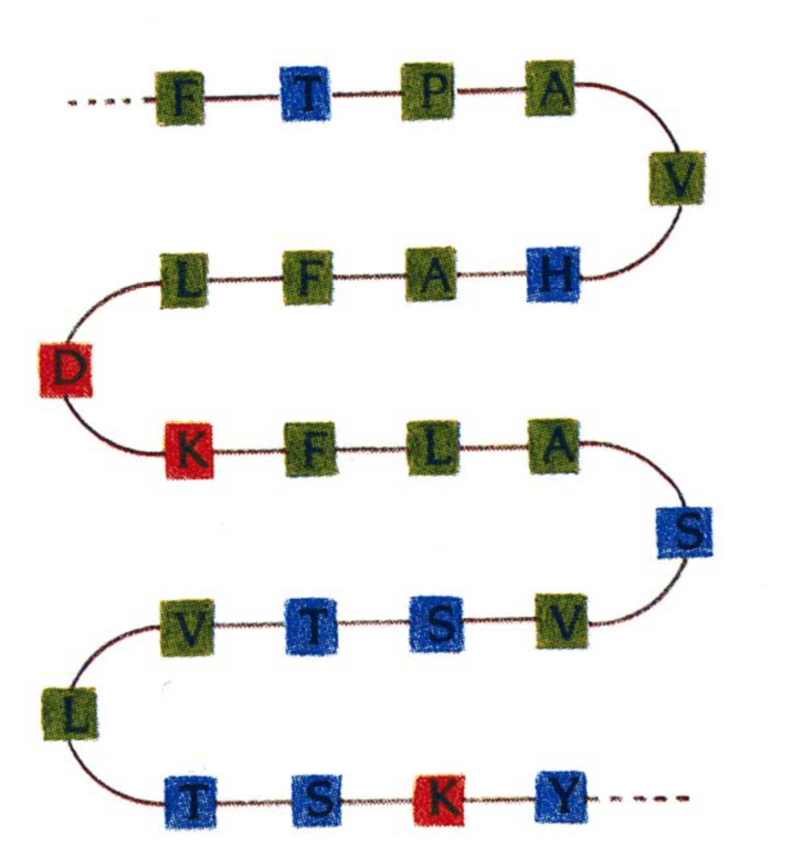
NMR-spectroscopy
of proteins in solution
- spectra and assignment

Peter Schmieder

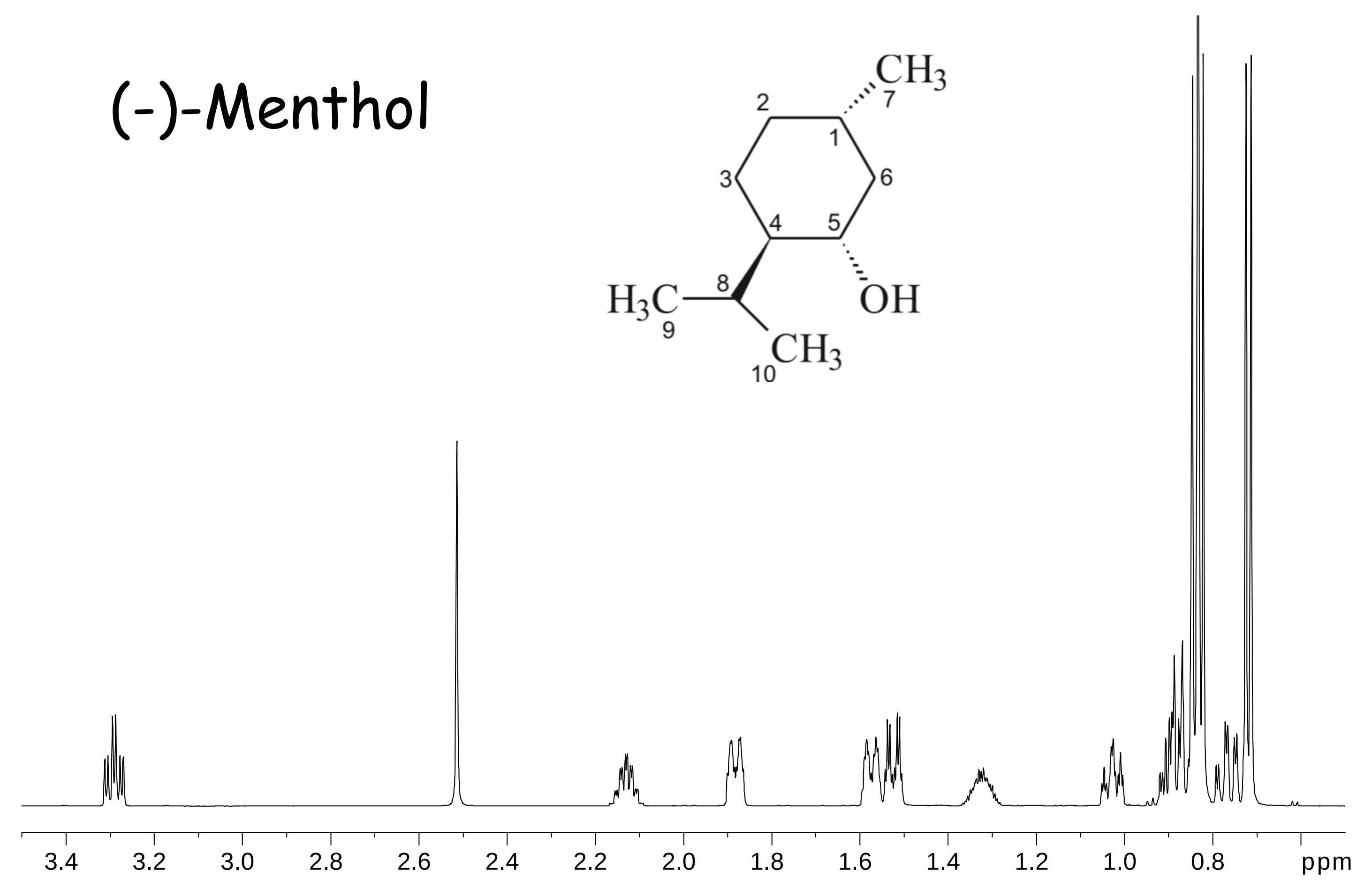
NMR-spectroscopy of proteins

NMR-spectroscopy of proteins

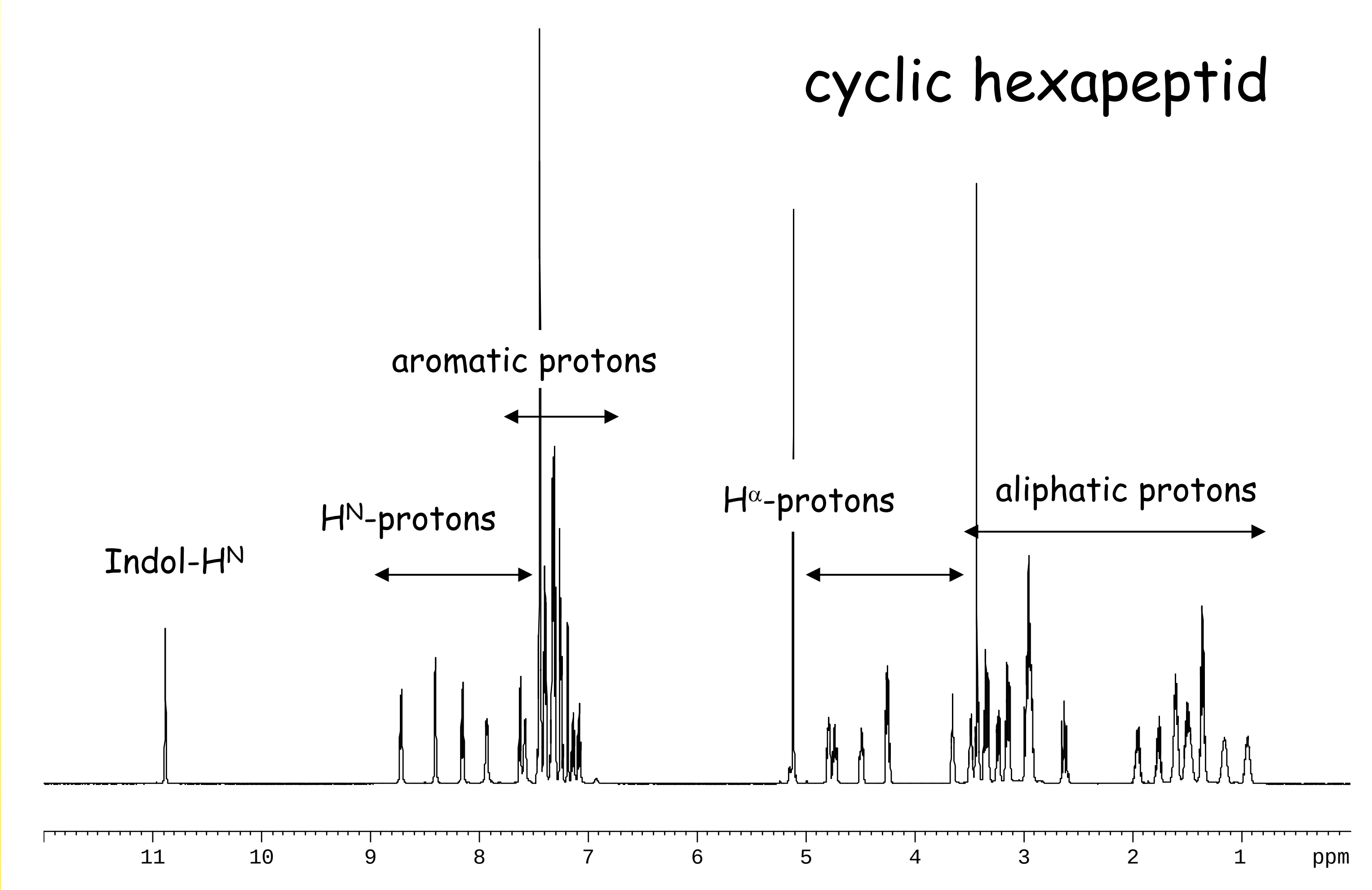


The main problem obtaining assignments of NMR-spectra of proteins result from the fact that a protein is a polymer, i.e. a repetition of identical units

