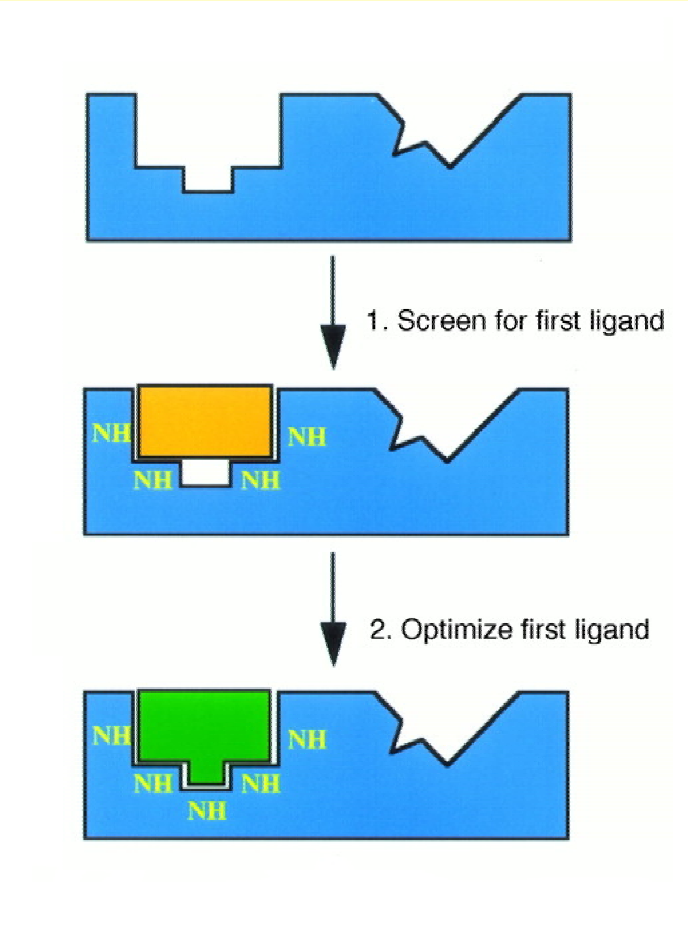
Ligand Screening



HSQC-specta 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

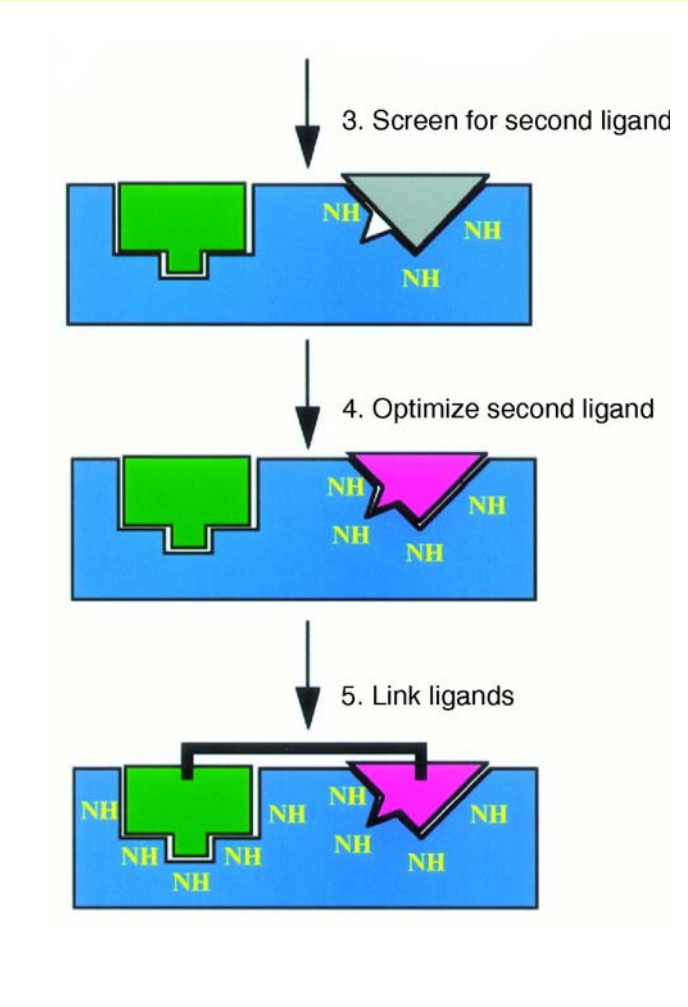
The method can be used in a „high-throughput" manner

