Practical course

„Molekulare und zelluläre Signaltransduktion"

NMR-spectroscopic investigation of proteins in solution

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NMR-spectroscopic investigation of proteins in solution

For an explicit understanding of the function of biological active macromolecules not only the chemical constitution but also the three dimensional structure, the dynamical properties and the interaction with other proteins and small molecules are of significant importance. The arrangement of functional groups in space and their mobility have essential influence on the specificity of interactions for example of ligands and proteins. Therefore besides functional studies characterization of the structure, dynamic and interactions of biomolecules are the prerequisite for an understanding of biological processes on an atomic level, which is the basic for a rational manipulation of these processes in terms of drug design.

With NMR structures can be determined in solution and in solid state. In solution the size of the protein and the solubility are limiting, in solid state low sensitivity and strong dipolar interactions. Nevertheless structure determination of proteins up to 50 kDa is possible, even for membrane proteins.

In an integrated structural biology approach techniques to determine three-dimensional structure of biomolecules can also be combined, e.g. cryo-electron microscopy and solid state NMR.

NMR-spectroscopy can also handle intrinsically disordered proteins (IDPs) and determine local structures.

Besides structure determination NMR is especial useful for investigating dynamics of biomolecules and for the study of protein ligand interactions. The latter are the actual key to the understanding of biological processes and therefore of special interest for pharmacology.

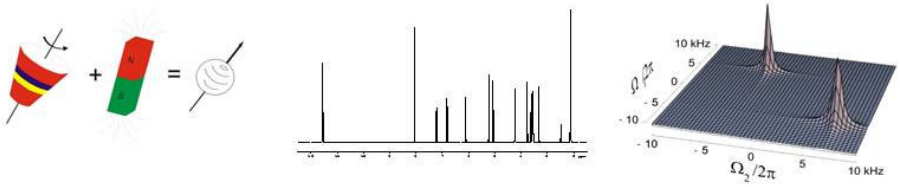
Prerequisite for structural and dynamic investigations using NMR is not only the production of sufficient amounts of pure and isotopically labeled protein but also the assignment of the resonances to the atoms of the protein.

This short practical course "NMR-spectroscopy" should give an insight into this assignment procedure of proteins in solution using triple resonance experiments.

The practical course "NMR-spectroscopy" consists of 4 parts:

NMR-spectrocopy of proteins in solution – an introduction

General and basic aspects, NMR-parameter, multidimensional NMR

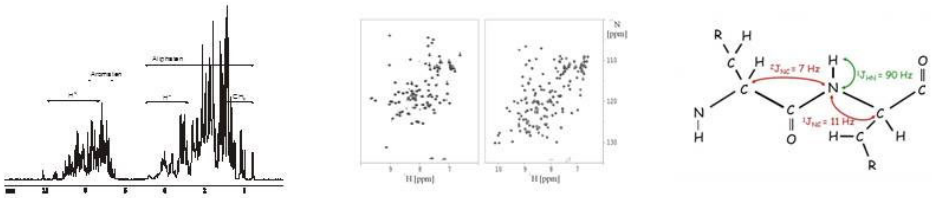


The NMR-spectrometer

spectrometer, shim, lock, 1D and 2D acquisition and processing

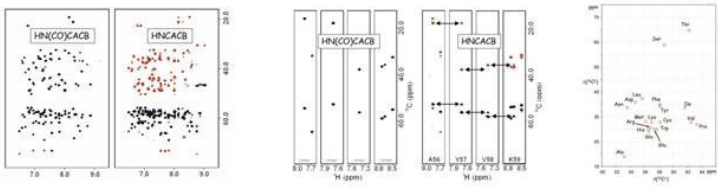
NMR-spectroscopy of proteins in solution – spectra and assignment

NMR-spectra of proteins, sequential assignment using triple resonance experiments



Application of sequential assignment

assignment of a stretch of the SH3 domain of α-spectrin using triple resonance techniques



http://schmieder.fmp-berlin.info/teaching/lehre_unis_berlin.htm

Nuclear Magnetic Resonance

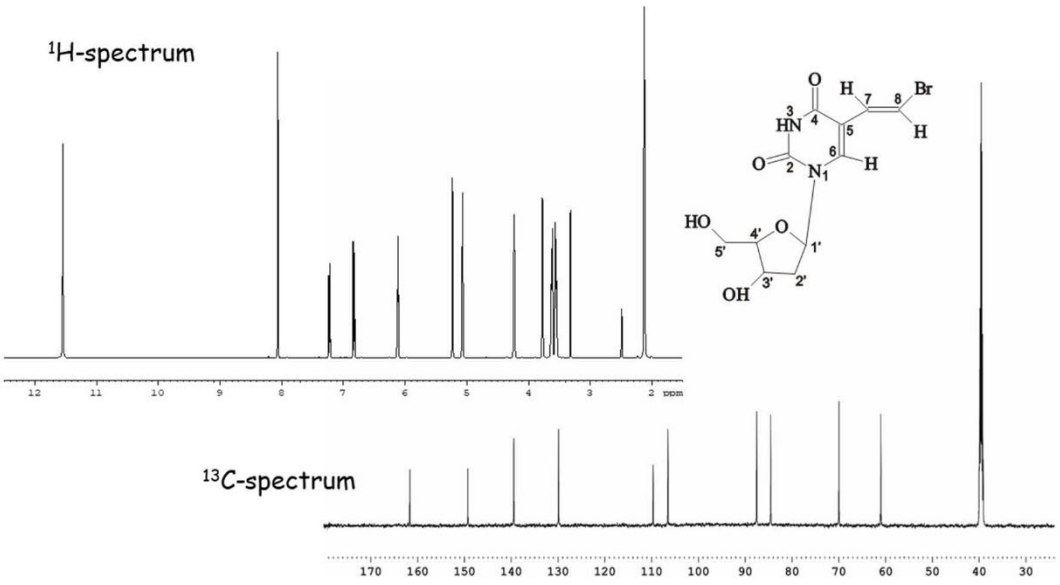
NMR-spectroscopy detects the resonance of a property of the atomic nuclei (the "spin") with radio waves. The effect is only readily observable in a strong magnetic field. Each nucleus is observed separately and interactions between nuclei can be observed as well.

The picture of a molecule provided by NMR thus corresponds well to the view of a chemist that is seeing molecules as atoms connected by bonds.

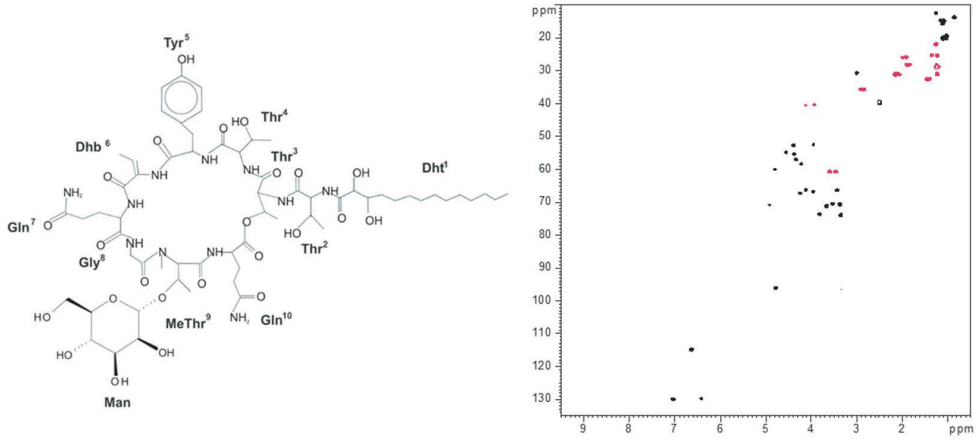
In the areas of biochemistry and structural biology NMR yields information on structure, ligand-interaction and mobility necessarily at atomic resolution.

NMR is the only high-resolution method that can observe hydrogens !