NMR-spectroscopy of proteins

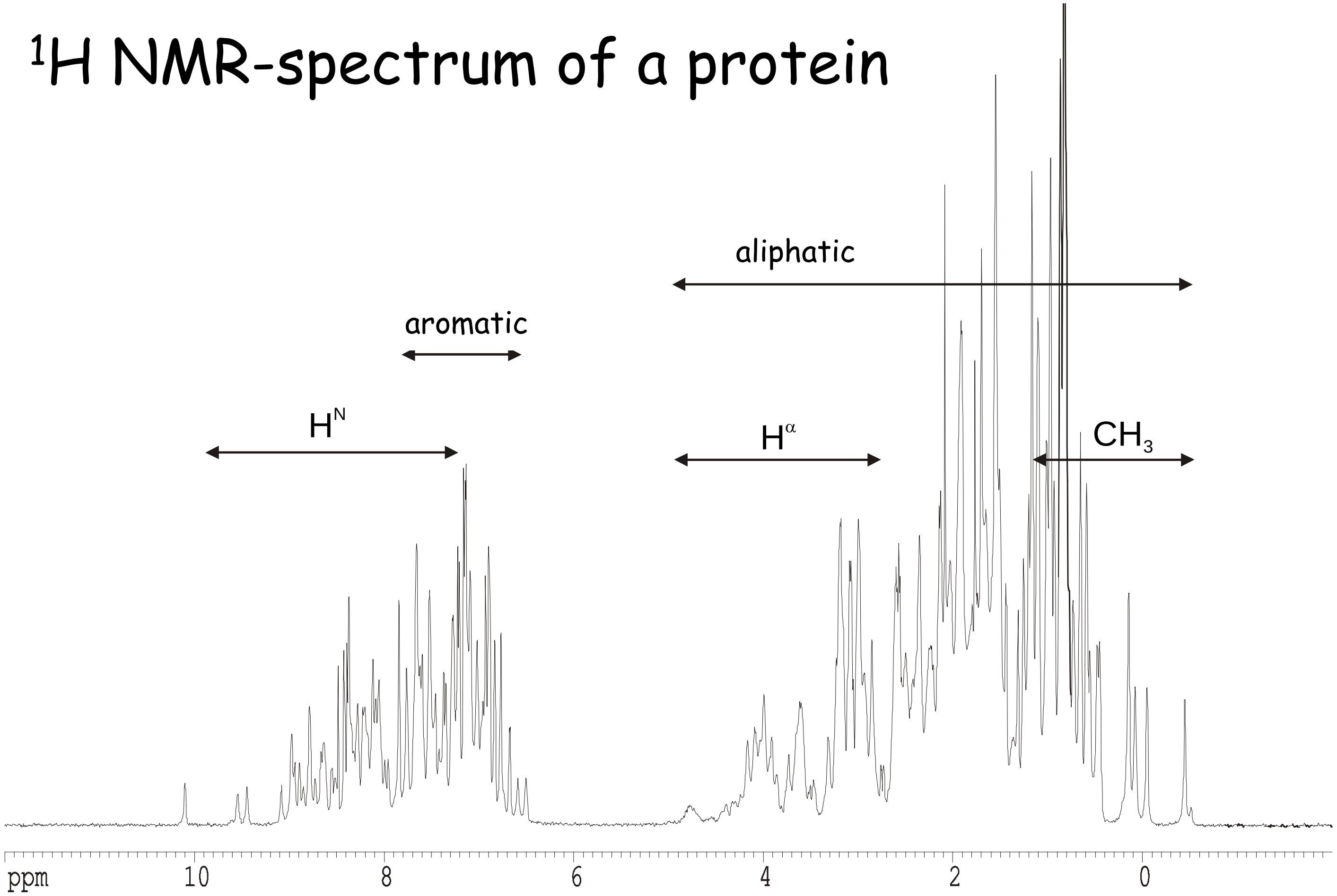


NMR-spectroscopy of proteins



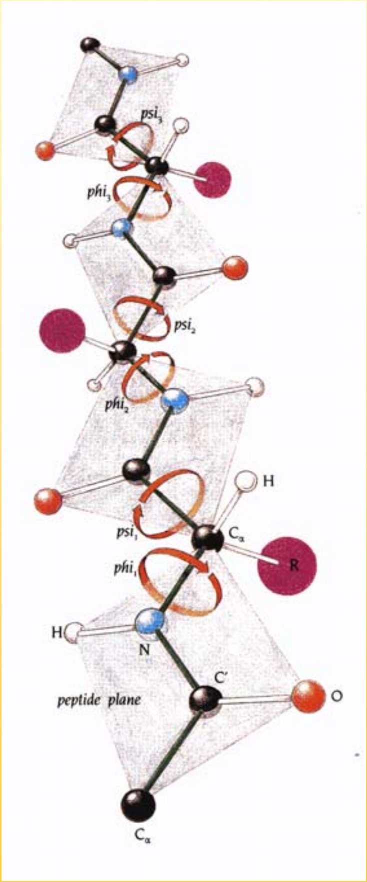
NMR-spectroscopy of proteins

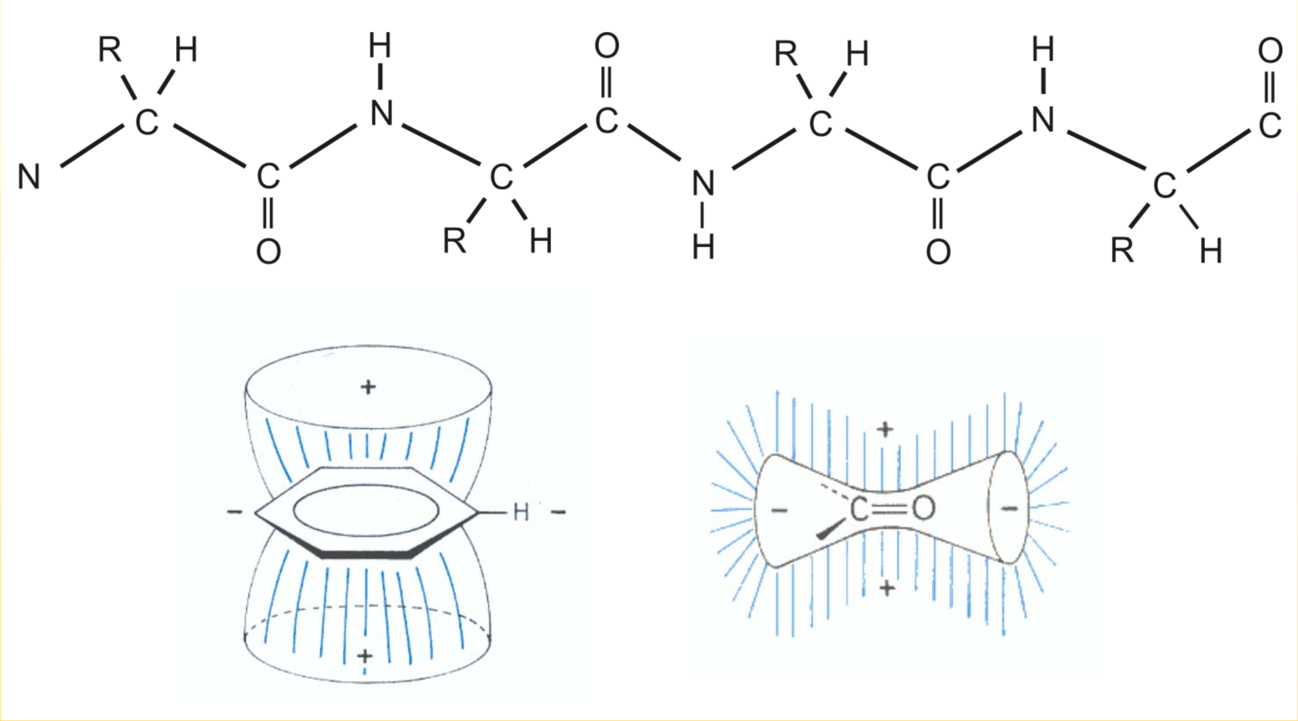
^{1}H NMR-spectrum of a protein



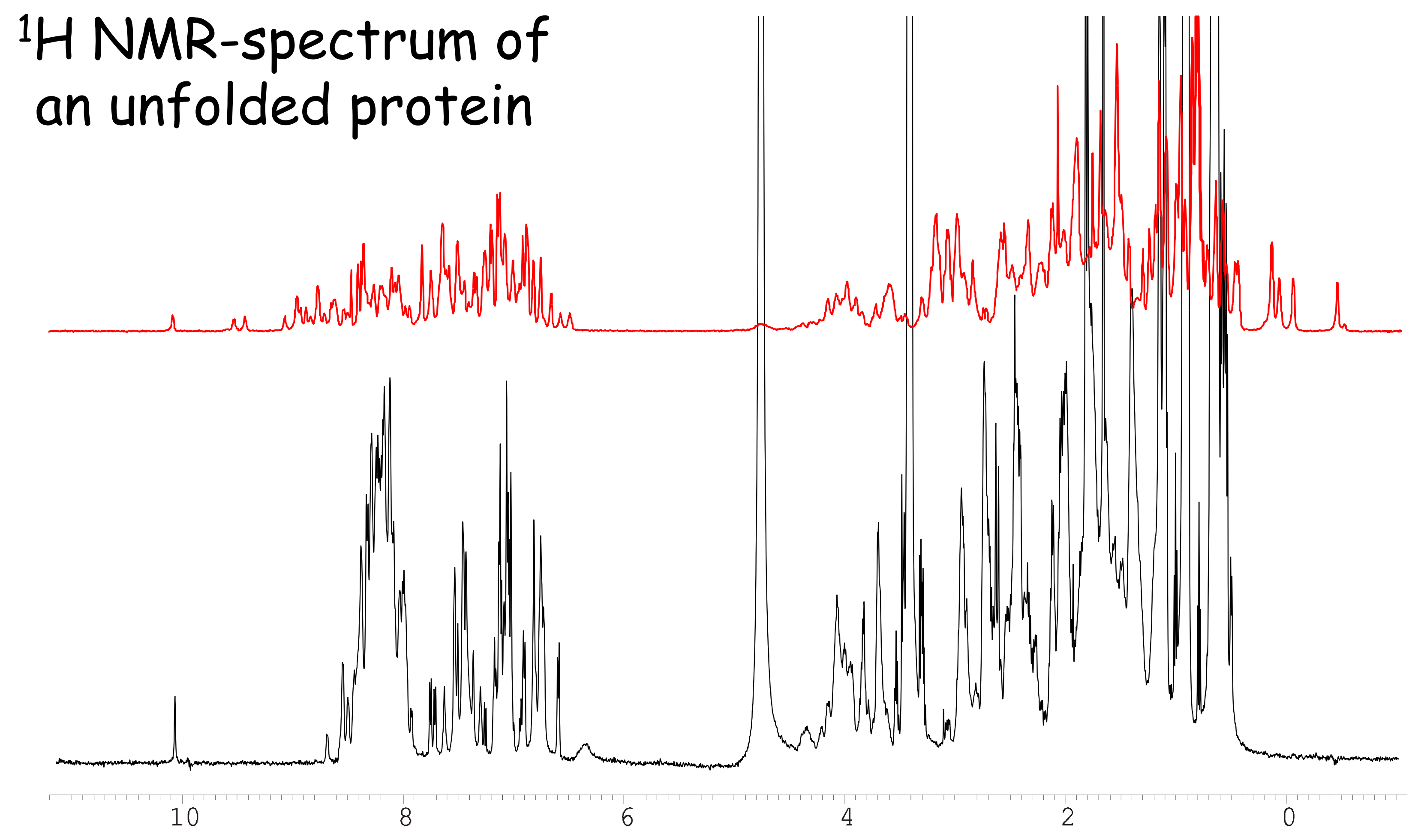
NMR-spectroscopy of proteins

Differences in the chemical shifts result from defined elements of secondary structure



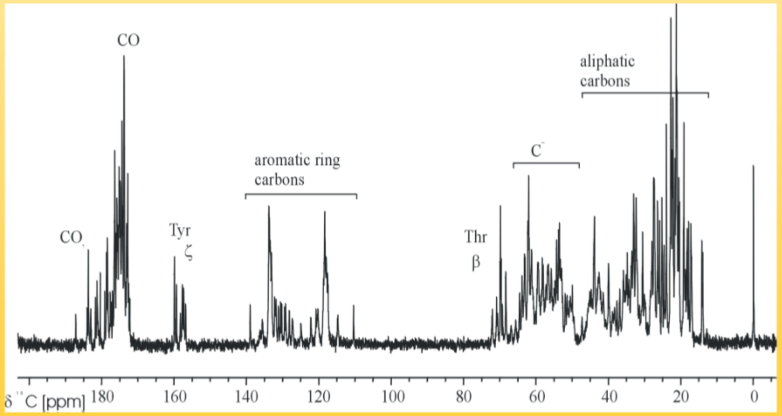


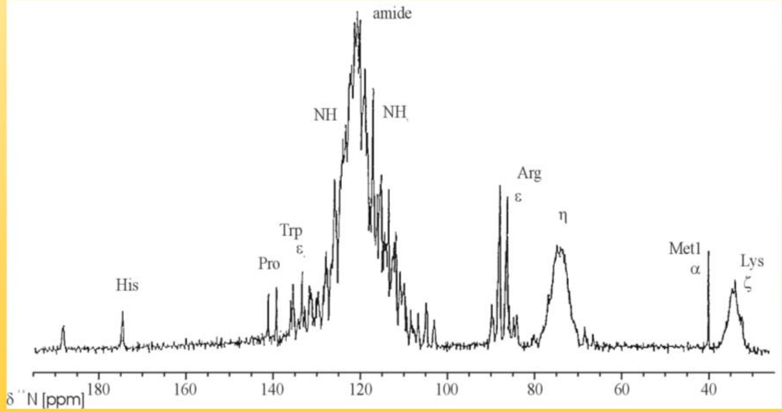
NMR-spectroscopy of proteins



NMR-spectroscopy of proteins

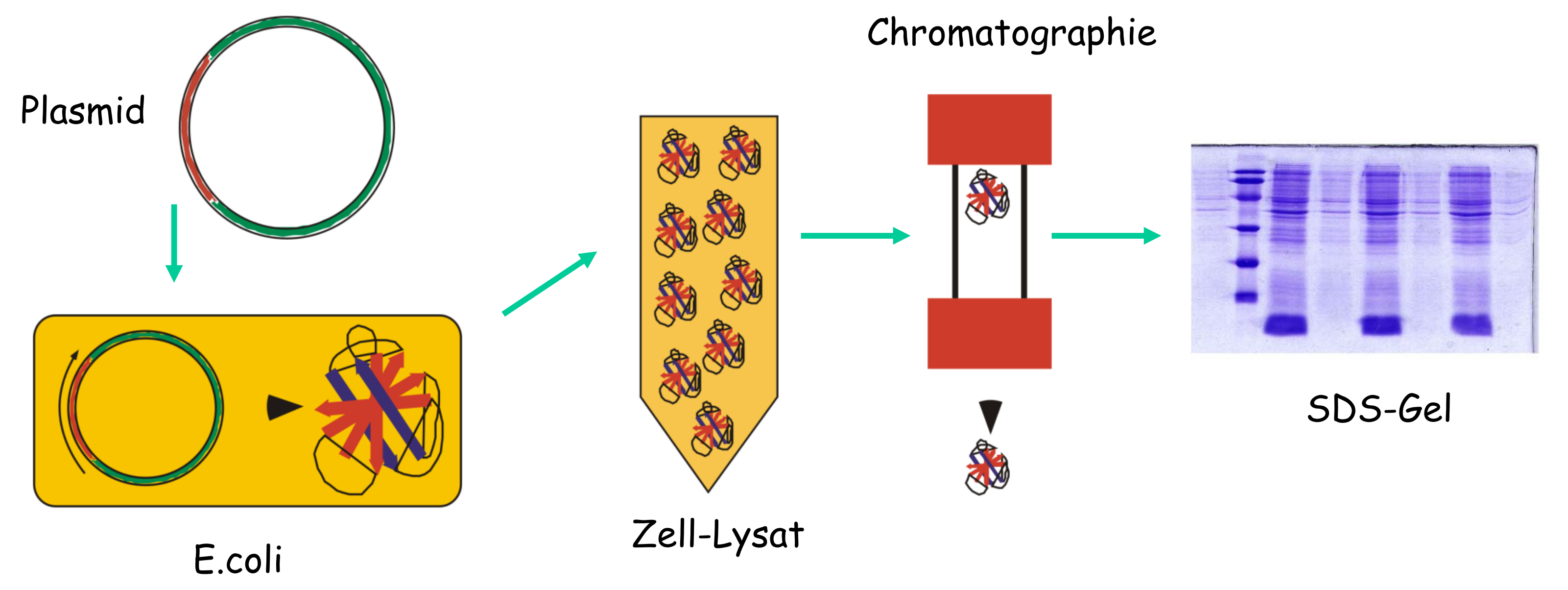
In addition to the necessity to used multidimensional spectra the superior dispersion of the spectra of carbon and nitrogen make heteronuclear experiments the methods of choice for protein NMR spectroscopy:





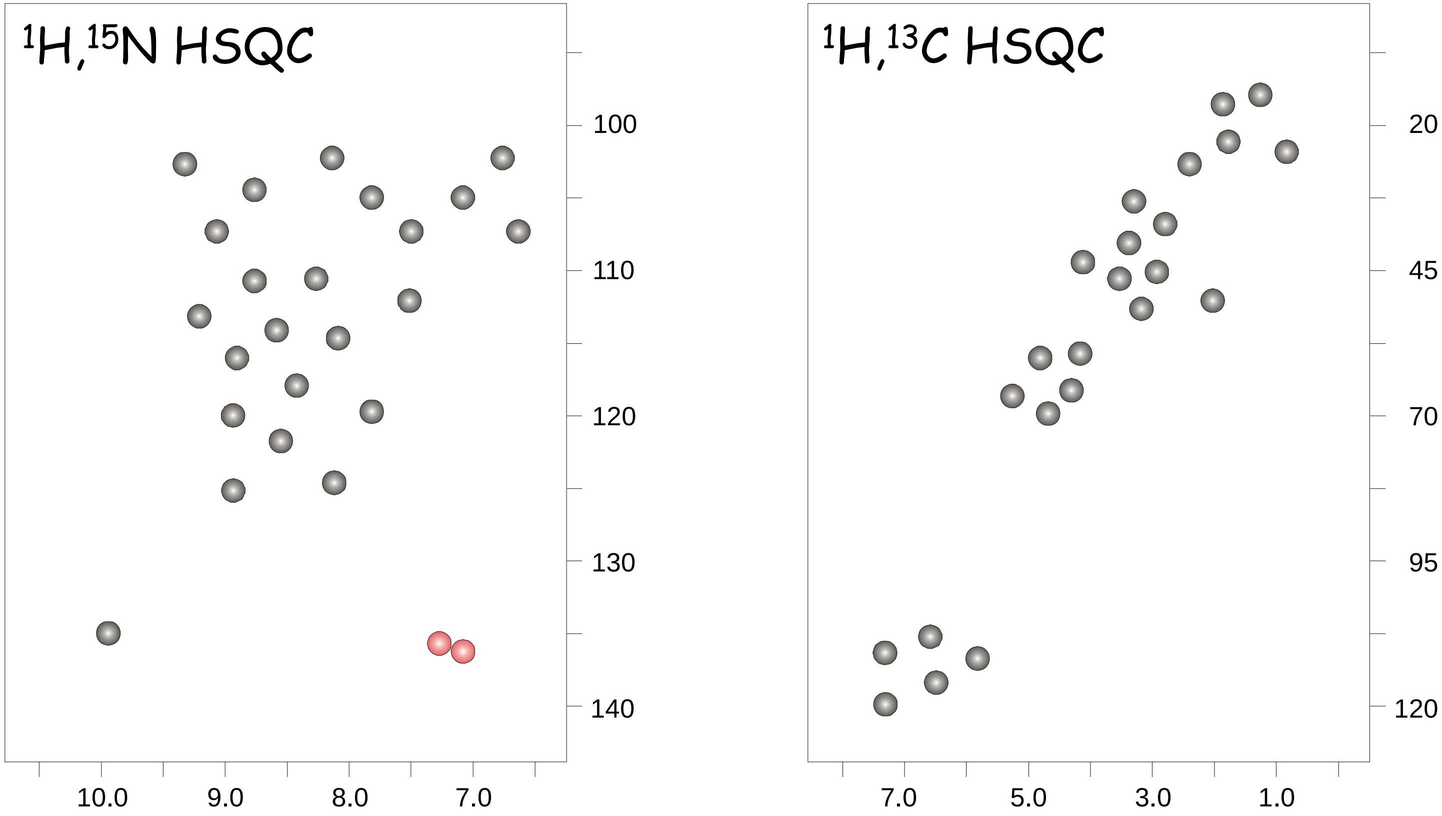
NMR-spectroscopy of proteins

Recording heteronuclearen spectra using the heteronucleus in natural abundance (^{13}C=1.1% and ^{15}N=0.4%) is not realistic with proteins. The proteins need to be labled when expressed.



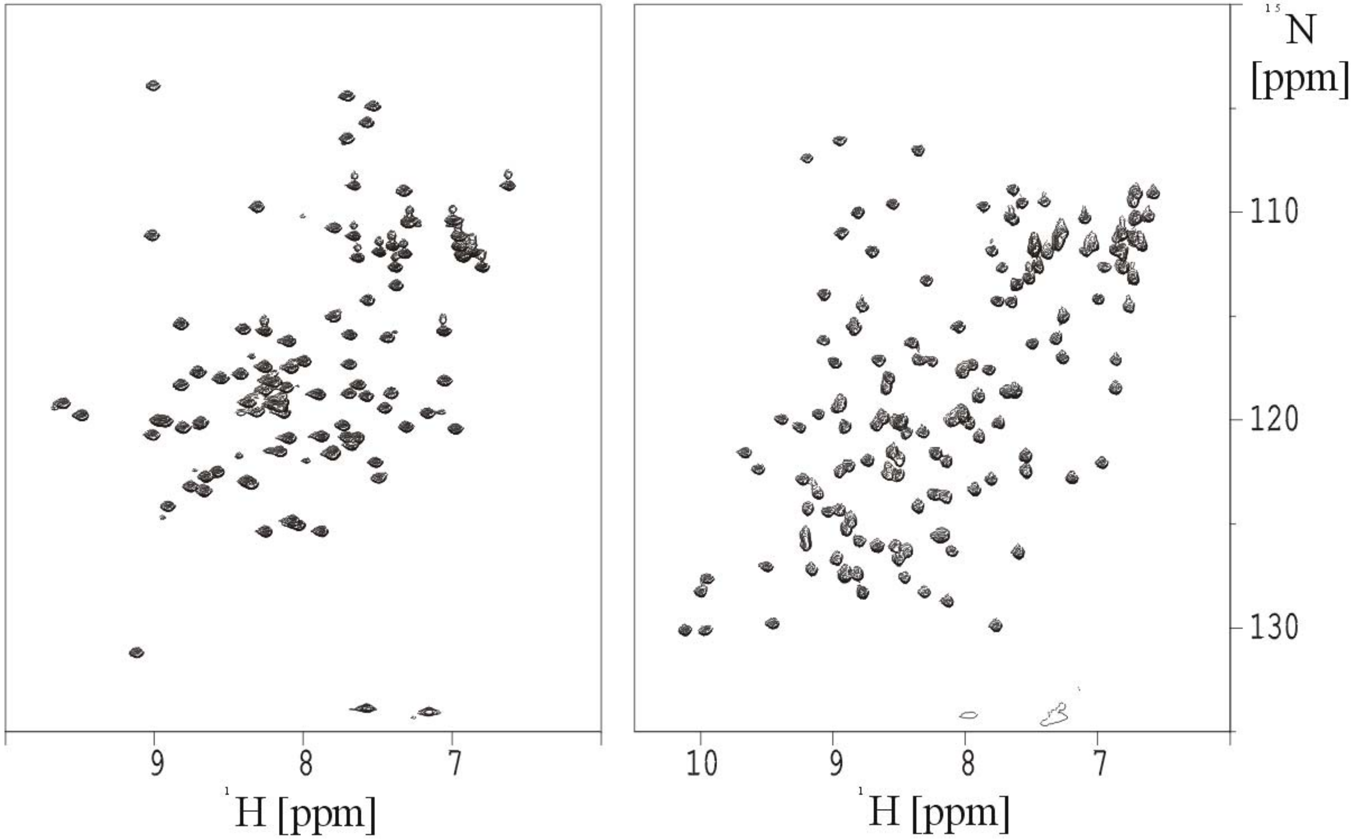
NMR-spectroscopy of proteins

^{1}H,^{15}N and ^{1}H,^{13}C HSQC-spectra of proteins (schematic)



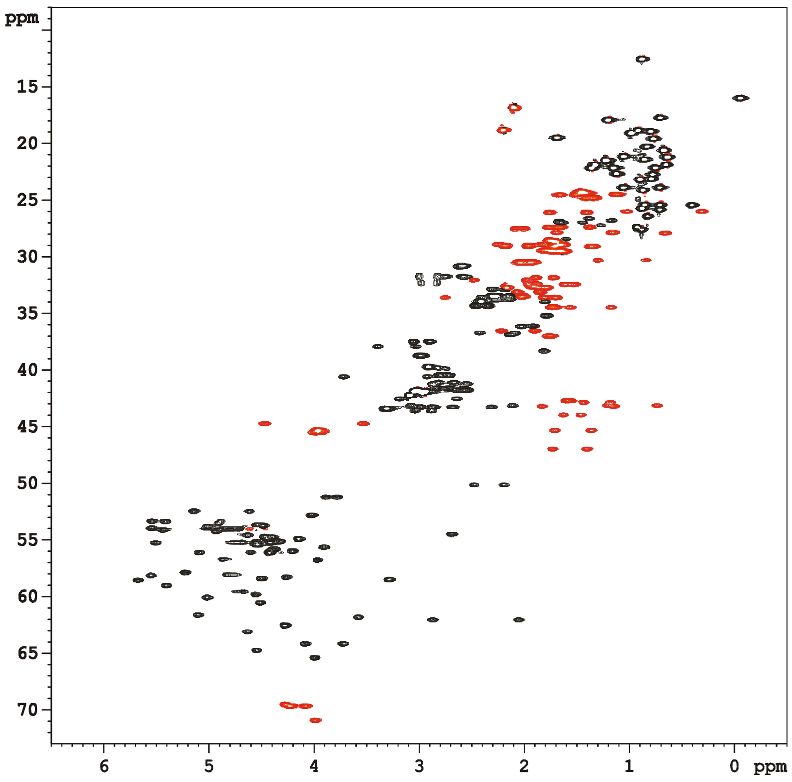
NMR-spectroscopy of proteins

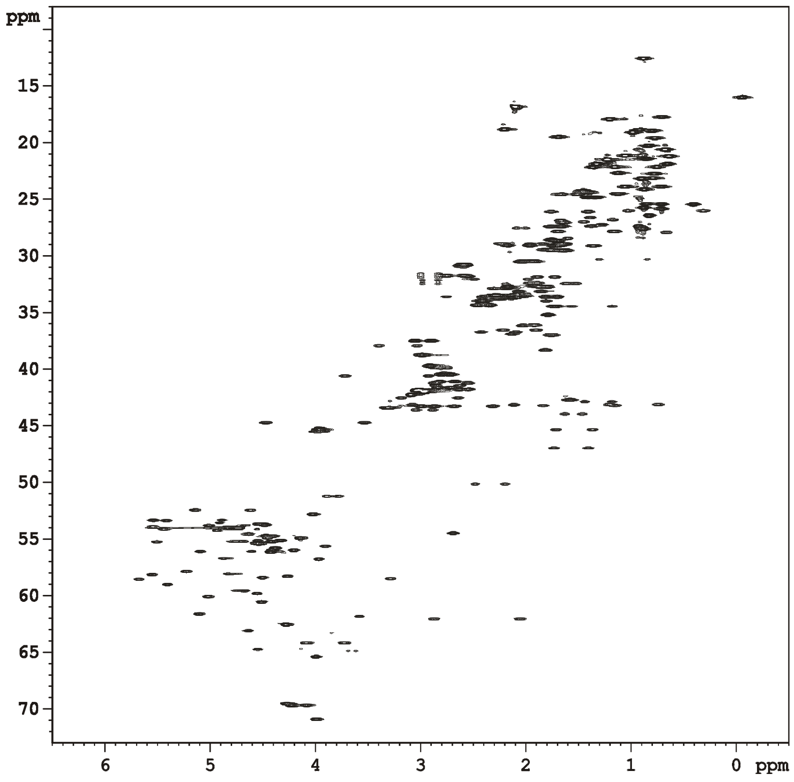
$^1H,^{15}N$ HSQC-spectra are „fingerprints“ of proteins



NMR-spectroscopy of proteins

^{1}H,^{13}C HSQC-spectra of proteins

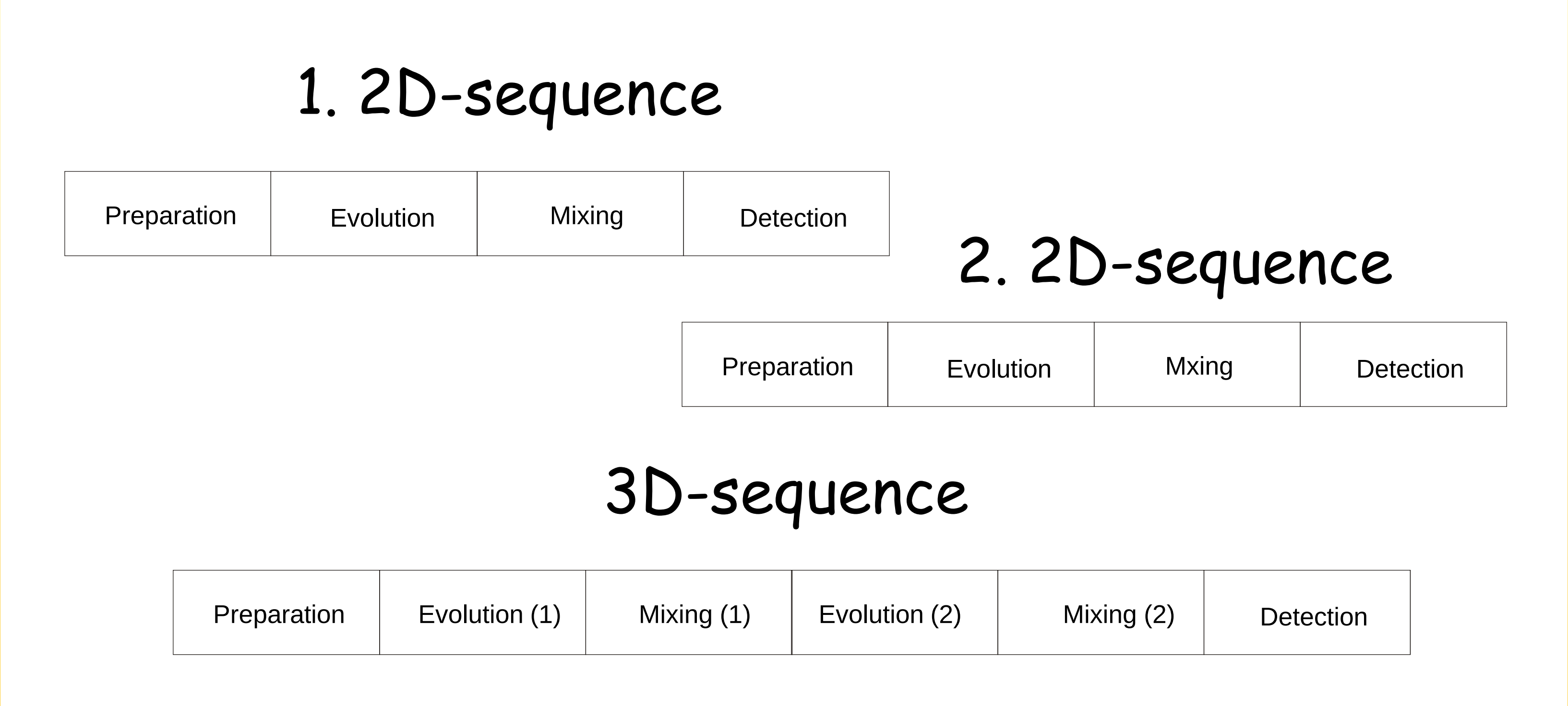




Multidimensional NMR-spectroscopy with more than two dimensions

Multidimensional NMR-spectroscopy

Formally a 3D experiment is a combination of two 2D experiments



1. 2D-sequence

Preparation	Evolution	Mixing	Detection

2. 2D-sequence

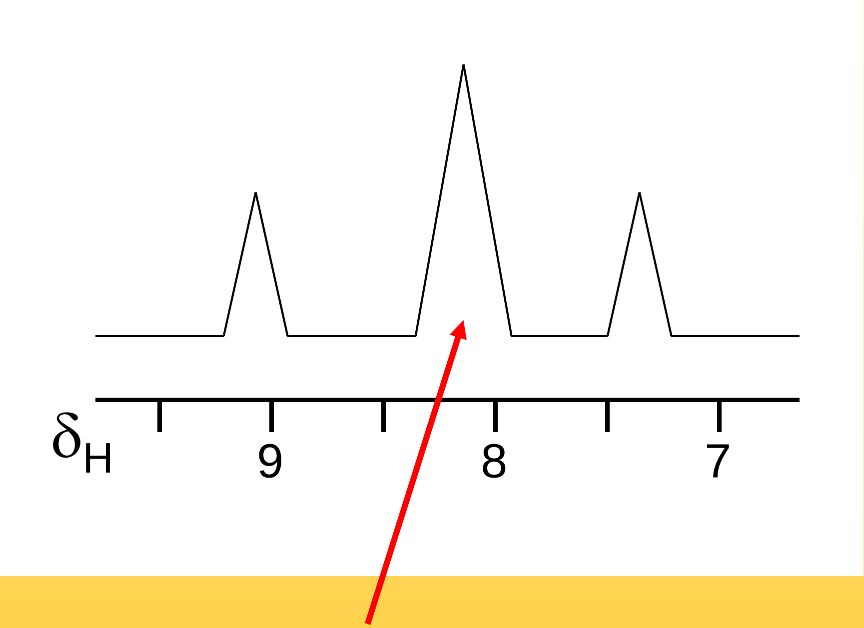
Preparation	Evolution	Mxing	Detection

3D-sequence

Preparation	Evolution (1)	Mixing (1)	Evolution (2)	Mixing (2)	Detection

Multidimensional NMR-spectroscopy

As a realistic example we look at an artificial region of amino protons, that are usually show overlap in the proton spectrum of a protein.