Ligand Screening



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

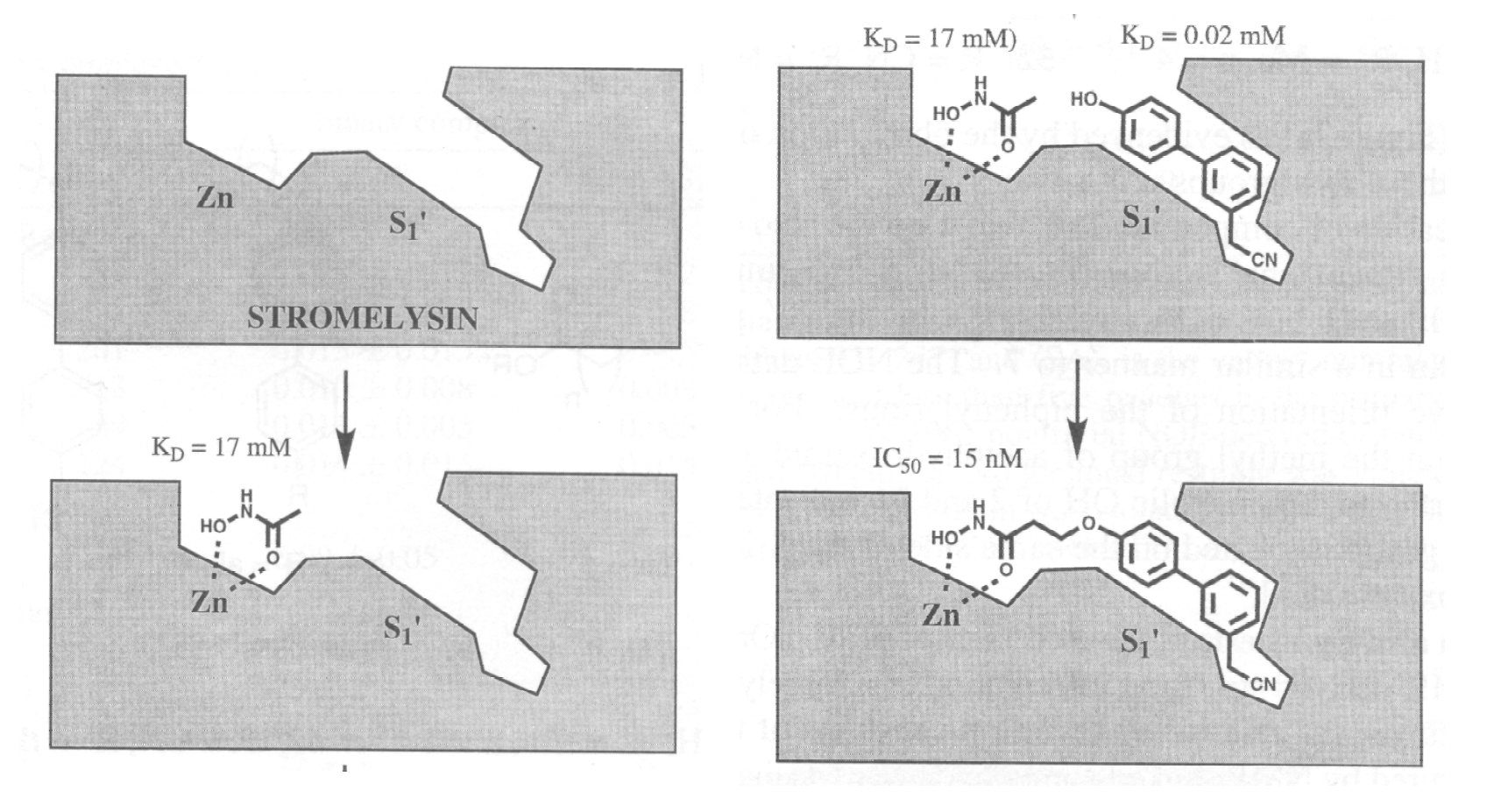
Ligand Screening



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

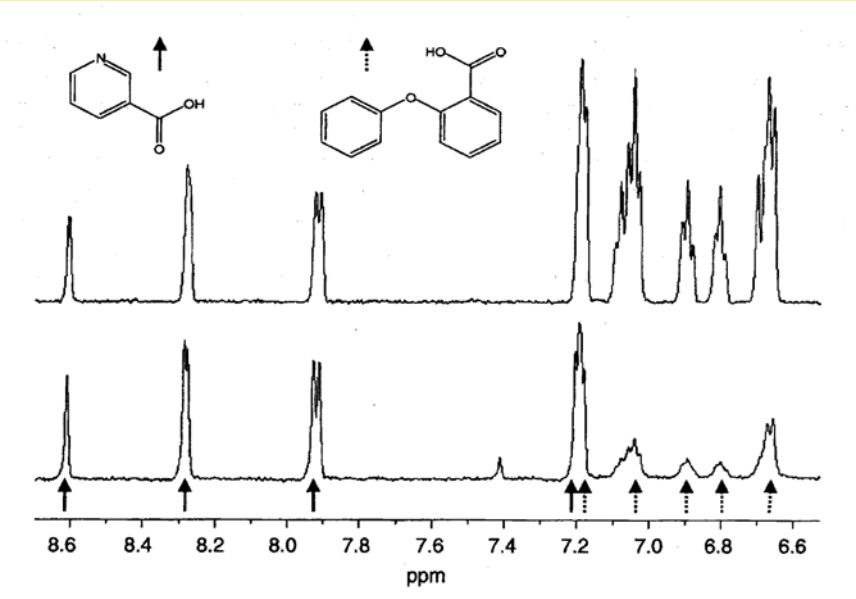
Ligand Screening

Application to the inhibition of stromelysin



Ligand Screening

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



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

Ligand Screening

Screening can be commercially interesting….

COMBINATURE
BIOPHARM AG

2007

Factsheet

NMR Fragment Screening

evotec

Tomorrow's Drugs Today™

Evotec offers a world leading, fully automated NMR fragment screening solution including protein production expertise, a 20,000 fragment library and extensive structure determination track record using NMR and X-ray crystallography.

The high sensitivity and robustness of NMR fragment screening enables the identification of particularly low molecular weight binders. Structural insight into sub-site occupancy is immediately obtainable for tens to hundreds of compounds. Artefacts through small molecule aggregation, protein or compound precipitation are revealed by gated 1H-NMR assays. NMR fragment screening is a complementary orthogonal approach to Evotec's proprietary biochemical fragment screening technology, which together form the uniquely positioned fragment based drug discovery (FBDD) technology platform, EVOlution™.

Fragment-based drug discovery by NMR

1. Screen for highly efficient fragment binders (blue)
2. Determine or model 3D protein-fragment structure
3. Analogue & determine binding sub-sites of substituents (orange) by HT NMR
4. Assay fragment leads in cellular / disease models

Data adapted from a protein-protein interaction inhibitor programme [Angew. Chem. Int. Ed. (2006) 45, 3790.]

Application

- Identification of novel chemotypes for targets requiring a high level of ligand specificity, such as proteases, kinases & phosphatases
- Leading approach for FBDD programmes where more than 50 mg of soluble protein is available
- Application to targets that failed in HTS or other biophysical assays
- Access to binding site information using Abbott's SAR-by-NMR™ assay technology

Our Capabilities

NMR-based Screening

- Automated sample preparation & data acquisition system with highly sensitive high field 600 MHz Cryo-Probehead™
- Worldwide license for Abbott's SAR-by-NMR™ technology delivering unique binding site information and robust 2D 1H-15N or 1H-13C HSQC assay results
- Ligand-detected NMR assays for unlabeled proteins without MW limit
- High success rate across numerous NMR screens