NMR as an analytic tool during synthetic work

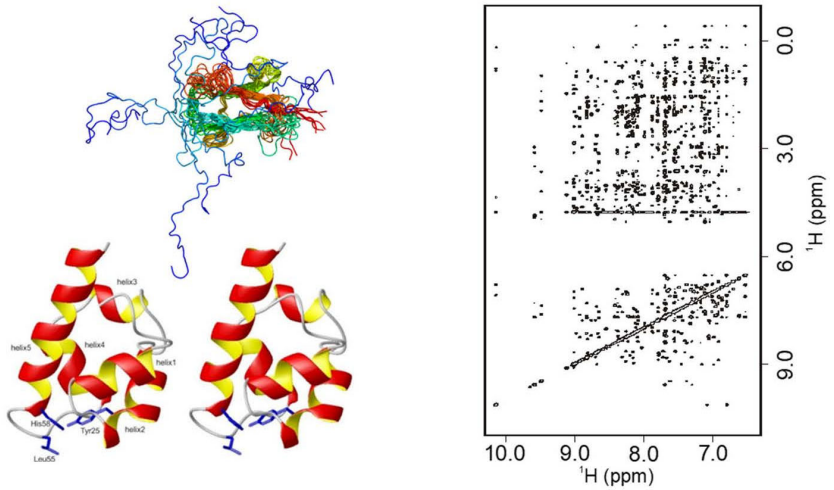


Determination of the constitution of natural products



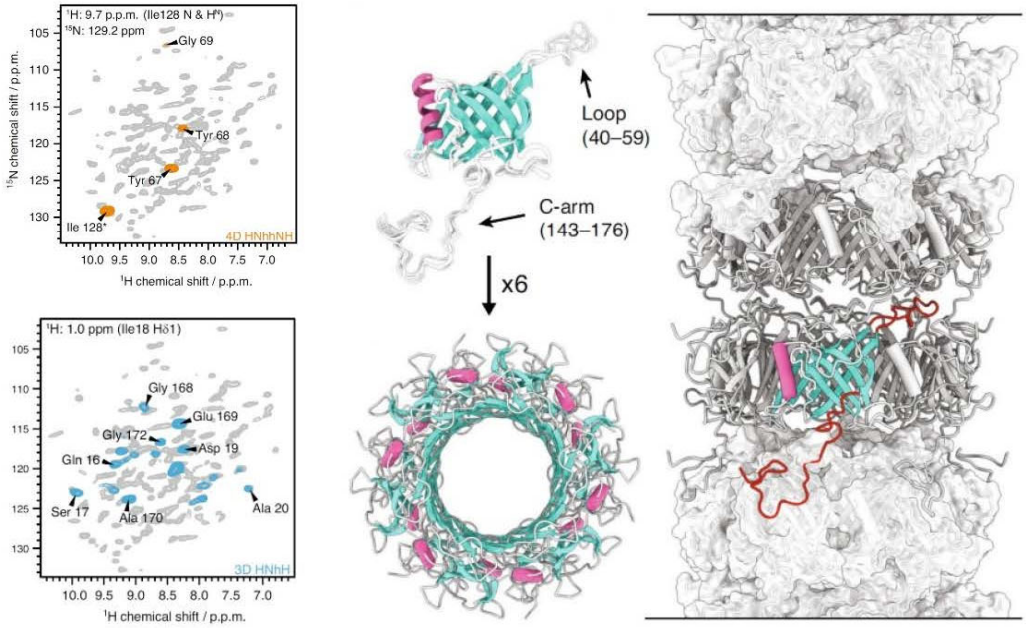
NMR is extremely powerful in determining the constitution of natural products, here 2D-NMR is indispensable.

Determination of 3D structures of proteins

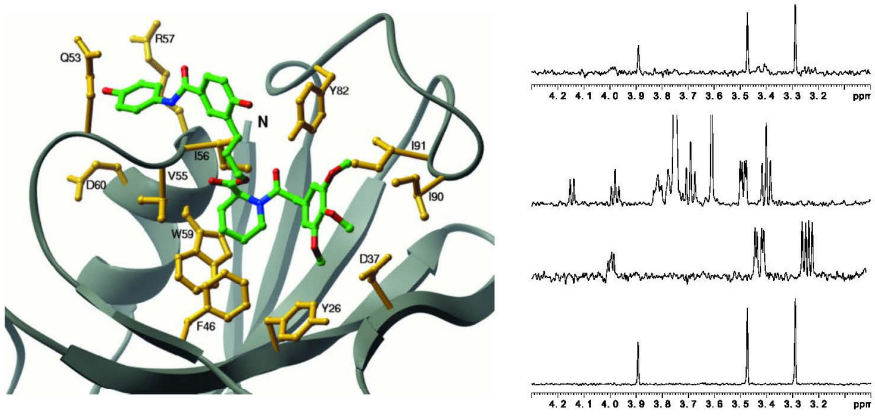


Using NMR the structure of proteins up to a certain limit can be determined using either solution or solid state NMR

Structure determination using „Integrated Structural Biology"

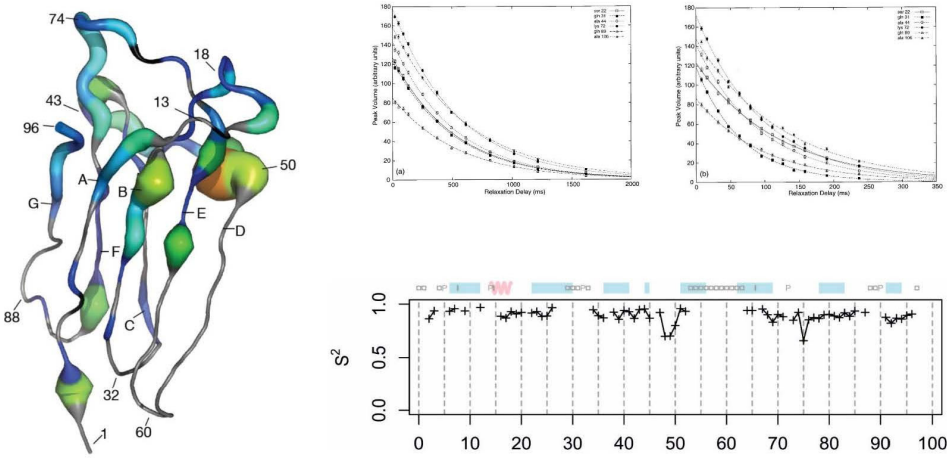


Detection of intermolecular interactions



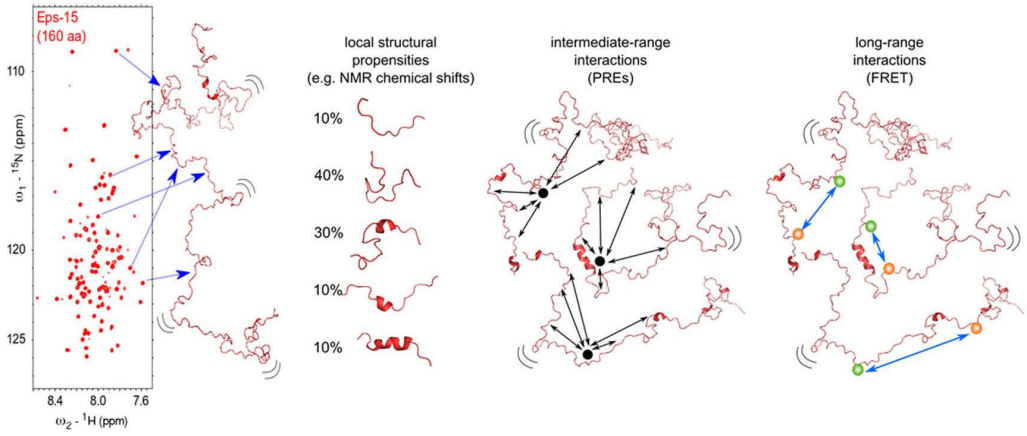
NMR can be used for the detection of protein-ligand interactions, either investigating a specific interaction or screening libraries of compounds

Investigation of the dynamics of biomolecules



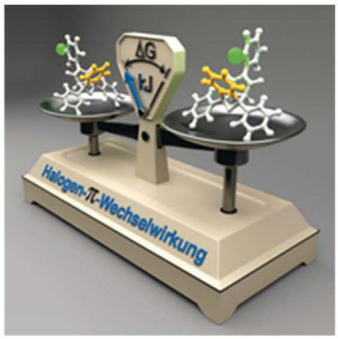
Using NMR the overall motion of proteins as well as their internal dynamics can be investigated

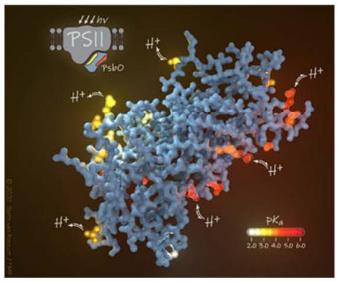
Investigation of IDPs

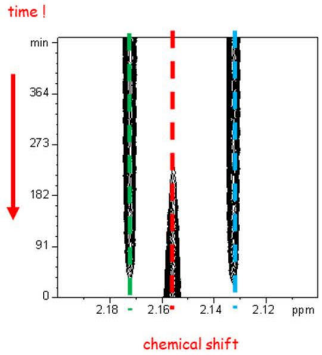


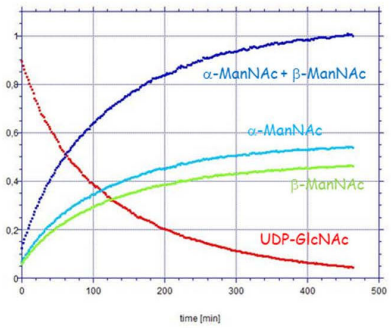
IDPs that are difficult to handle with methods for structure determination can be investigated by NMR combined with optical techniques