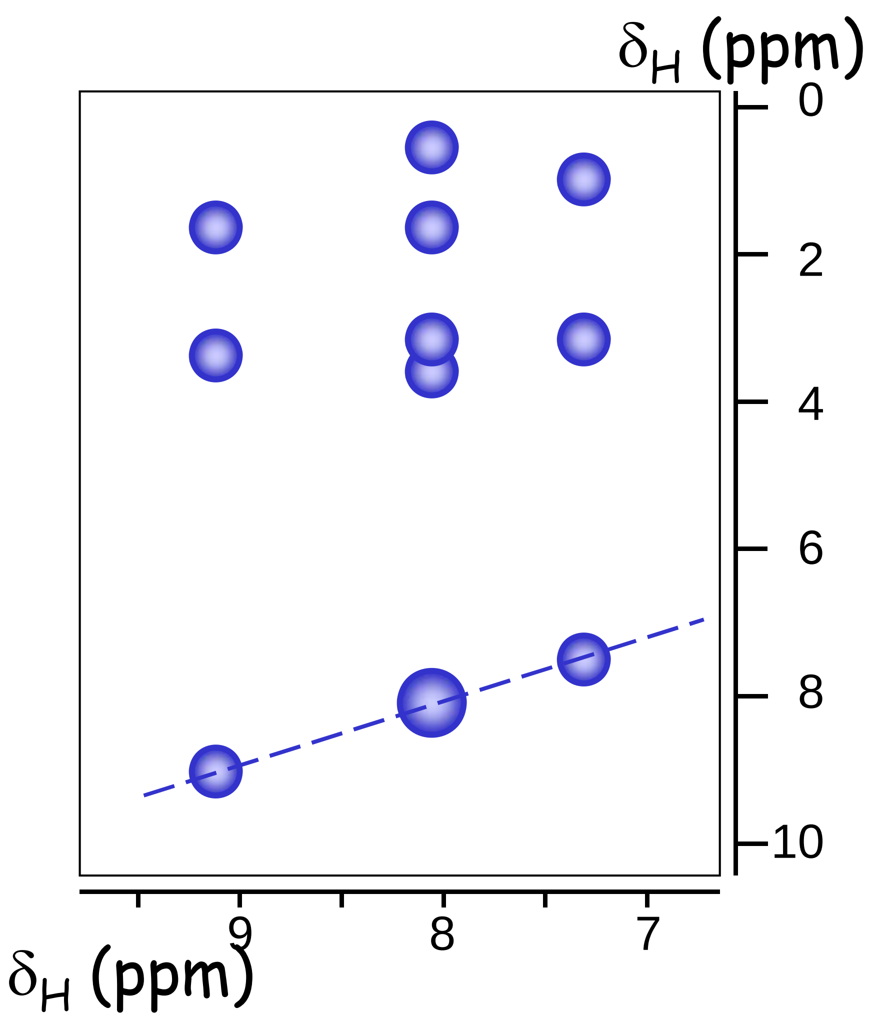


2 signals

1D-^{1}H-spectrum:

Region of the amino protons, 4 signals can be found, two of which overlap.

Multidimensional NMR-spectroscopy

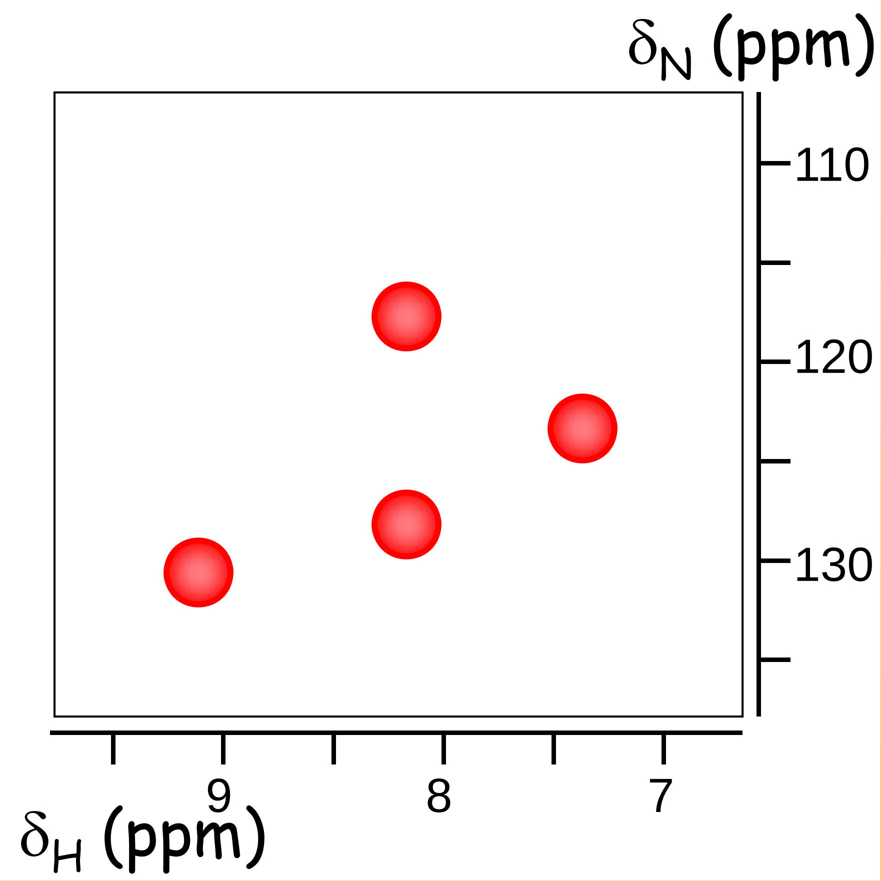


Homonuclear

^1H-^1H spectrum:

The overlap of the two amino protons prevent a distinction of the otherwise resolved protons in the aliphatic region

Multidimensional NMR-spectroscopy



Heteronuclear

^1H-^{15}N-spectrum:

The overlap of the

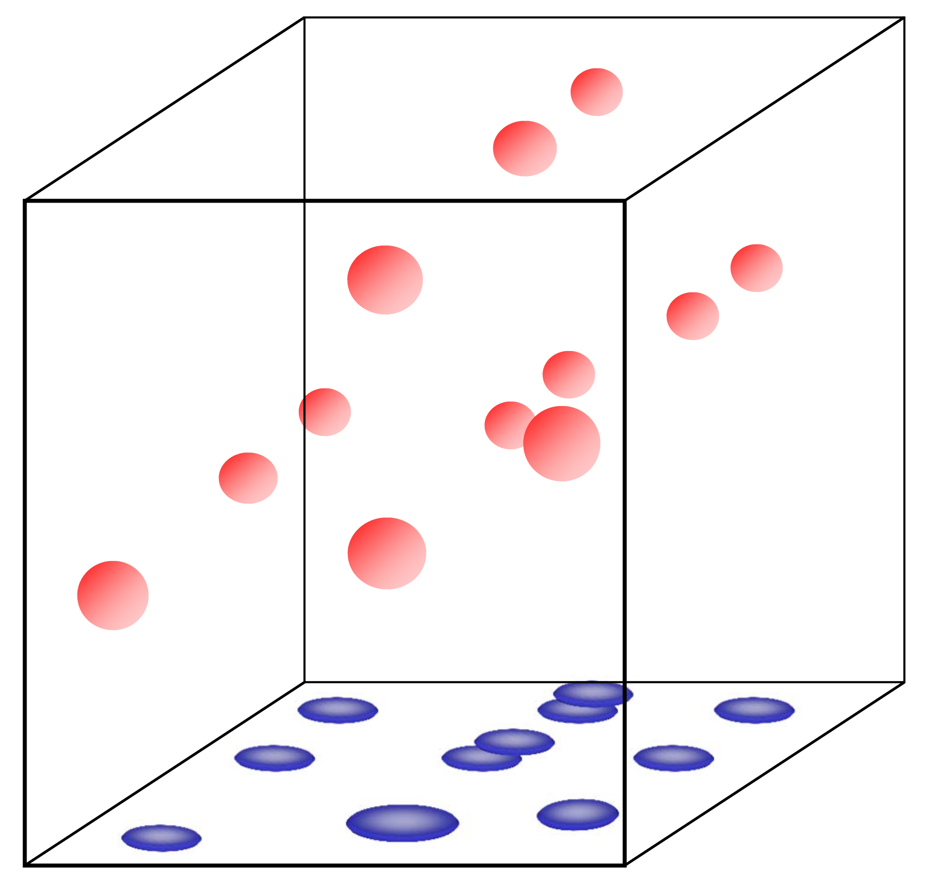
two amino protons is

resolved due to

different 15N

chemical shifts.

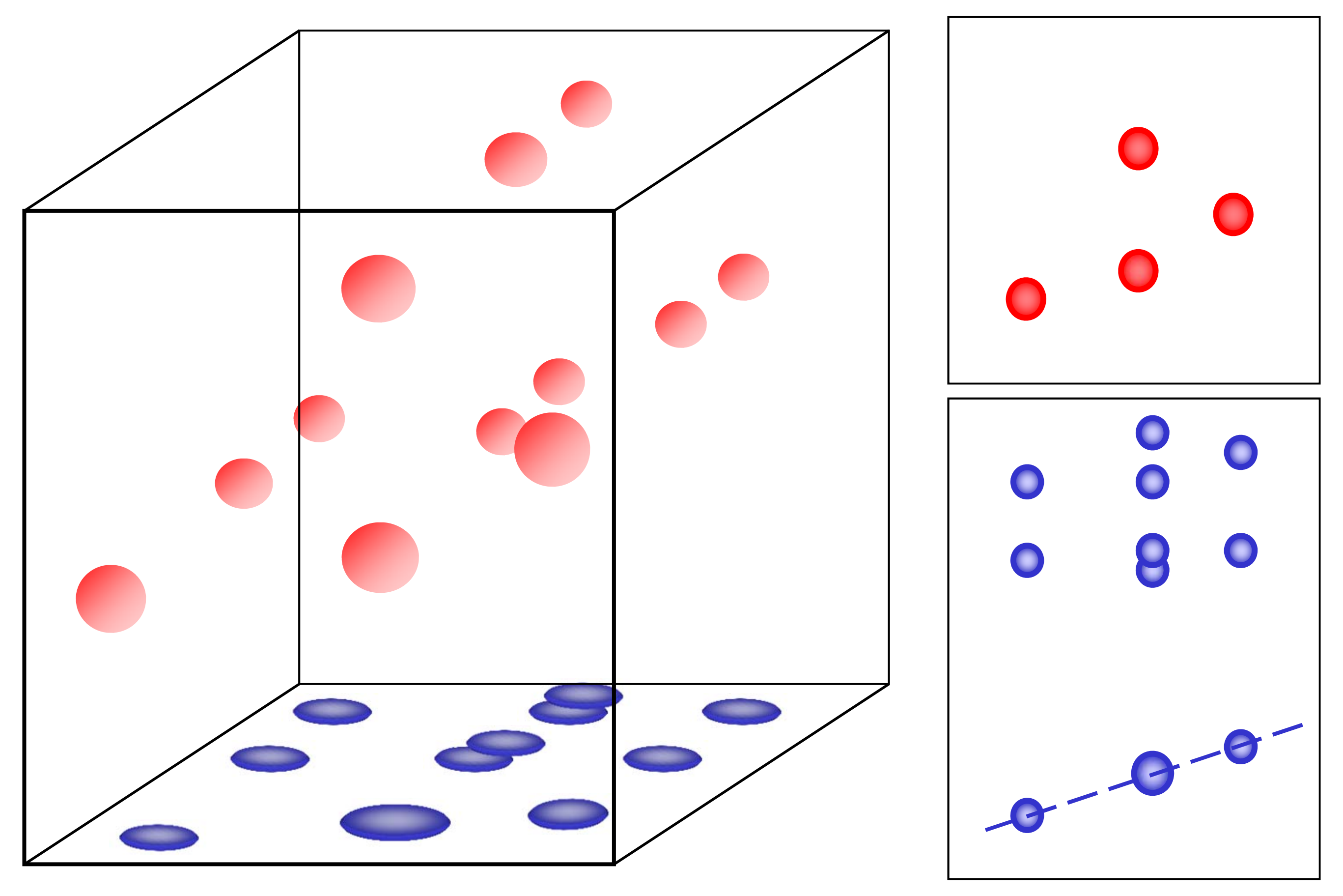
Multidimensional NMR-spectroscopy



In an ^{1}H-^{1}H-^{15}N-3D-spectrum all overlap is resolved. Two of the faces of the cuboid correspond to the original 2D spectra.