Library of 20,000 Fragments

- World's largest NMR screening library optimised for diversity and lead-likeness using proprietary software
- Solubility for each fragment tested experimentally
- All fragments reviewed by medicinal chemists for their scaffold-like attributes and suitability for analoging by parallel chemistry

Protein Production

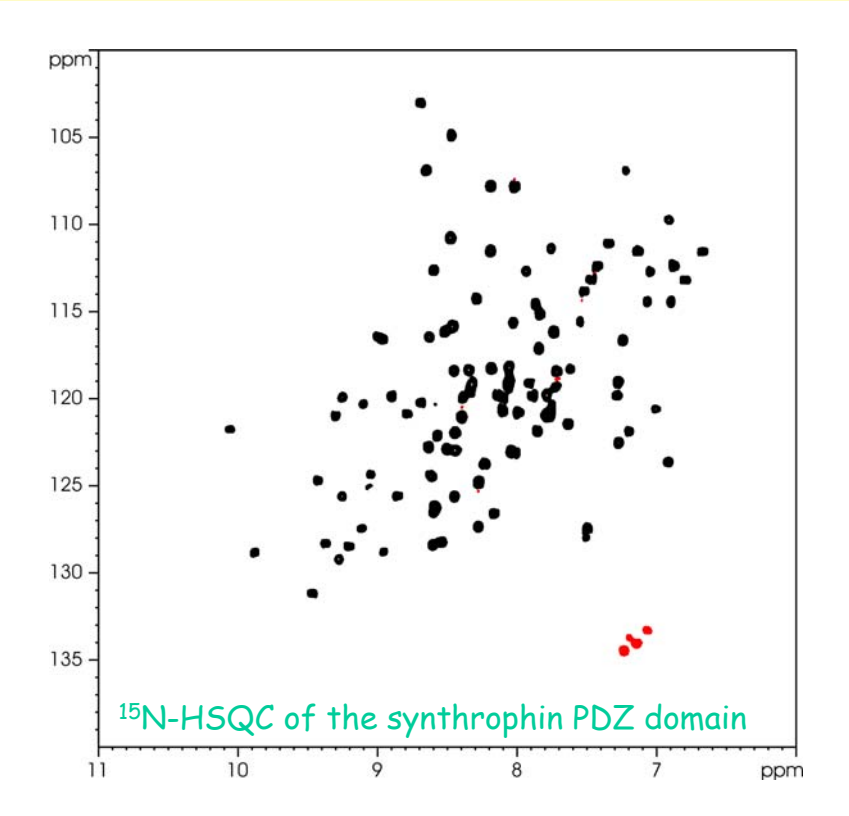
- Demonstrated expertise in multimilligrams production of isotope-labeled proteins for NMR studies
- Proprietary systems for construct design and protein purification
- Experience of more than 70 protein productions in bacterial, insect and mammalian expression systems