Determination of thermodynamic and kinetic parameters



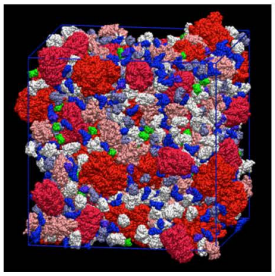


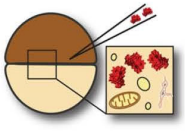


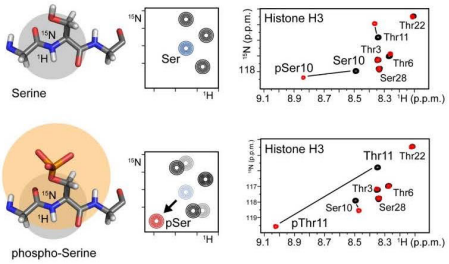


Using NMR we can follow reaction, determine equilibrium constants and pKa values

Detection of processes in living cells

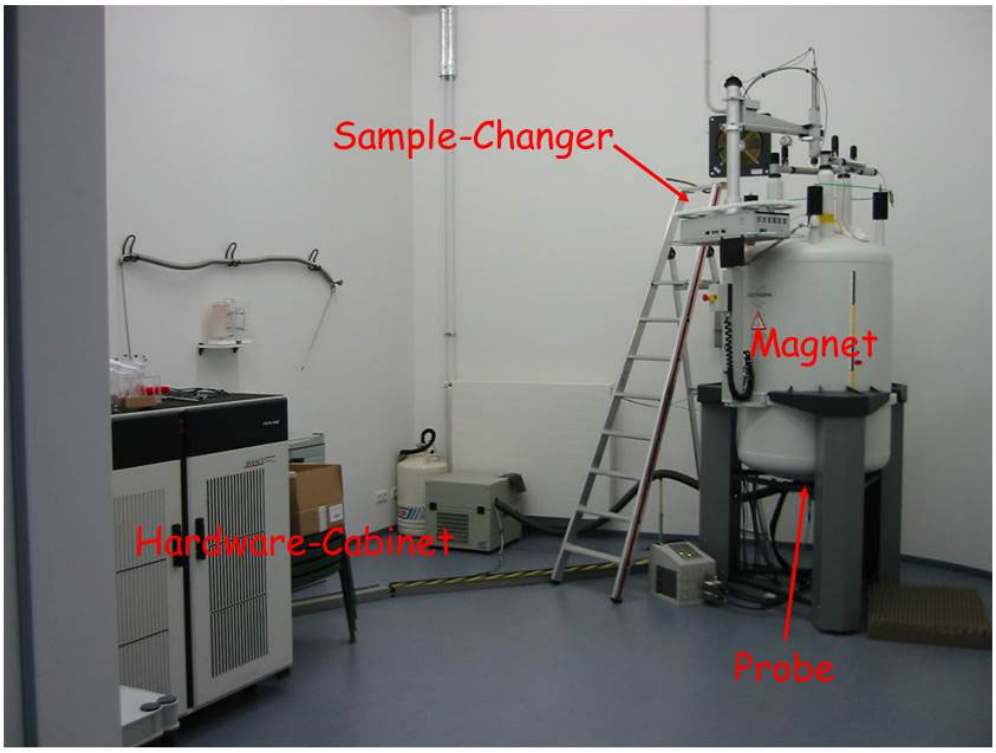






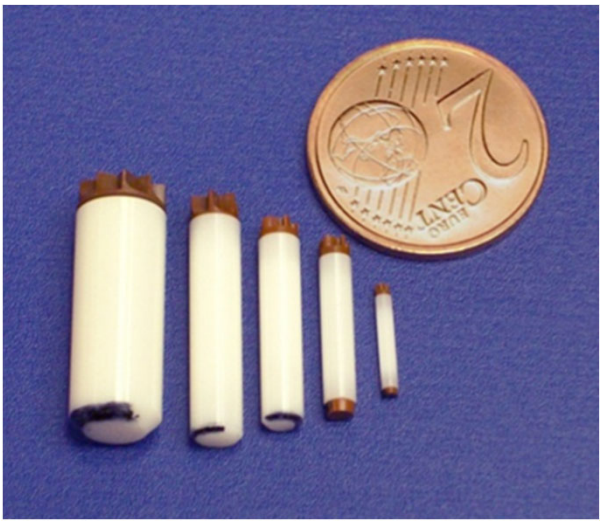
Using in-cell-NMR changes and processes within living cells can be visualized