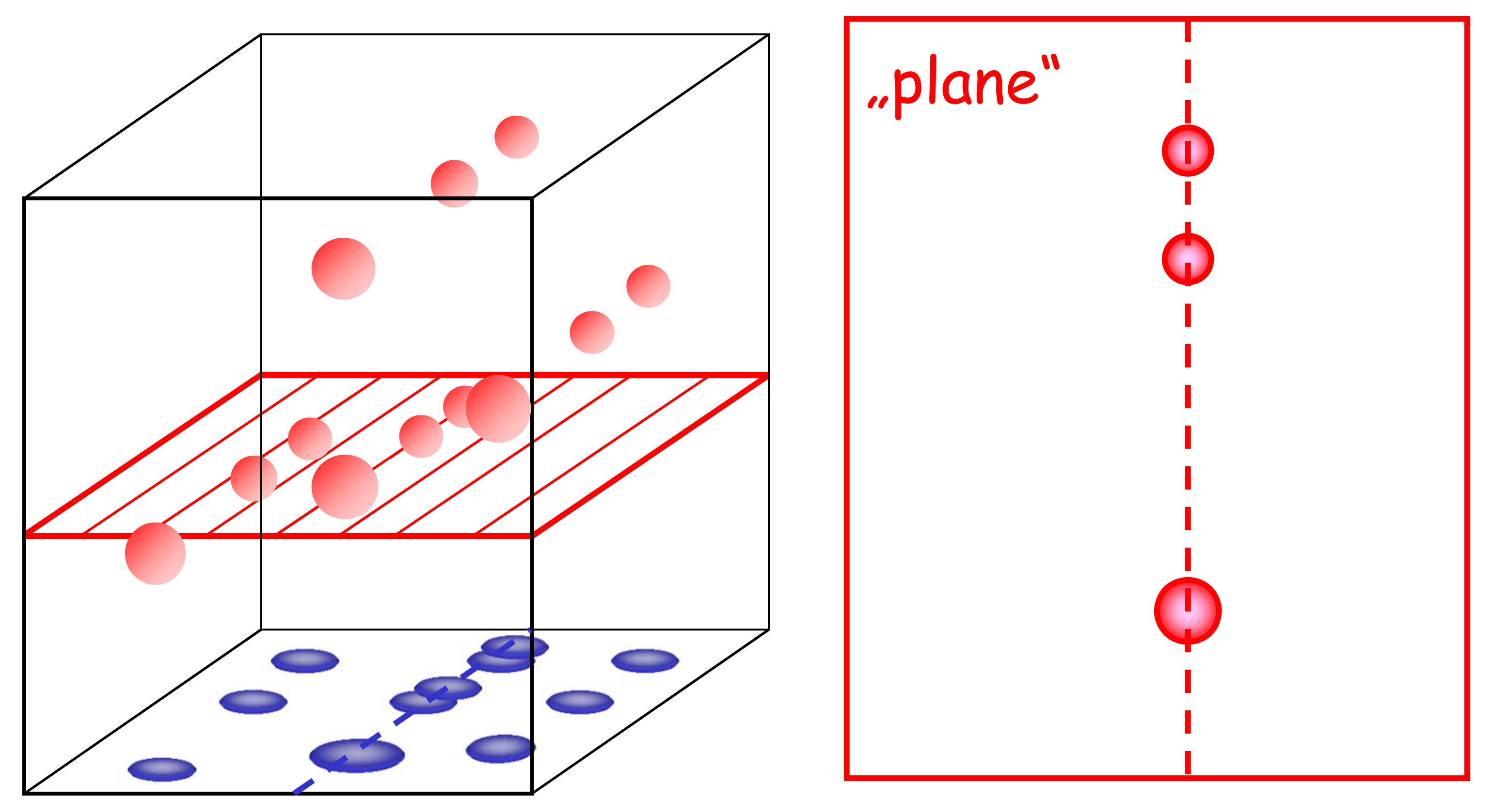
Multidimensional NMR-spectroscopy

Those faces are called the "projections" of the 3D



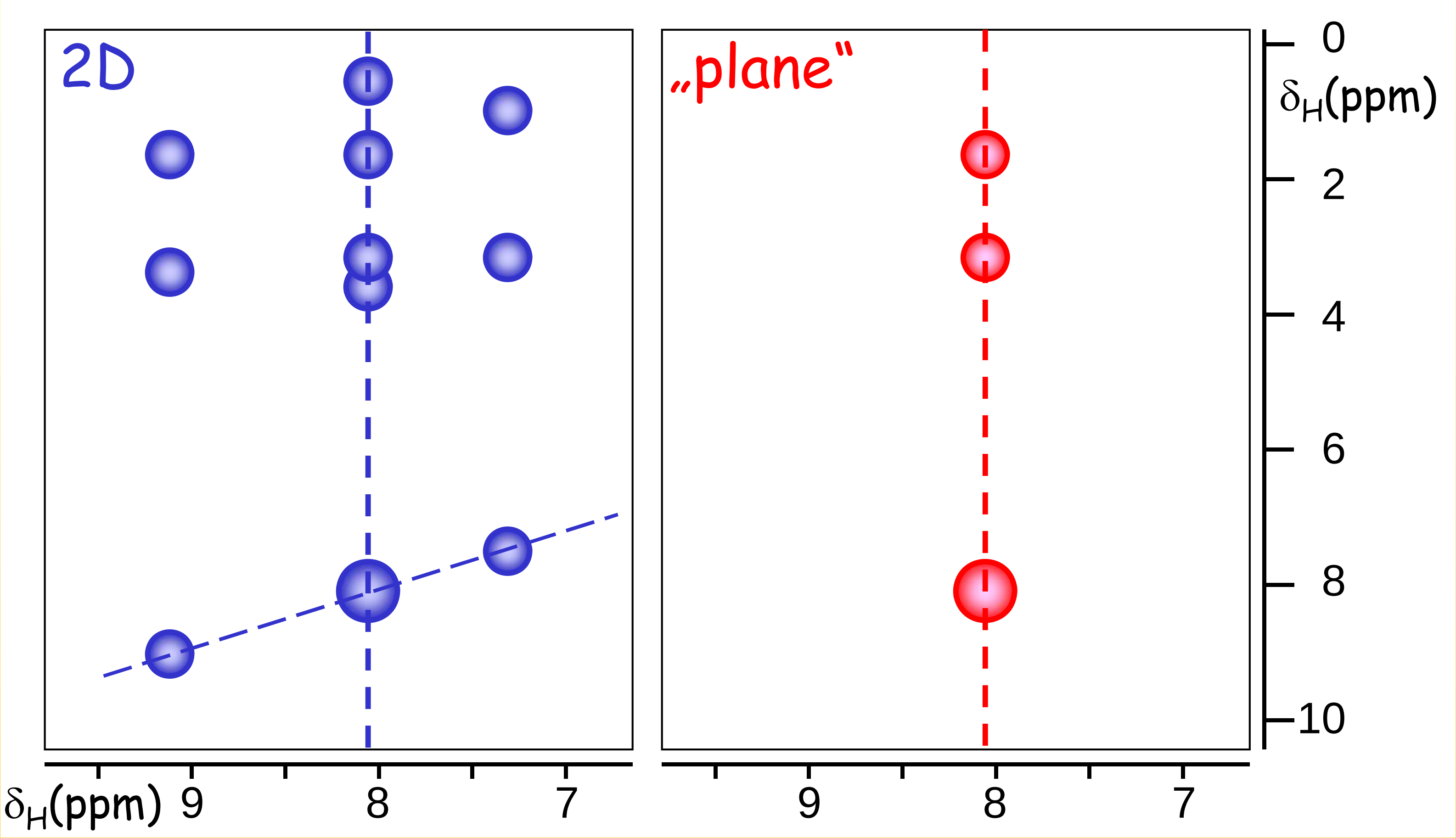
Multidimensional NMR-spectroscopy

Now we cut a „plane“ at a certain 15N chemical shift out of the cuboid.

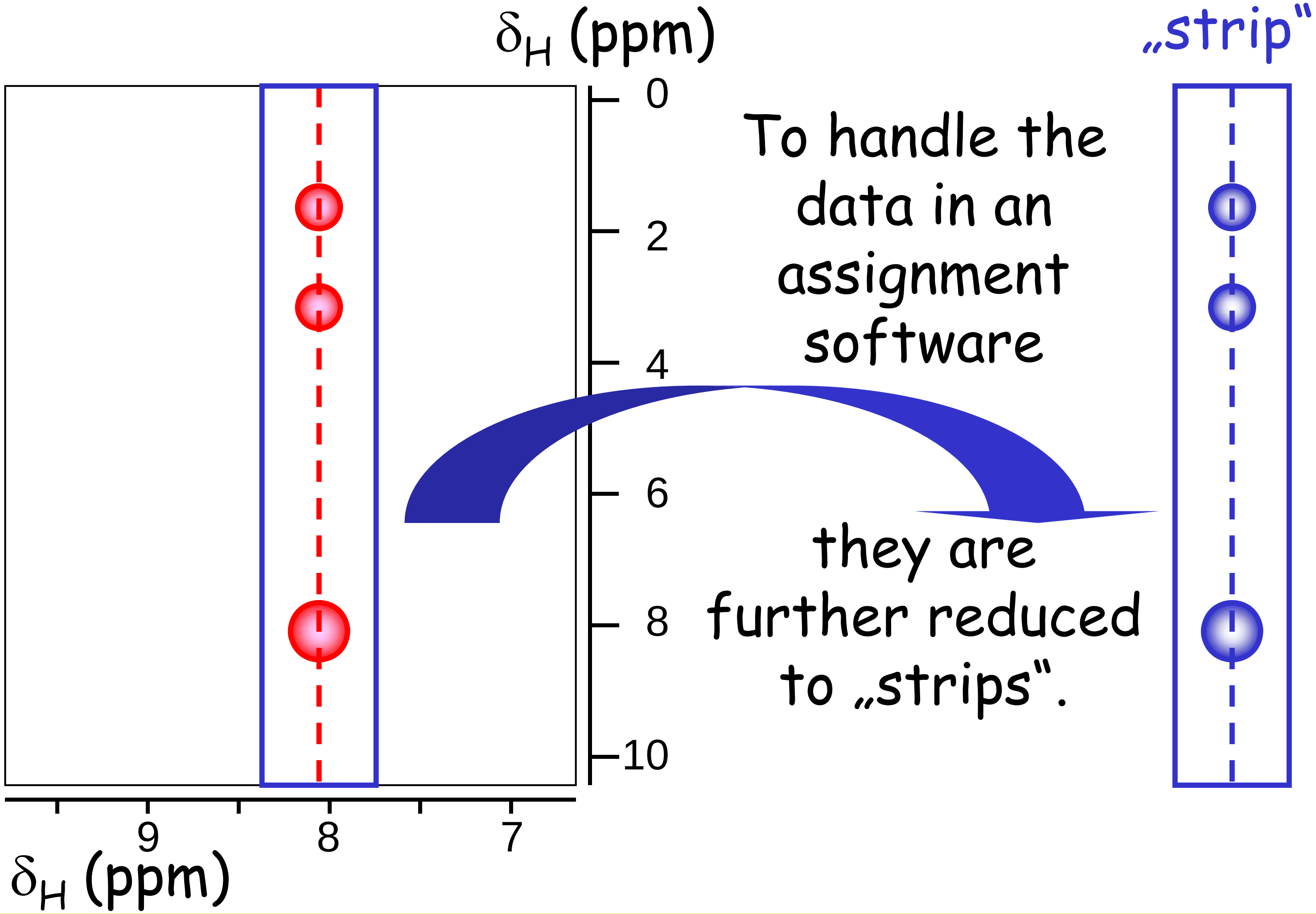


Multidimensional NMR-spectroscopy

The overlap has been removed from the ^1H-^1H-2D spectrum



Multidimensional NMR-spectroscopy

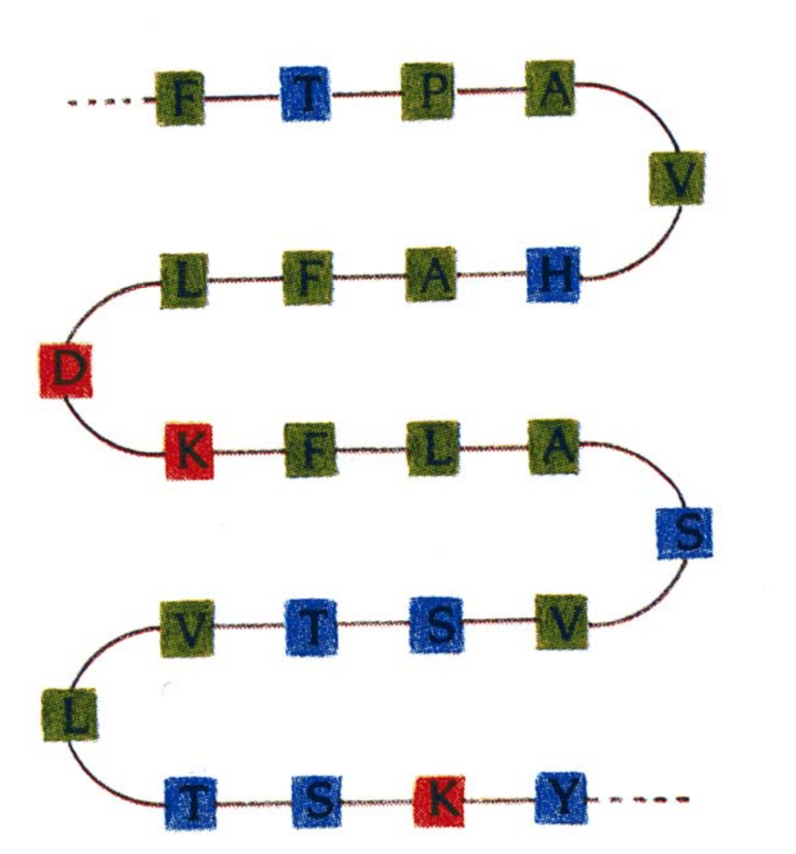


To handle the data in an assignment software

they are further reduced to „strips“.