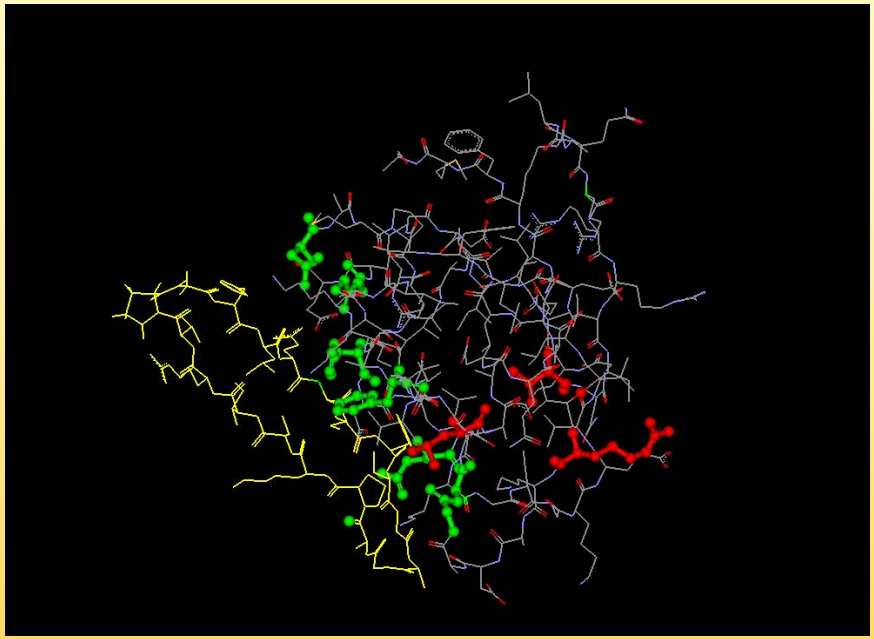
Structure Determination

- In-house X-ray crystallography diffractometer with access to the world's premium synchrotron facilities
- Access to 6 NMR systems (frequency ≥600MHz) dedicated to structure elucidation
- Leading network of academic consultants including the Oxford / Berlin biomolecular structure environment

This offering is available as a standalone service as well as being fully integrated into Evotec's unique and proprietary fragment-based drug discovery (FBDD) technology platform EVOlution™. For more information on Evotec's Innovation Centre for FBDD, EVOlution™ or business opportunities, please contact our commercial team at info@evotec.com or visit our website at www.evotec.com.

Ligand Screening





…but it can also be used to detect specific interations

Summary

using protein-NMR-spectroscopy it is possible

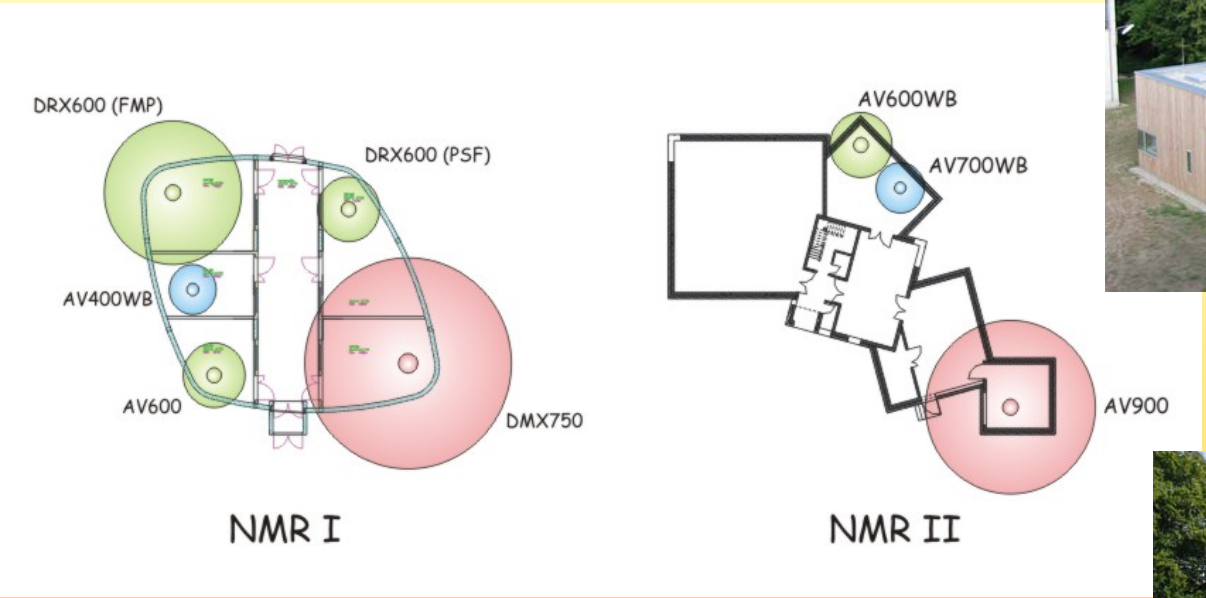
to determine the structure of small to medium sized proteins

to study protein-protein or protein-ligand interactions

Interested ?

Want to know more ?

The NMR facility



That's it

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