NMR-spectrometer



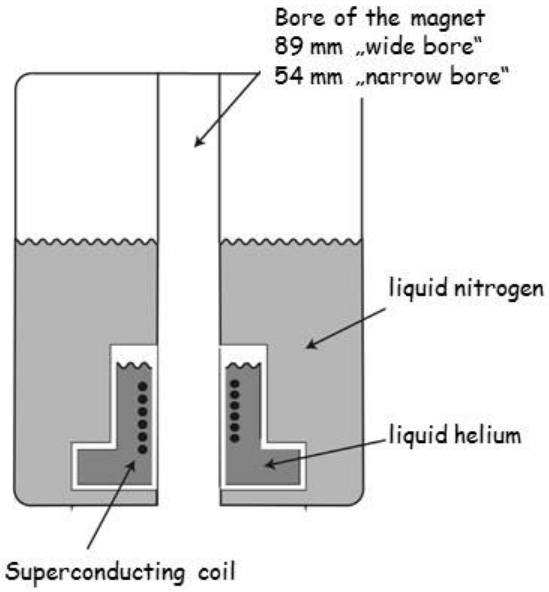
NMR-samples

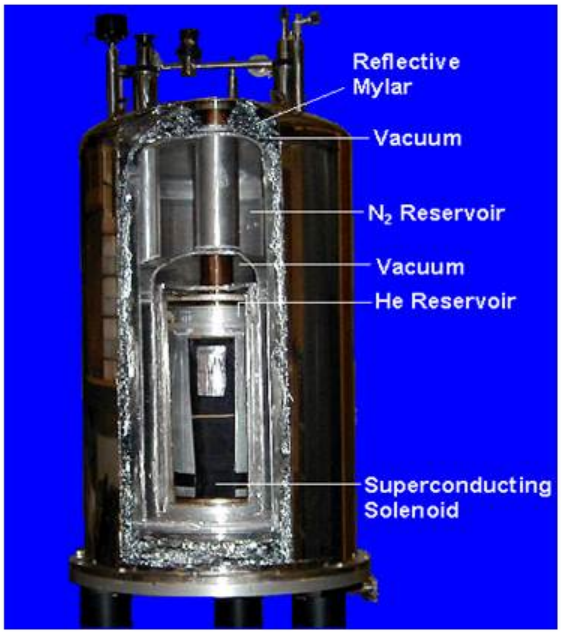
solution-state



solid-state

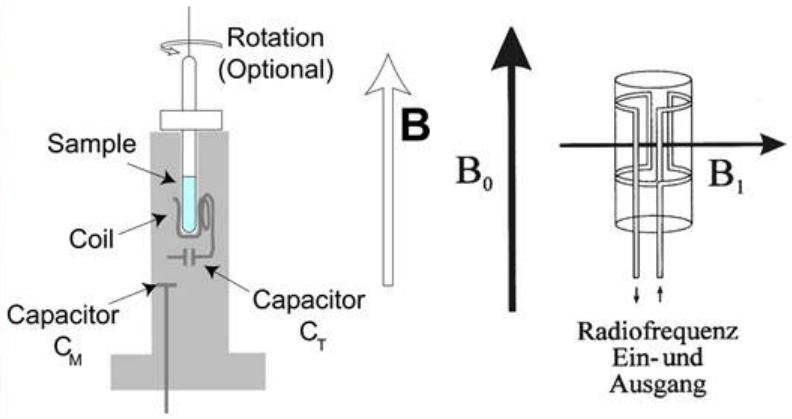
Magnet



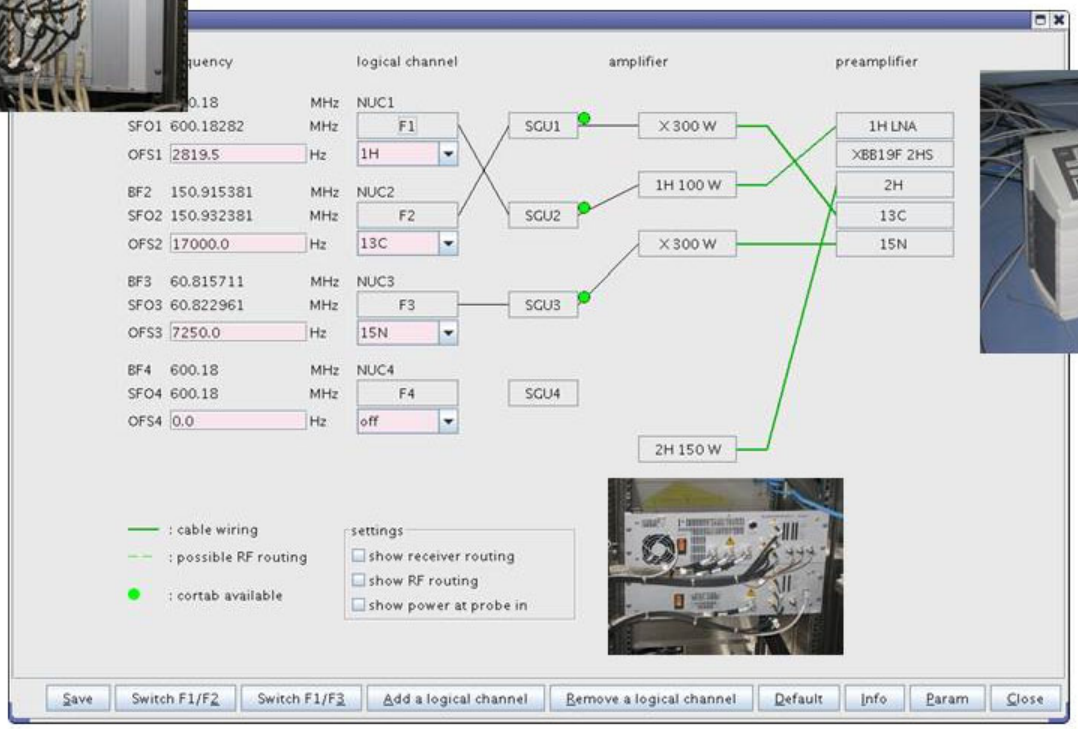


The sample in the probe

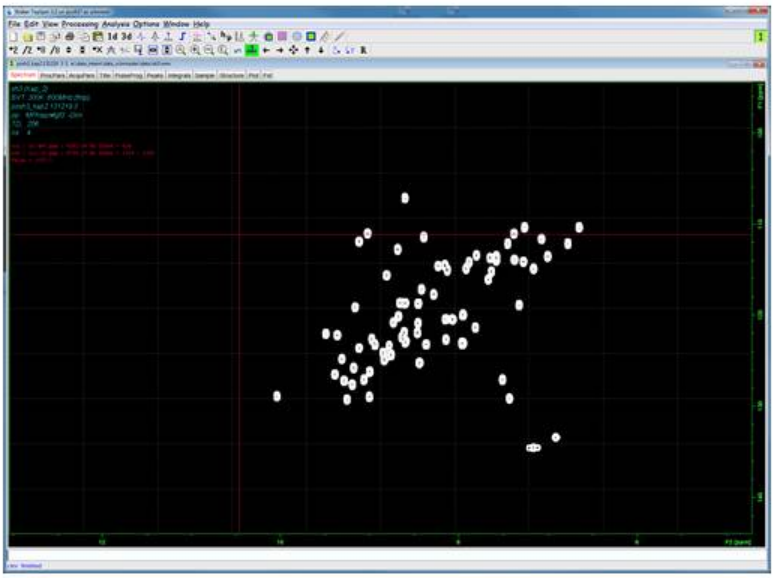
Helmholtz-Spule



Hardware

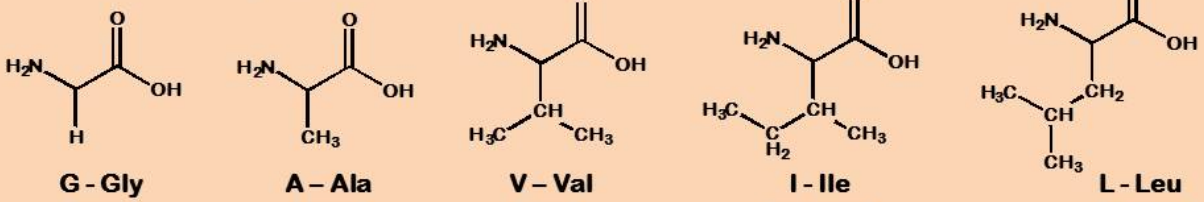


Software



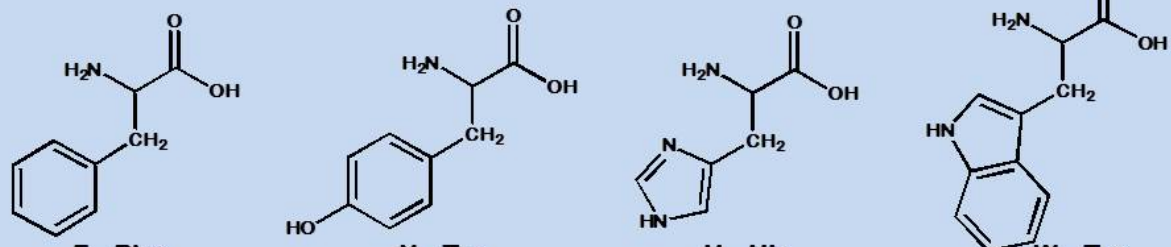
```
;zgdc
;avance-version (03/04/17)
;1D sequence with decoupling

#include <Avance.incl>

"d11=30m"

1 ze
  d11 pl12:f2
2 30m do:f2
  d11 cpd2:f2
  d1
  p1 ph1
  go=2 ph31
  30m do:f2 mc #0 to 2 F0(zd)
exit


ph1=0 2 2 0 1 3 3 1
ph31=0 2 2 0 1 3 3 1
```

without functional groups



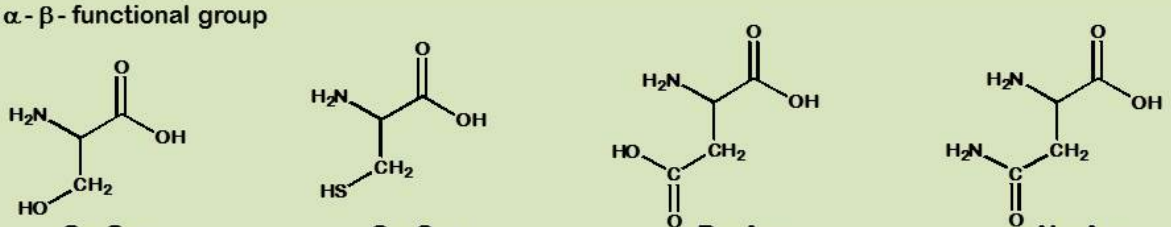
G - Gly Glycine | **A – Ala** Alanine | **V – Val** Valine | **I - Ile** Isoleucine | **L - Leu** Leucine

with aromatic side chains



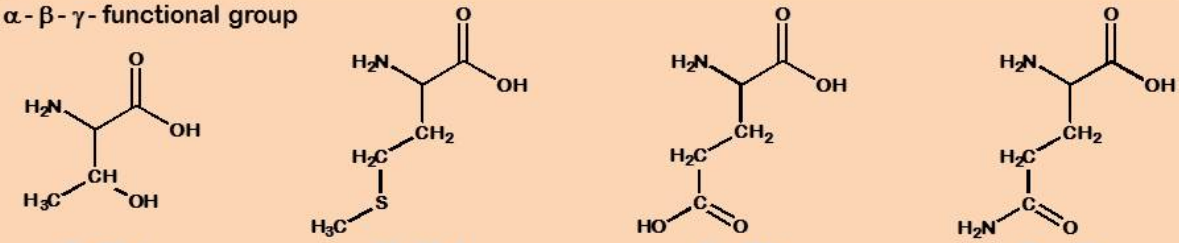
F – Phe Phenylalanine | **Y – Tyr** Tyrosine | **H – His** Histine | **W – Trp** Tryptophan

α - β - functional group



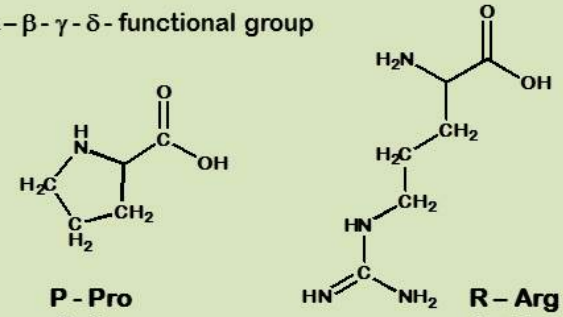
S – Ser Serine | **C – Cys** Cysteine | **D – Asp** Aspartate | **N – Asn** Asparagine

α - β - γ - functional group



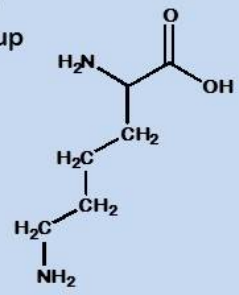
T - Thr Threonine | **M - Met** Methionine | **E – Glu** Glutamate | **Q – Gln** Glutamine