Sequence specific assignment of proteins using tripel-resonance-techniques

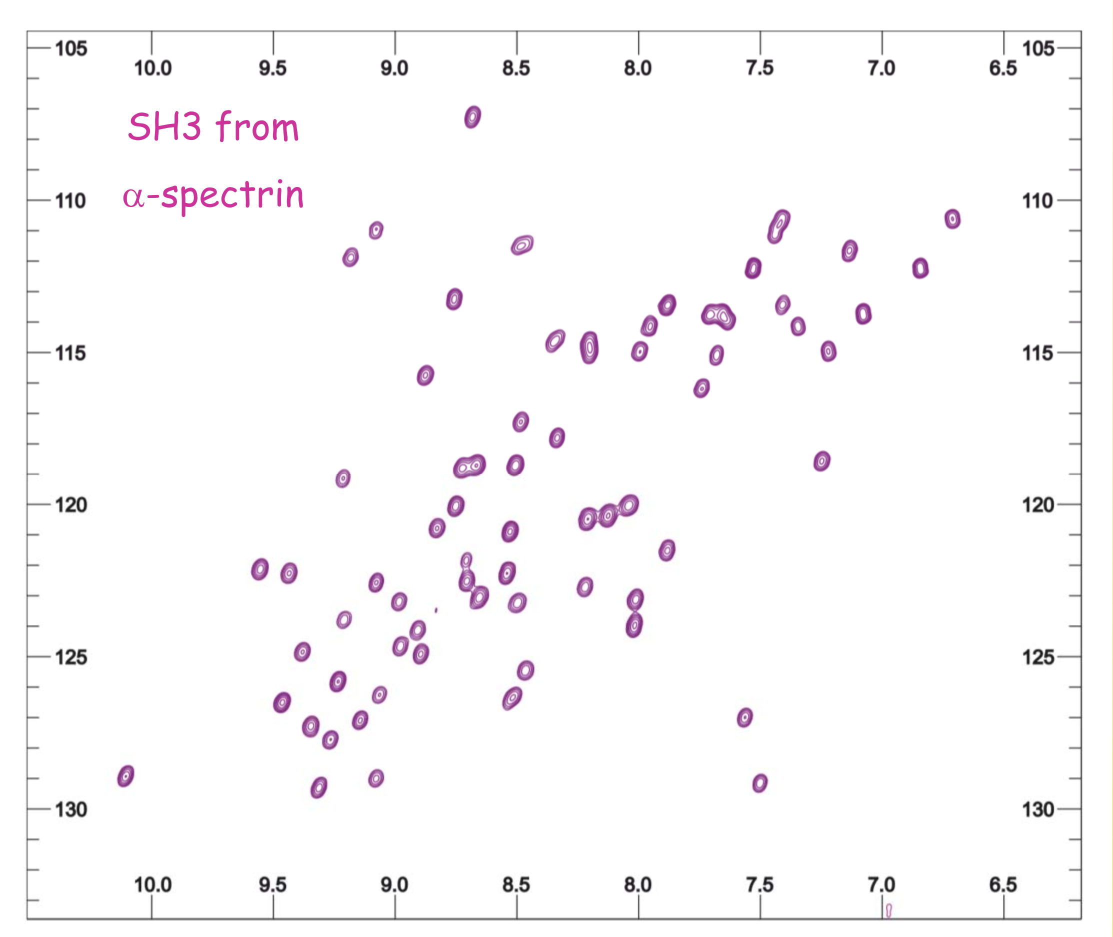
Sequence specific assignment of proteins



As with all other molecules the first step of an extraction of information is the assignment of resonances – but we have that „polymer-problem".

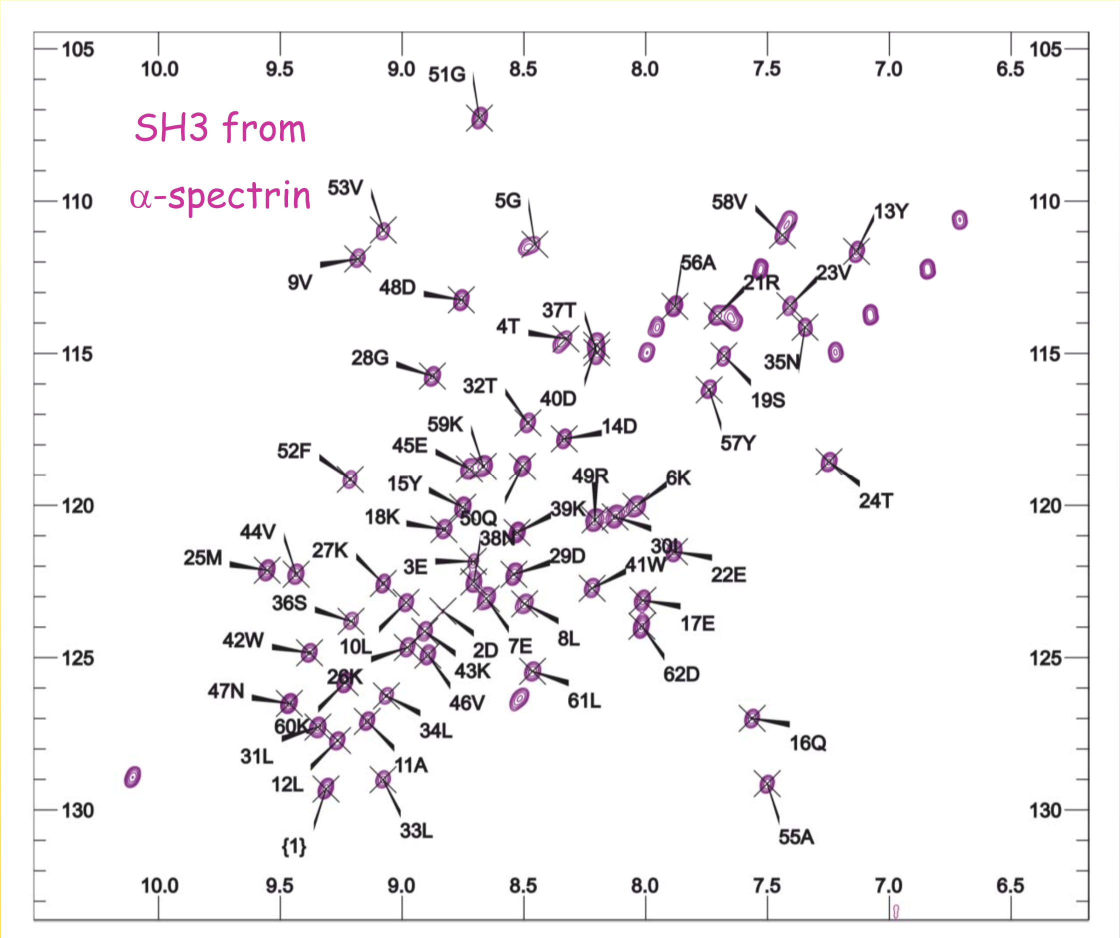
Sequence specific assignment of proteins

We need to get from here ....



Sequence specific assignment of proteins

to here ....

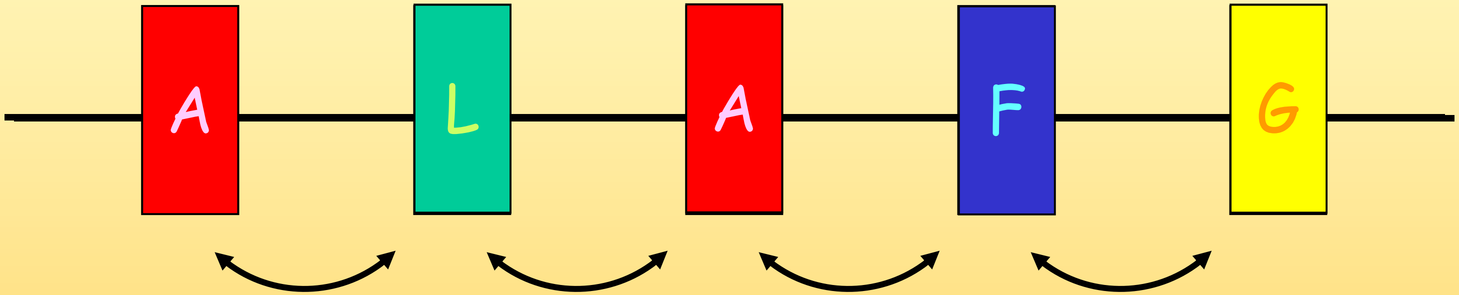


SH3 from α-spectrin

Sequence specific assignment of proteins

The solution for the assignment problem is the

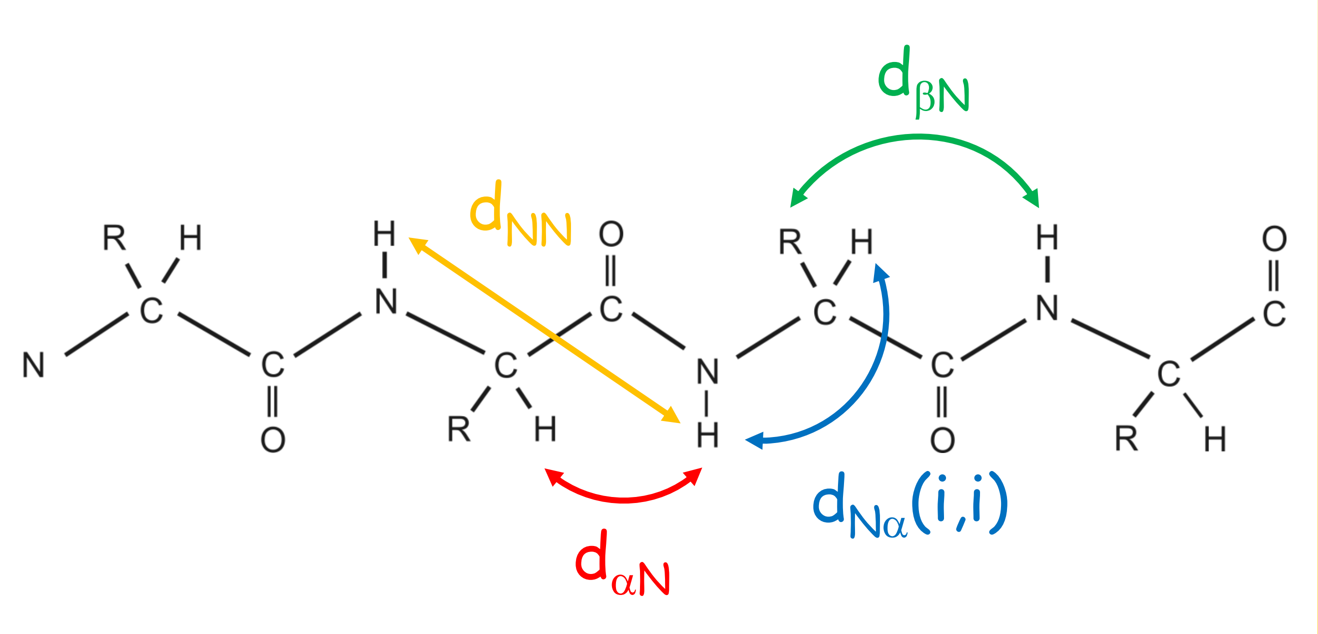
sequence-specific assignment



1. Which type of amino acid is present (color)
2. Which aa is next to which (neighborhood)
3. Comparison with the protein sequence to assign
4. The order of (1) and (2) is irrelevant

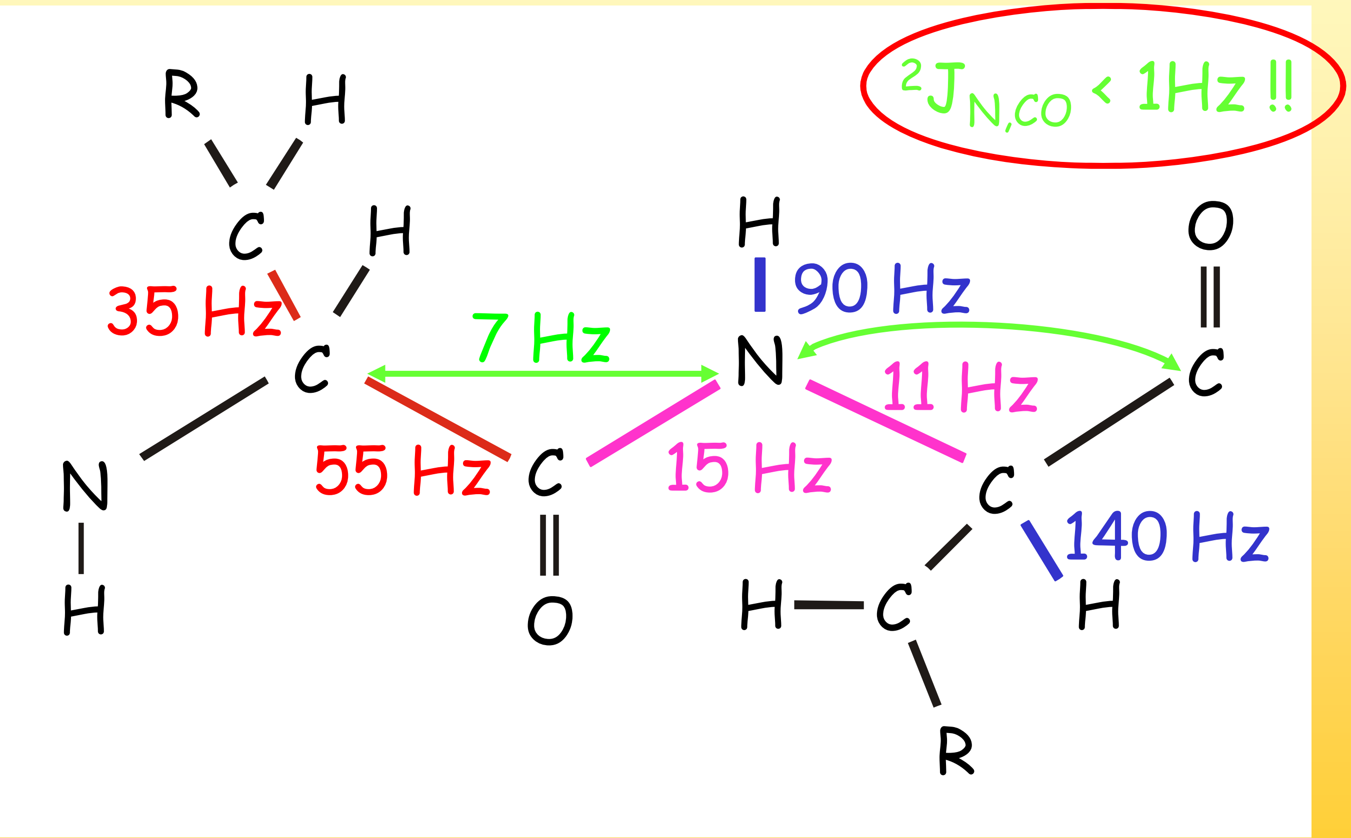
Sequence specific assignment of proteins

The „classic“ way of obtaining a sequence specific assignment works using proton-proton interactions, i.e. homonuclear spectra. It fails if proteins get larger and has therefore been replaced by a strategy based on triple-resonance spectra.