α – β - γ - δ - functional group



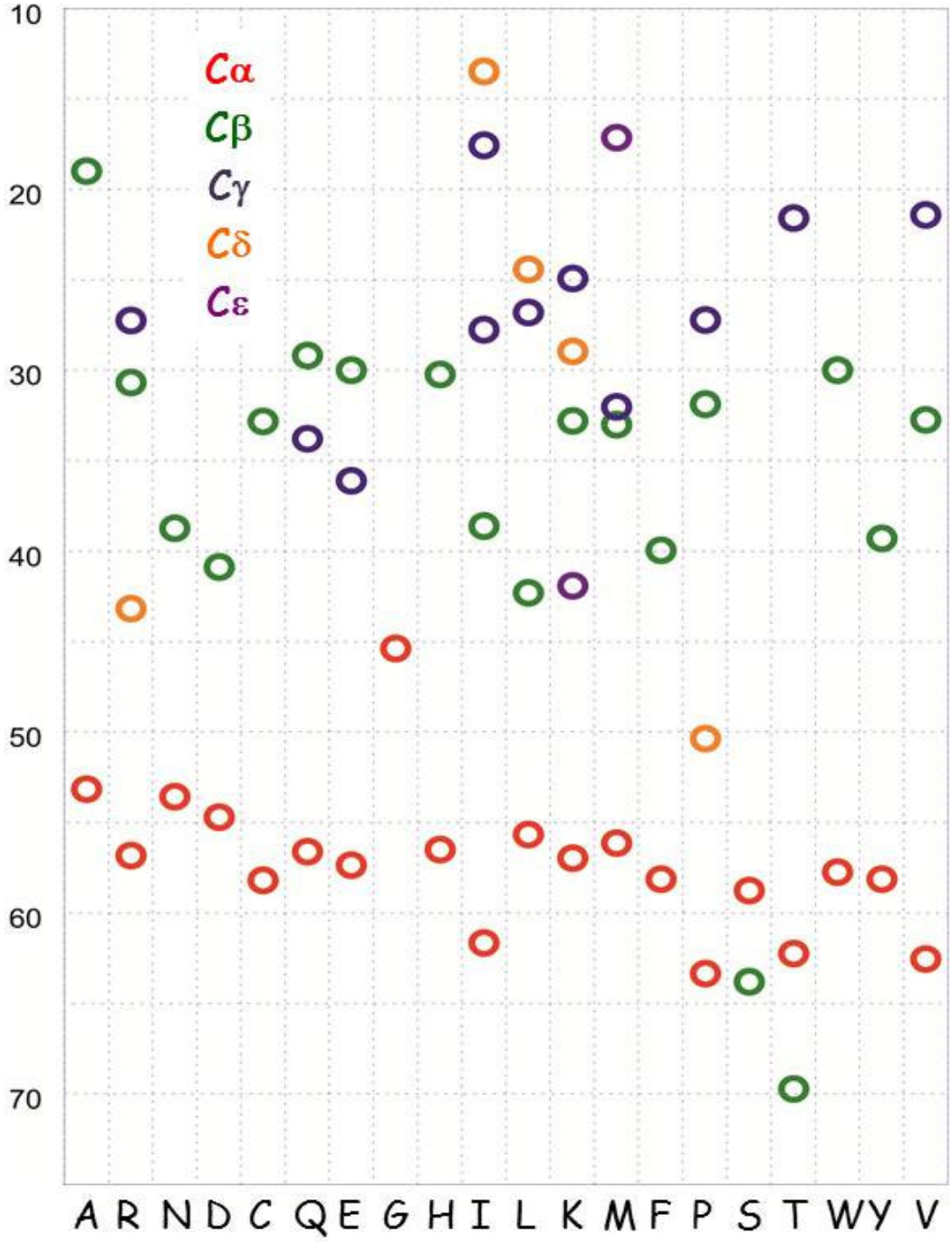
P - Pro Proline | **R – Arg** Arginine

α – β - γ - δ – ε - functional group

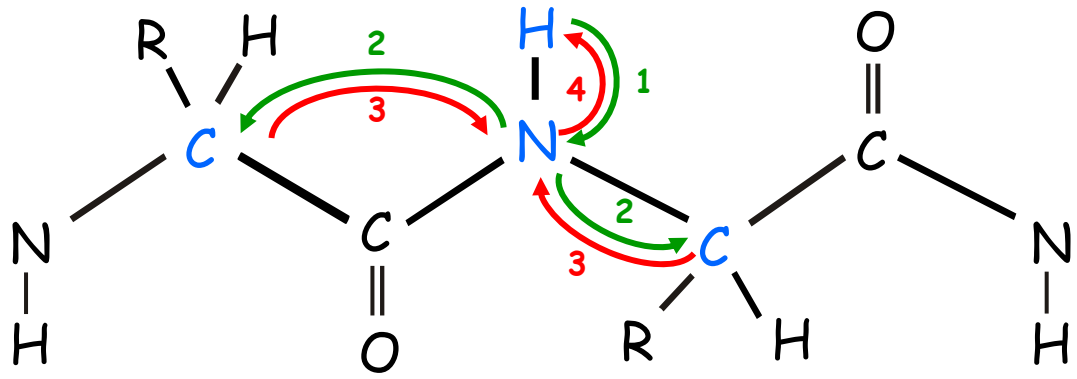


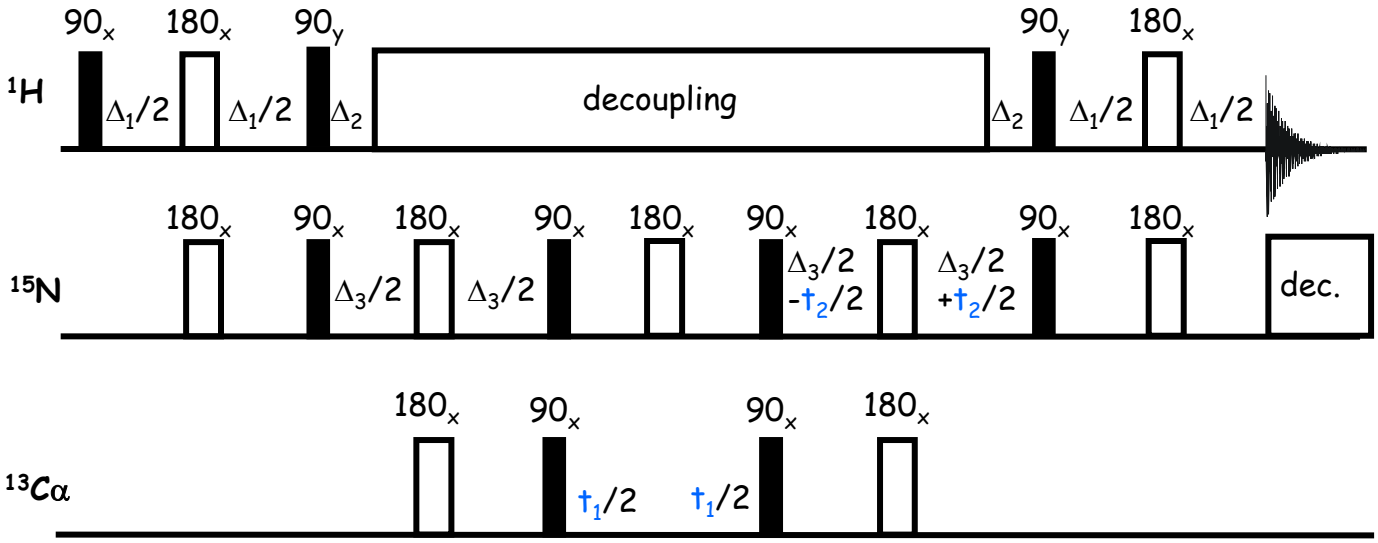
K – Lys Lysine

Average chemical shift of side chain carbons
of proteinogenic amino acids

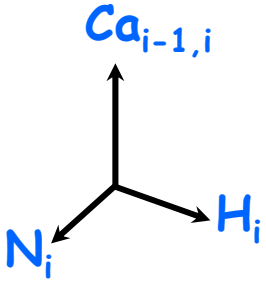


HNCA



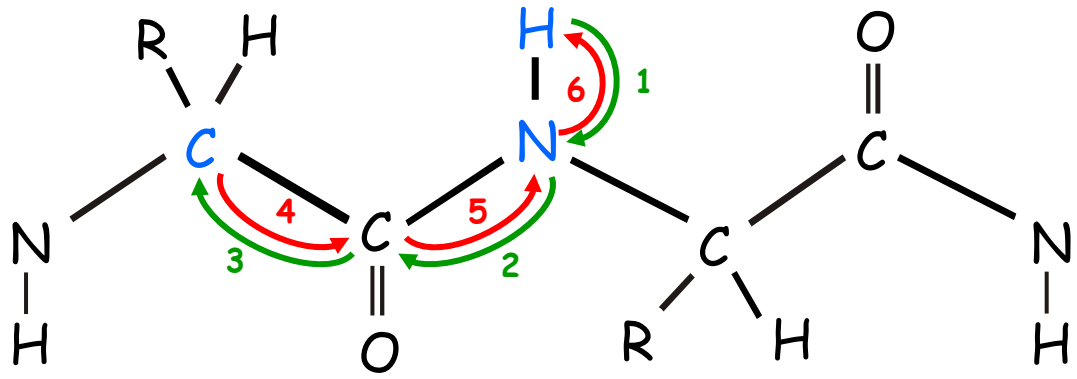


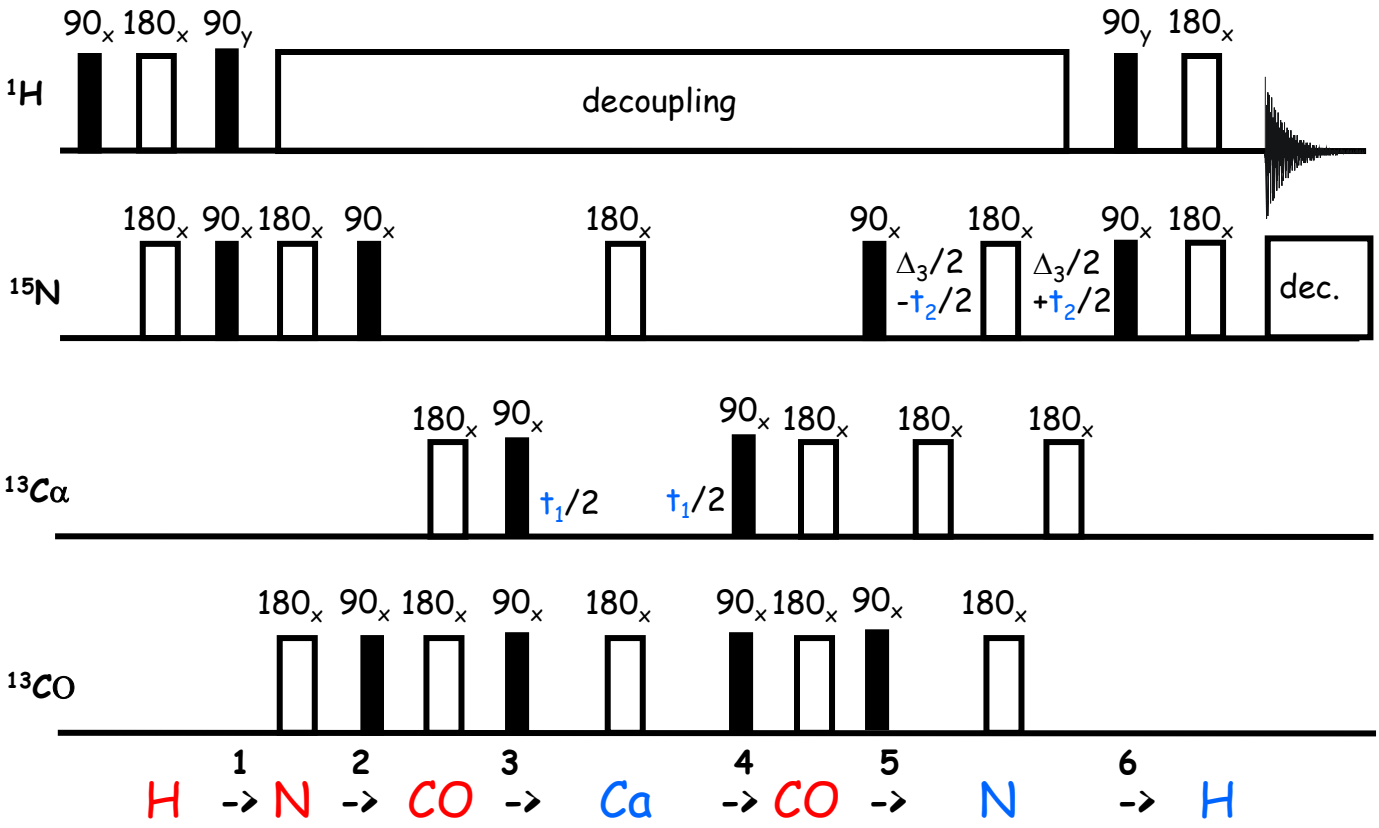