Sequence specific assignment of proteins

J-couplings between heteronuclei in proteins



Sequence specific assignment of proteins

Because of the differences between the aliphatic and the carbonly carbons in proteins both can be treated separately spectroscopically:

1. Chemical shift

   δ_{CO} ~ 170-180 ppm

   $\delta_{C\alpha/\beta}$ ~ 10-70 ppm

2. Scalar carbon-carbon coupling

   J(CO,Cα) ~ 55 Hz

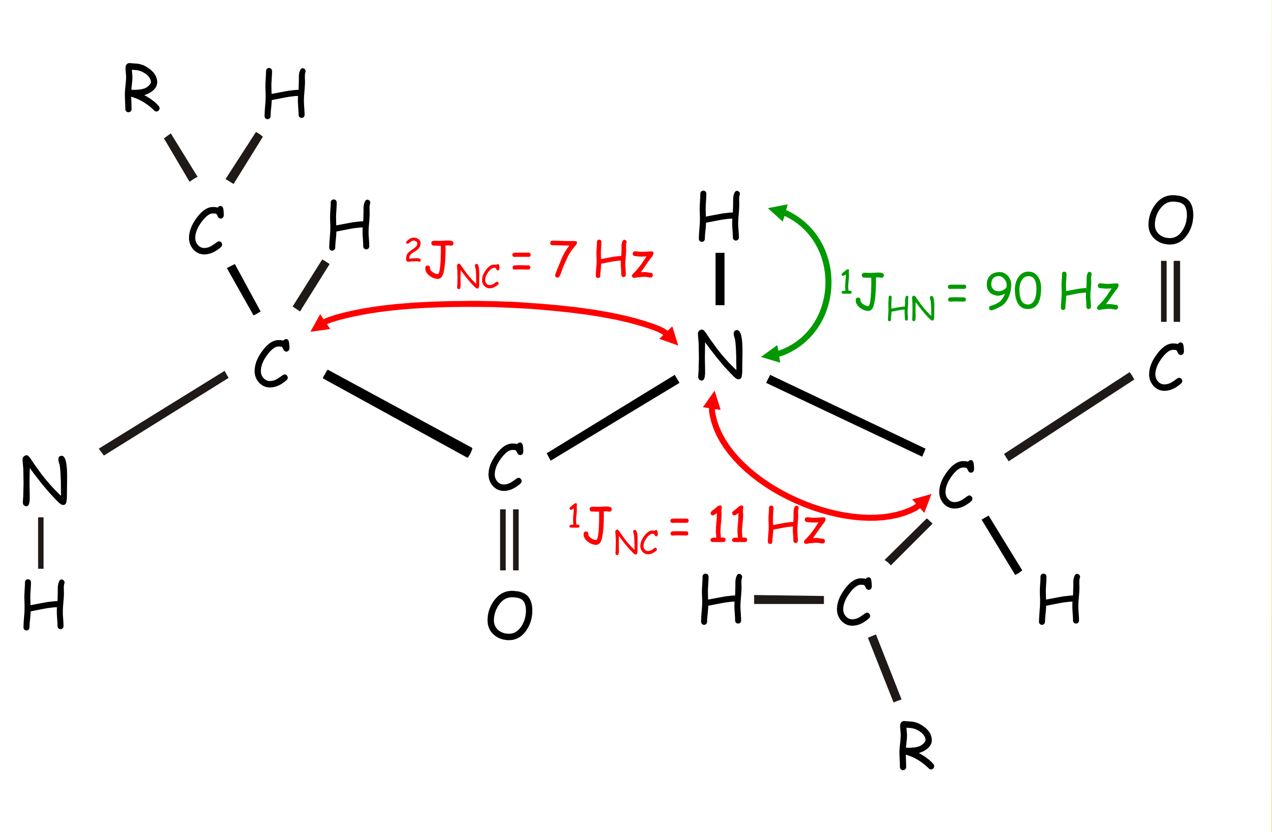
   J(C,C) ~35 Hz

3. Carbonyl carbons have no protons attached.

Carbonyl carbons are a “fourth” type of nucleus.

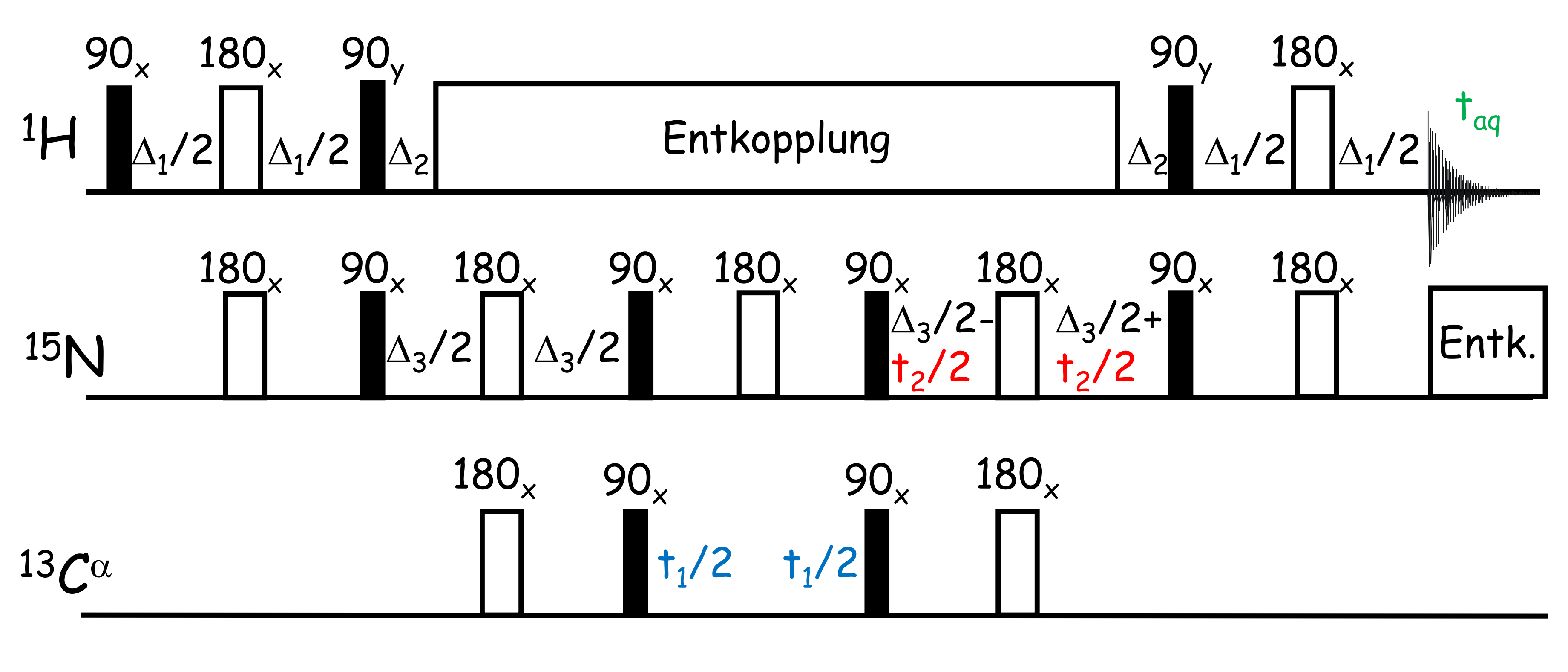
Sequence specific assignment of proteins

HNCA



Sequence specific assignment of proteins

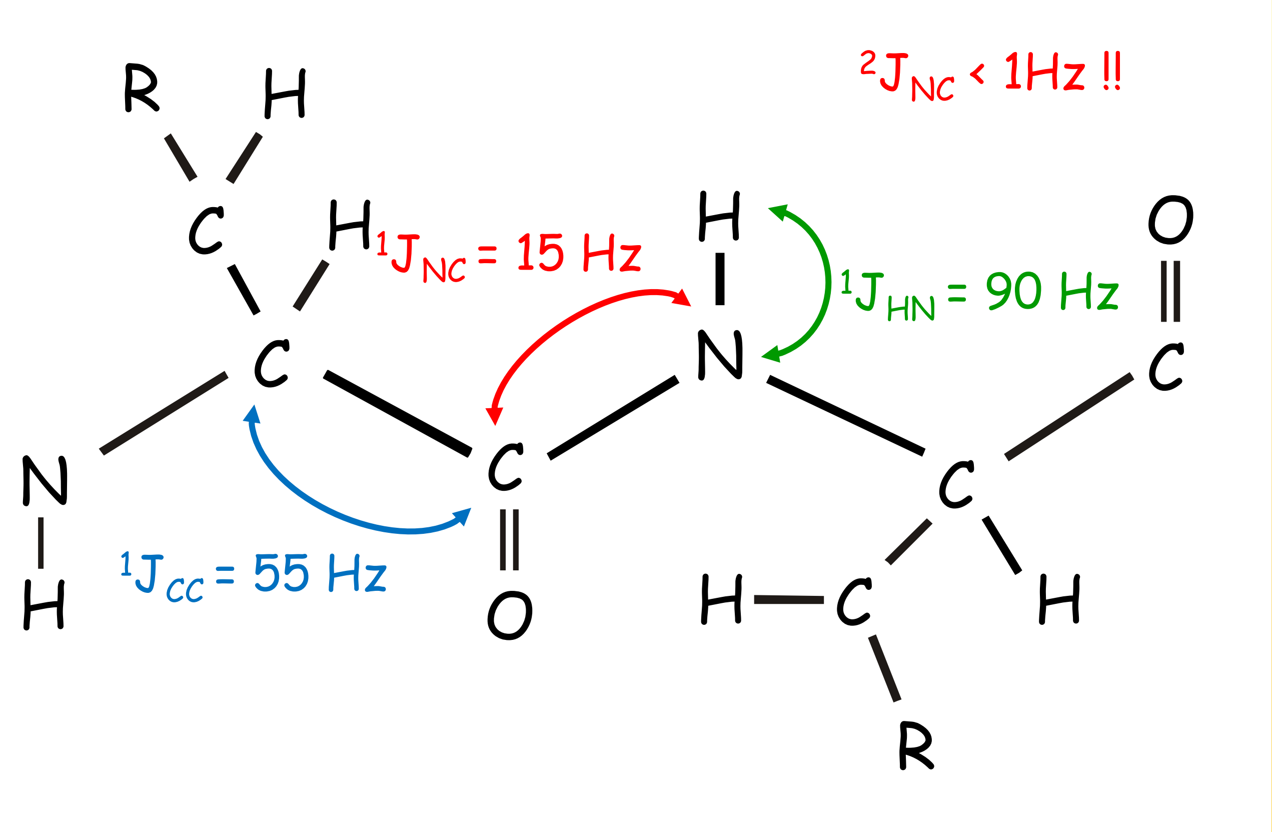
The pulse sequence of the HNCA



$H \longrightarrow N \longrightarrow C^\alpha \longrightarrow N \longrightarrow H$

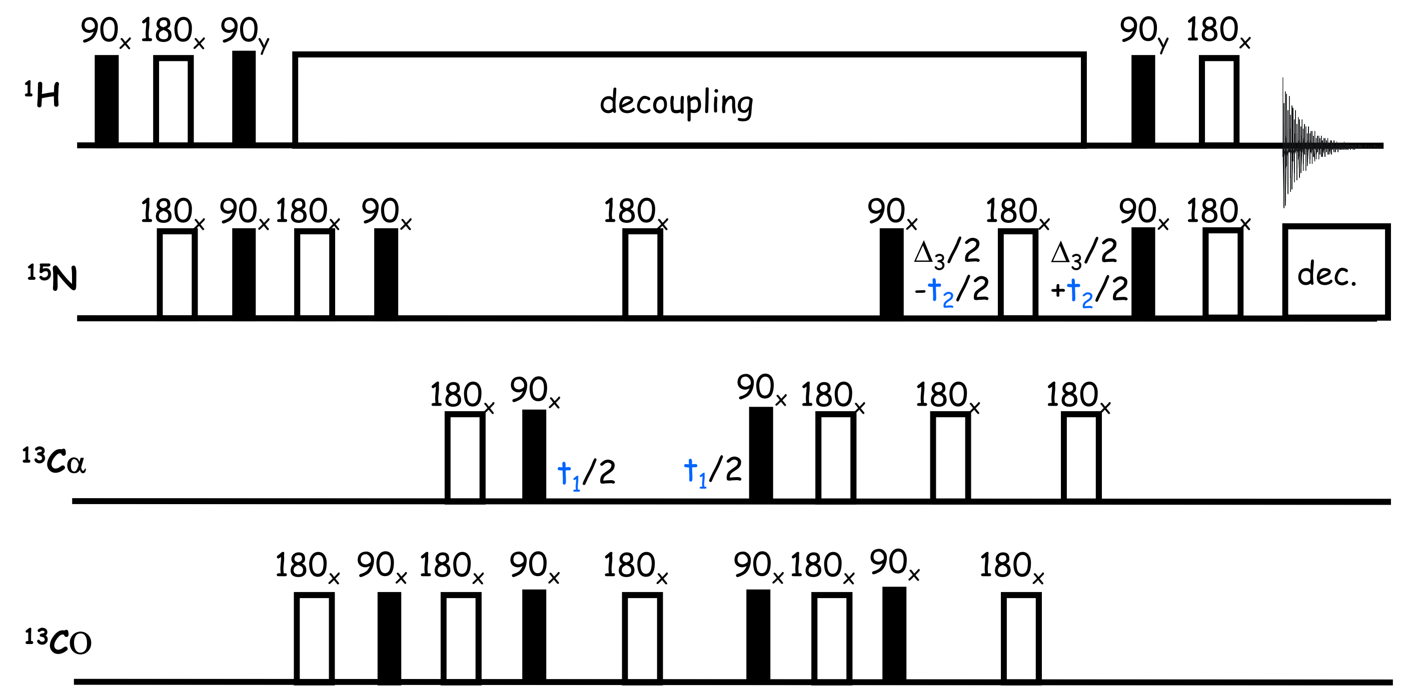
Sequence specific assignment of proteins