H 1 -> N 2 -> Ca 3 -> N 4 -> H

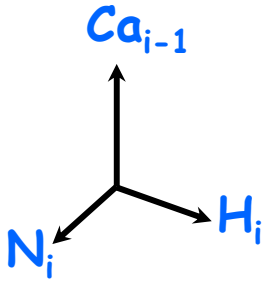


Ca-Experiment with 2 Ca-Signals per amino acid. Related Experiment with only one Ca-Signal (of amino acid i-1) is HNCOCAi.

HNCOCAi

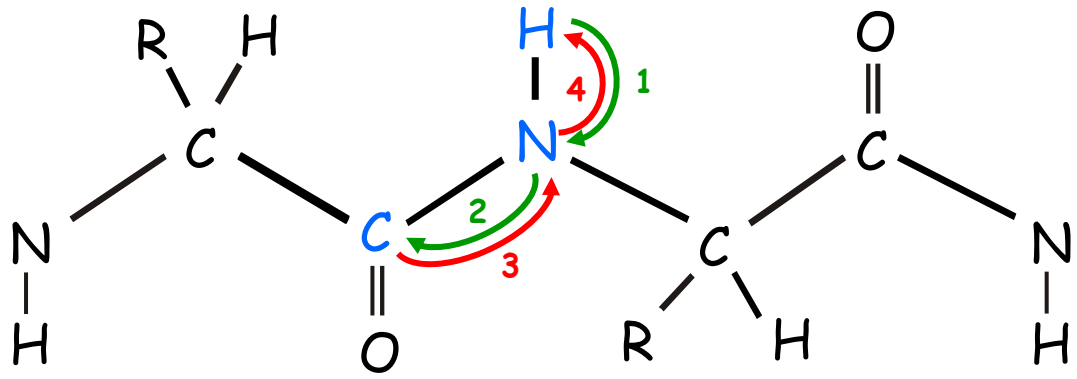


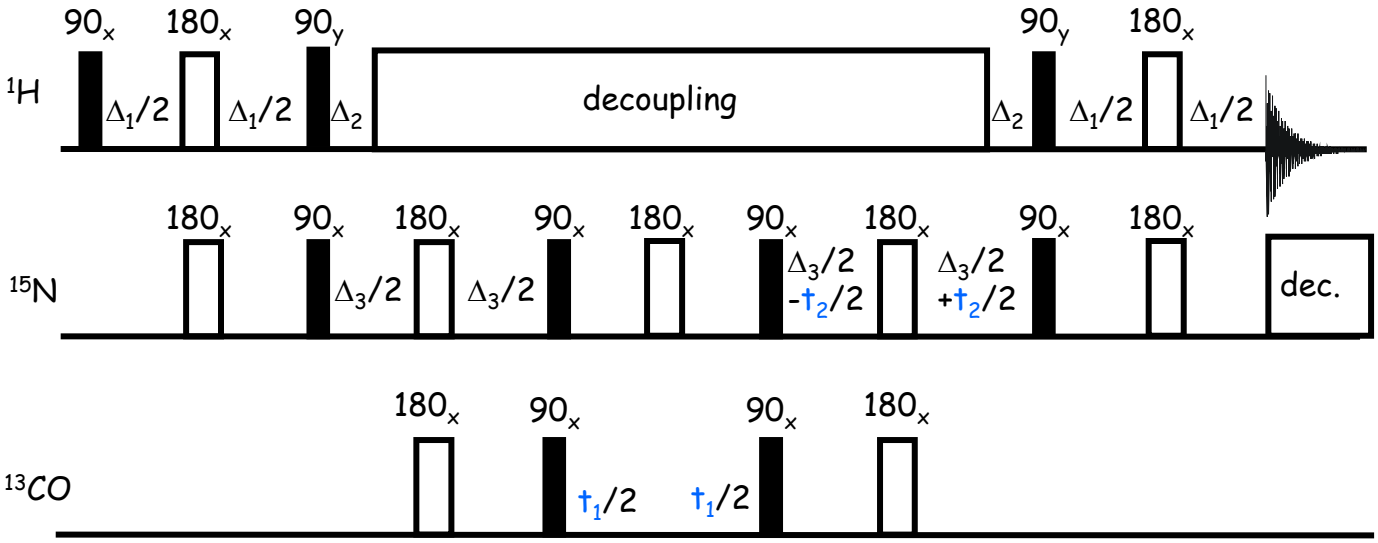




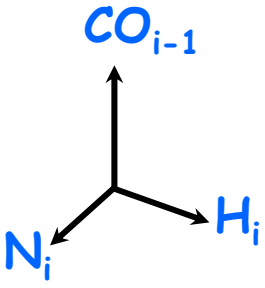
Ca-Experiment with one Ca-Signal per amino acid. Related Experiment with 2 Ca-Signals (of amino acid i and i-1) is HNCA.

HNCO



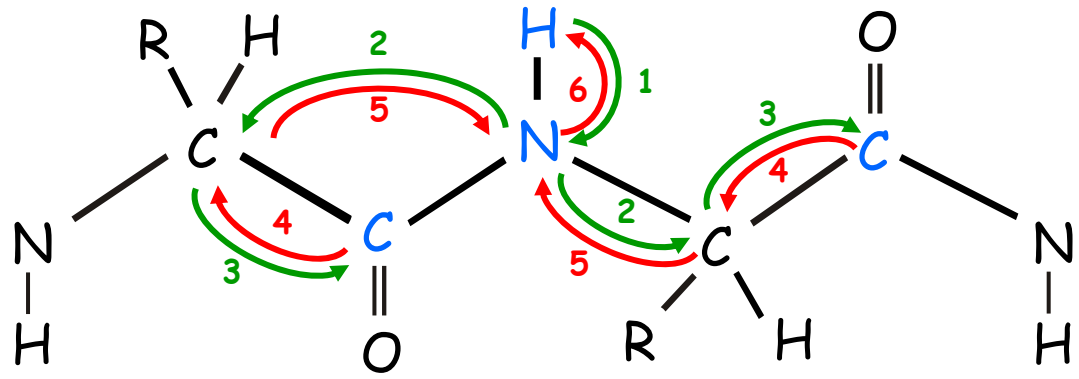


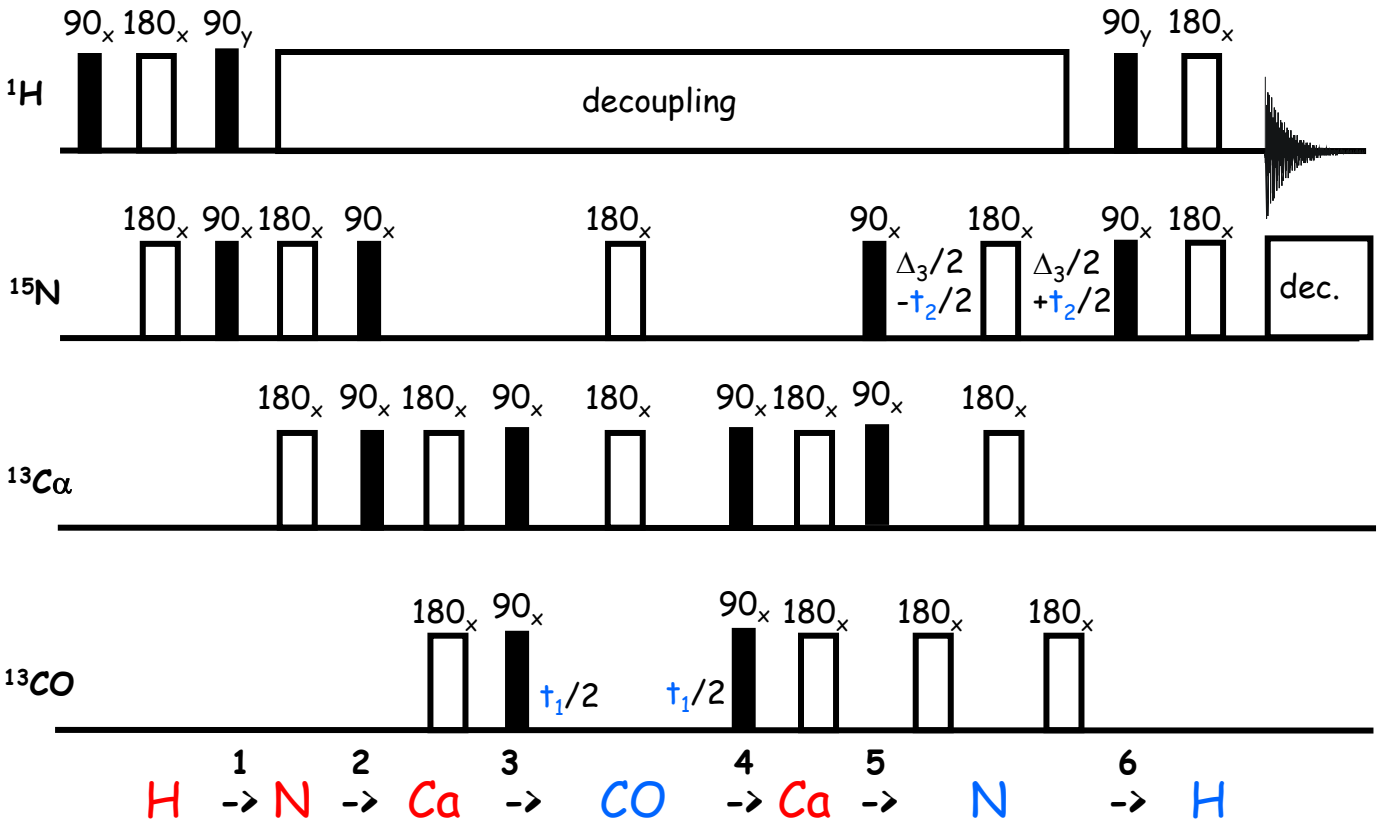
H **1** -> N **2** -> CO **3** -> N **4** -> H

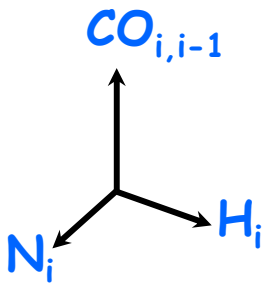


CO-Experiment with one Cα-Signal per amino acid. Related Experiment with 2 CO-Signals (of amino acid i and i-1) is HNCACO.

HNCACO



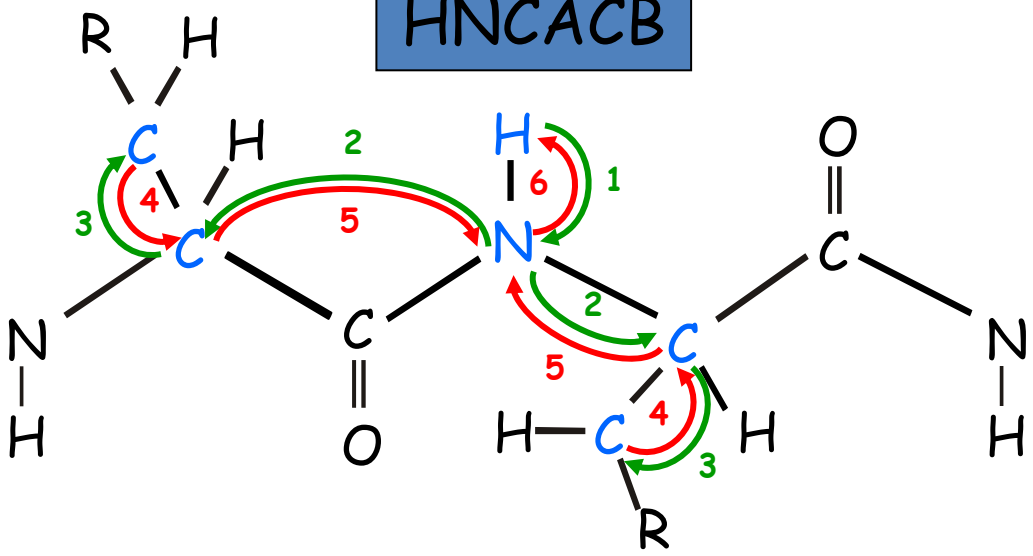


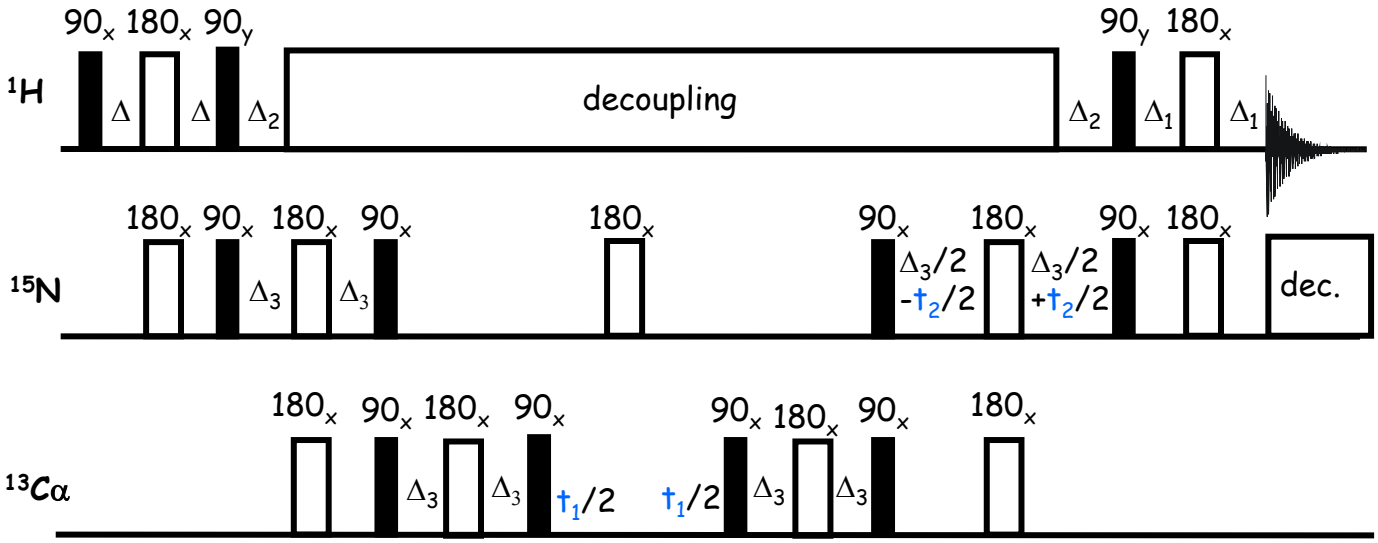


CO-Experiment with 2 CO-Signals per amino acid. Related Experiment with only one CO-Signal (of amino acid i-1) is HNCO.

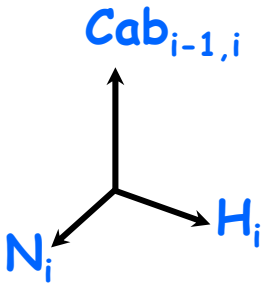
M. Beerbaum
P. Schmieder
NMR-Facility

HNCACB



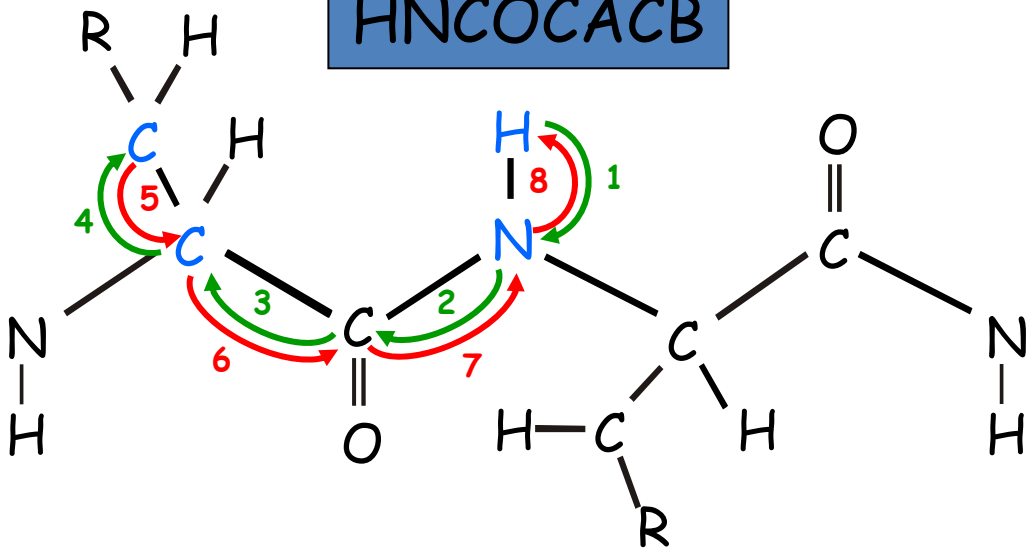


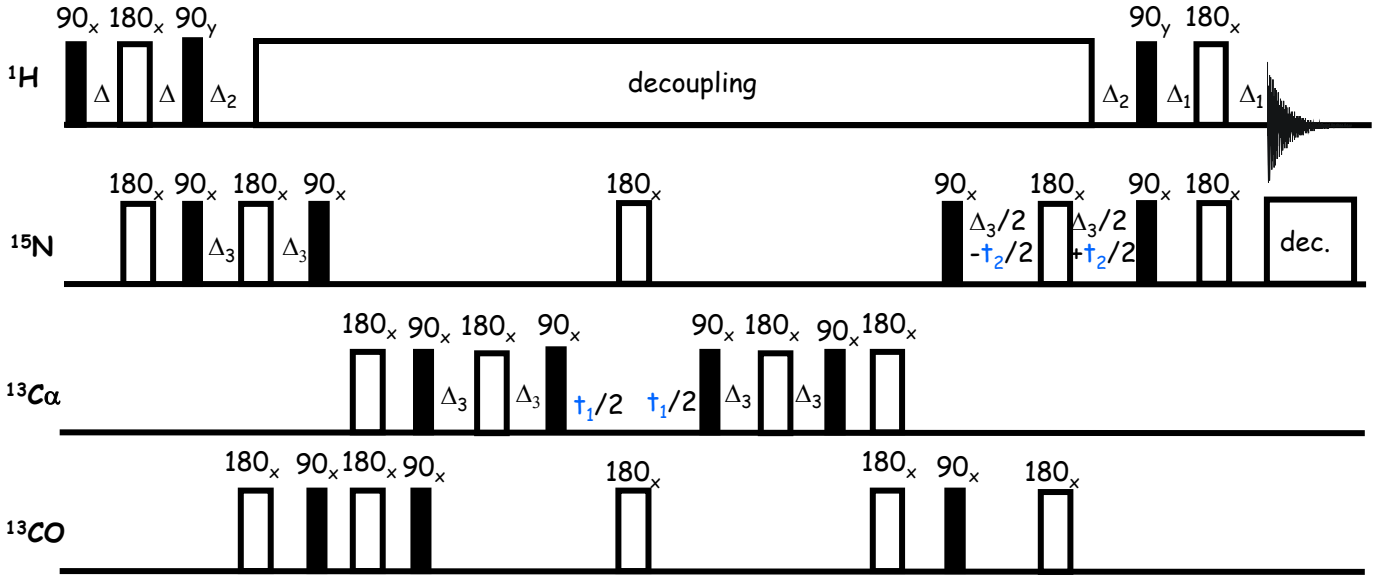
H -1-> N -2-> Ca -3-> CaCb -4-> Ca -5-> N -6-> H



Cab-Experiment with 2 Ca and 2 Cb-Signals per amino acid. Gives the same spectrum as a CBCANNH. Related Experiment with only 2 Cab-Signals (of amino acid i-1) are HNCOCACB or CBCACONNH.

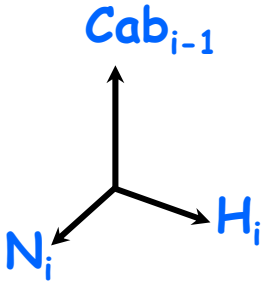
HNCOCACB





1 2 3 4 5 6 7 8

H -> N ->CO-> Ca ->CaCb-> Ca -> CO -> N -> H



Cab-Experiment with 1 Ca and 1 Cb-Signal per amino acid. Gives the same spectrum as a CBCACONNH. Related Experiment with 4 Cab-Signals (of amino acid i-1 and i) are HNCACB or CBCANNH.

Assignment exercise

The full assignment of protein spectra can last days up to weeks. Normally 3D and 4D spectra are used. In this practical course the principle procedure should be practiced by the assignment of a stretch of an SH3 domain. The sequence to assign consists of 9 amino acids. The aim of the exercise is to analyze which part of the SH3 sequence it is. The spectra that are used are a 15N-HSQC and 2D projections of 3Ds of this part of SH3, they have no 15N axis and are thus 1H-13C correlations.

Sequence: **MDETG KELVL ALYDY QEKSP REVTM KKGDI**
LTLLN STNKD WWKVE VNDRQ GFVPA AYVKK LD

Spectra:

- 15N-HSQC (fingerprint spectrum necessary for dynamic and interaction studies)
- HNCACB (H-C projection)
- HNCOCACB (H-C projection)
- HNCACO (H-C projection)
- HNCO (H-C projection) (spectra to enable the assignment of the fingerprint region)

Procedure:

1. group spectra in strips of the eight amino acids
2. arrange strips in sequential order due to their correlation peaks to the previous amino acid
3. derive the amino acid type of the eight amino acids from their Cα and Cβ chemical shifts
4. match the amino acids to the sequence
5. fill the shift table and transfer the assignment to the 15N-HSQC

Amino Acid	Hn shift / ppm	Nh shift / ppm	Cα shift / ppm	Cα shift / ppm	CO shift /ppm
	-	-			