HN(CO)CA



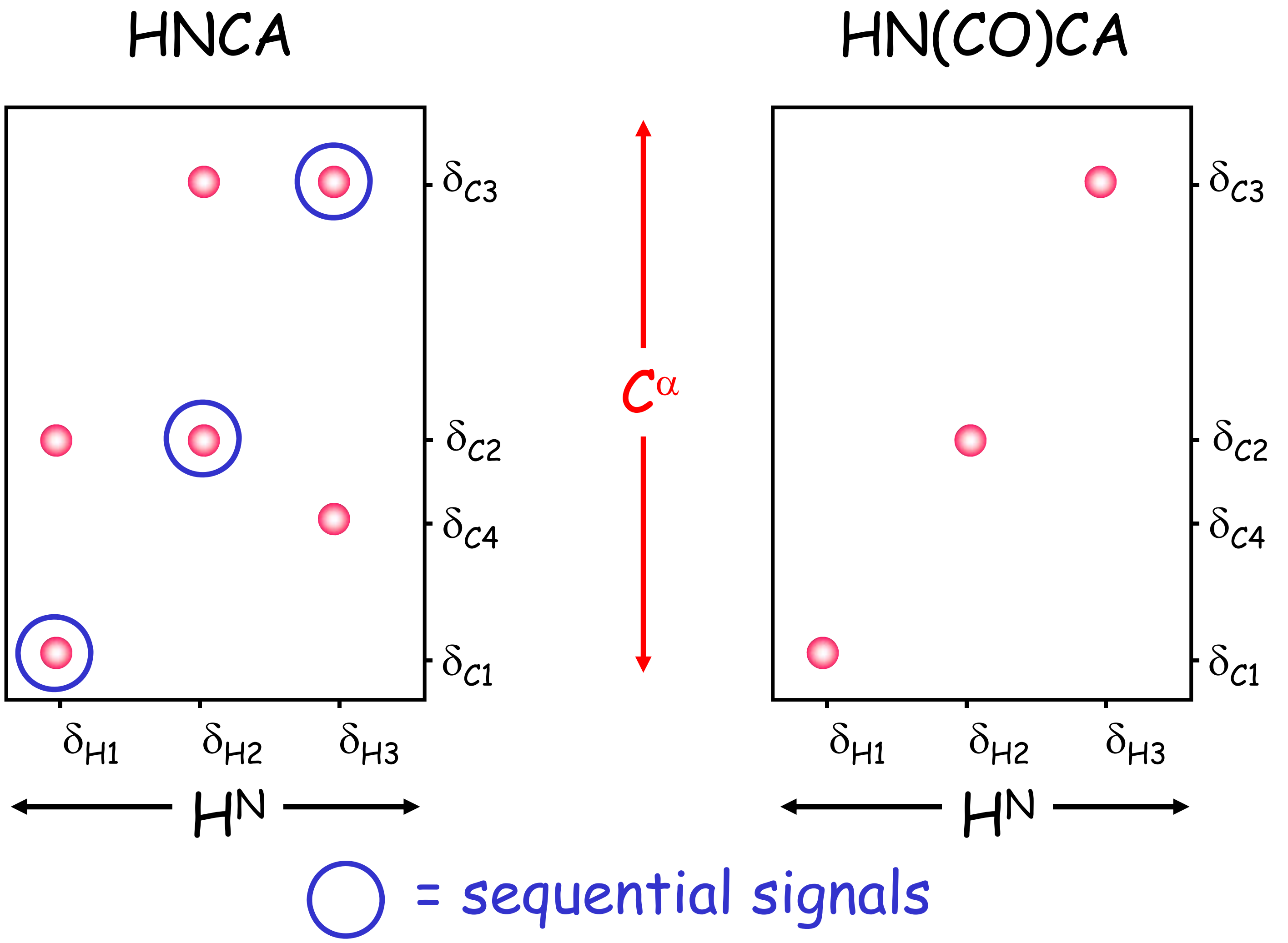
Sequence specific assignment of proteins

The pulse sequence of the HN(CO)CA



$$H \longrightarrow N \longrightarrow CO \longrightarrow C^{\alpha} \longrightarrow CO \longrightarrow N \longrightarrow H$$

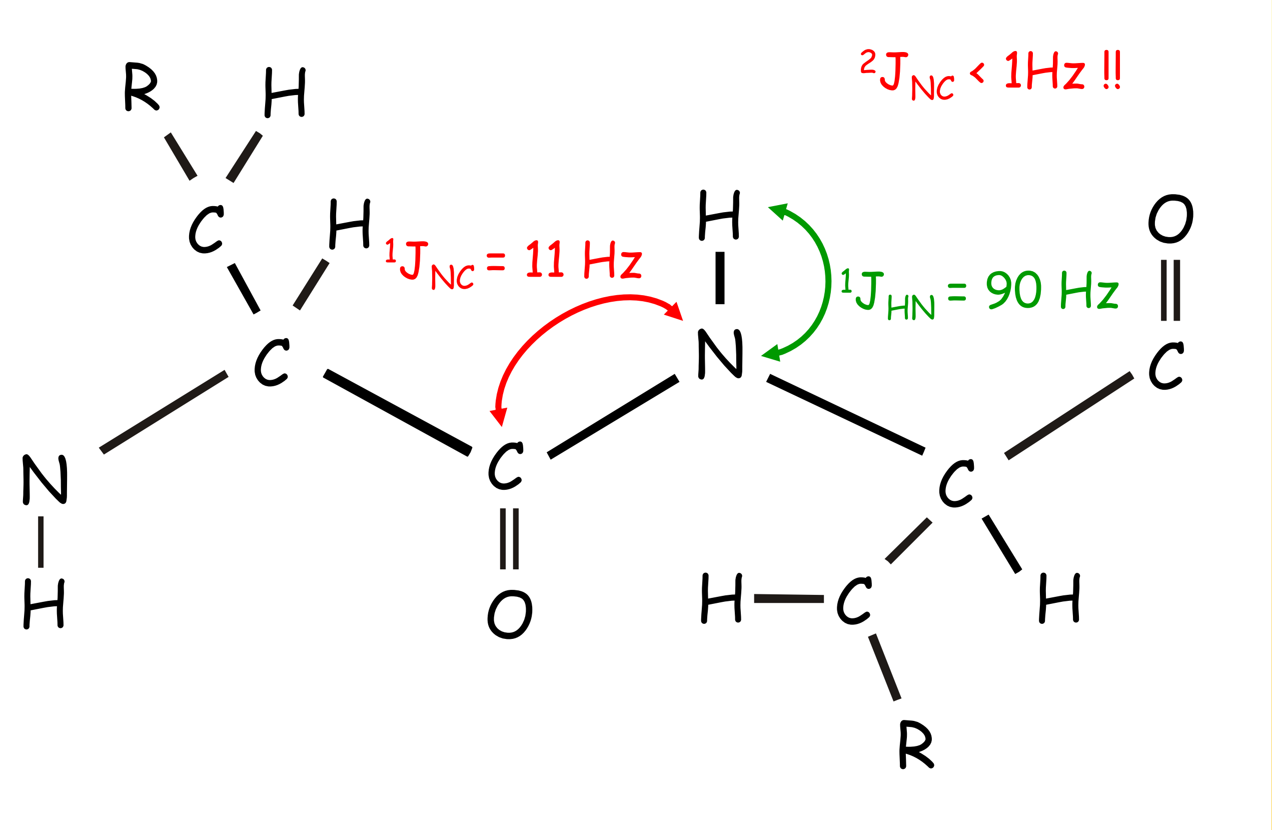
Sequence specific assignment of proteins



○ = sequential signals

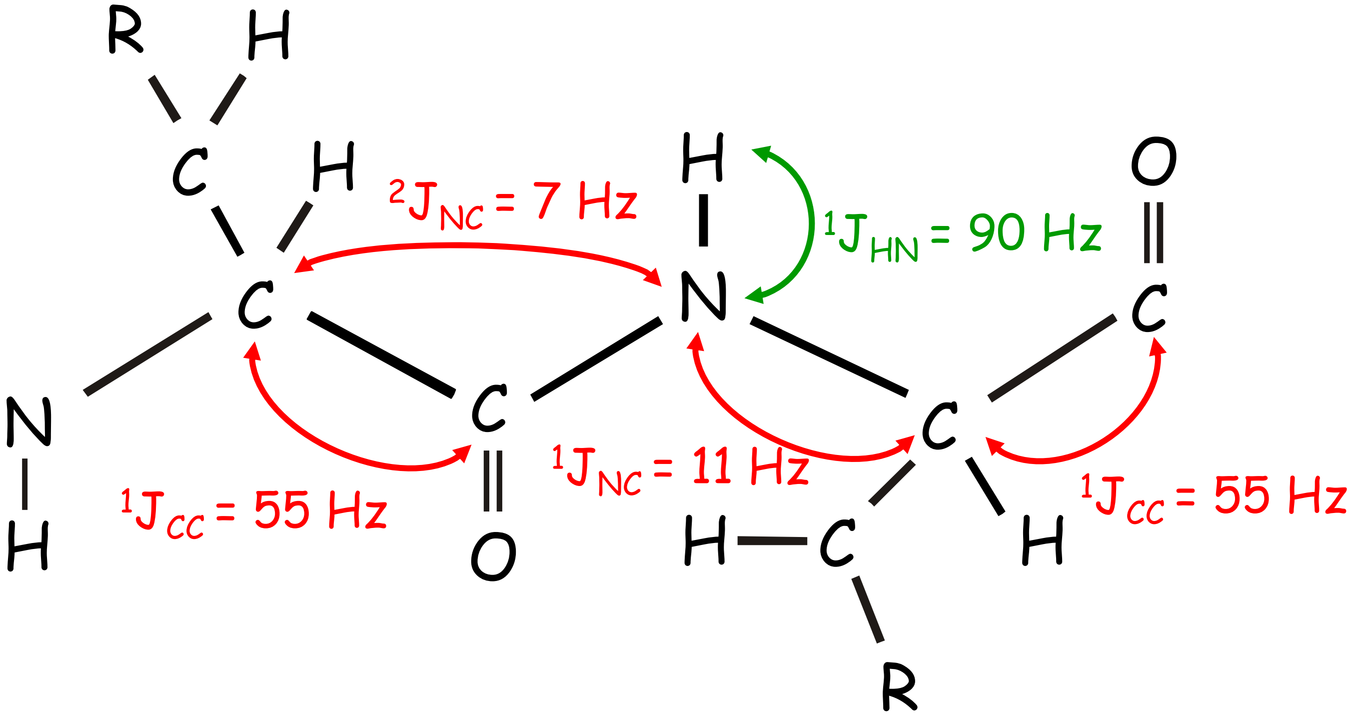
Sequence specific assignment of proteins

HNCO

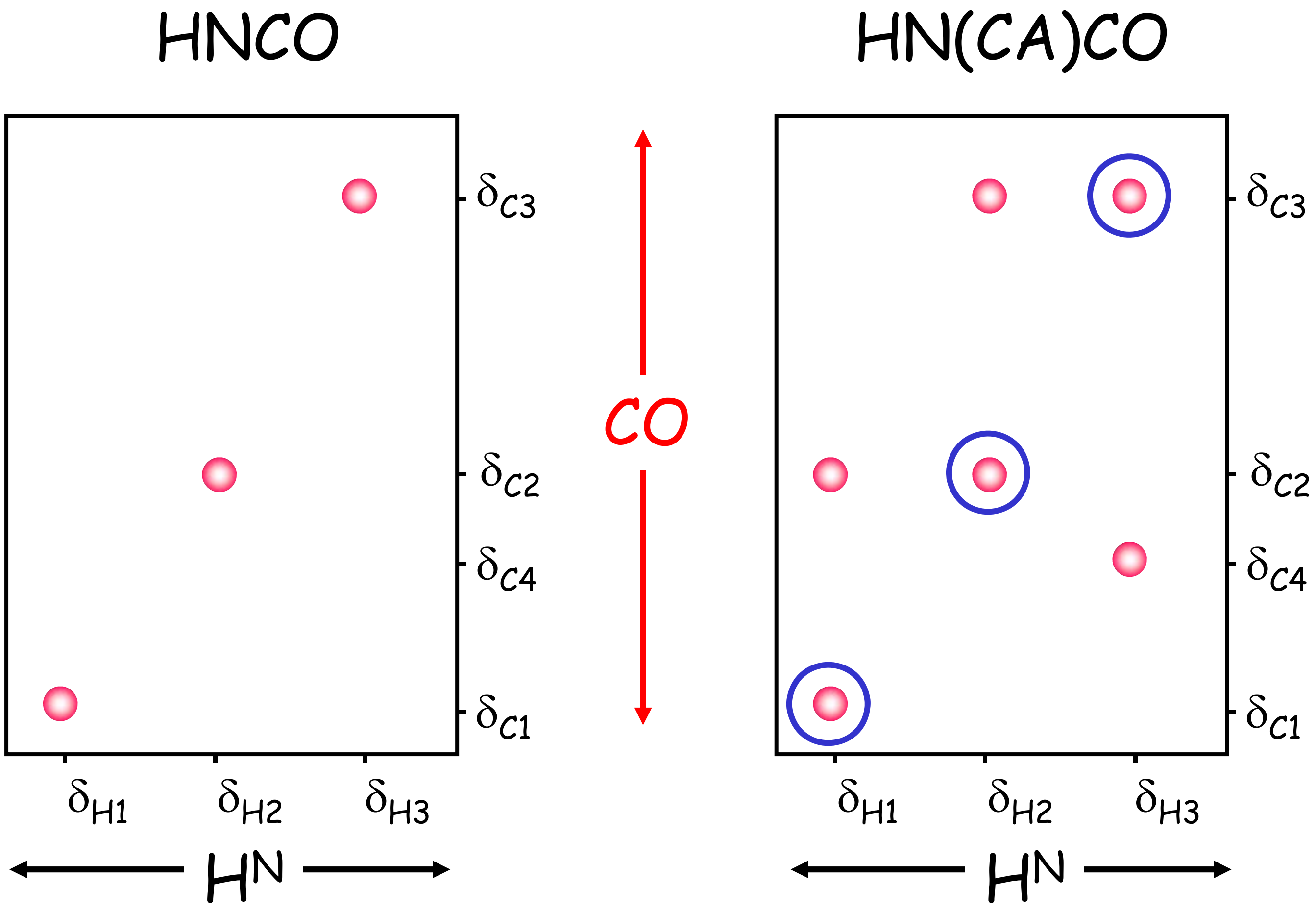


Sequence specific assignment of proteins

HN(CA)CO



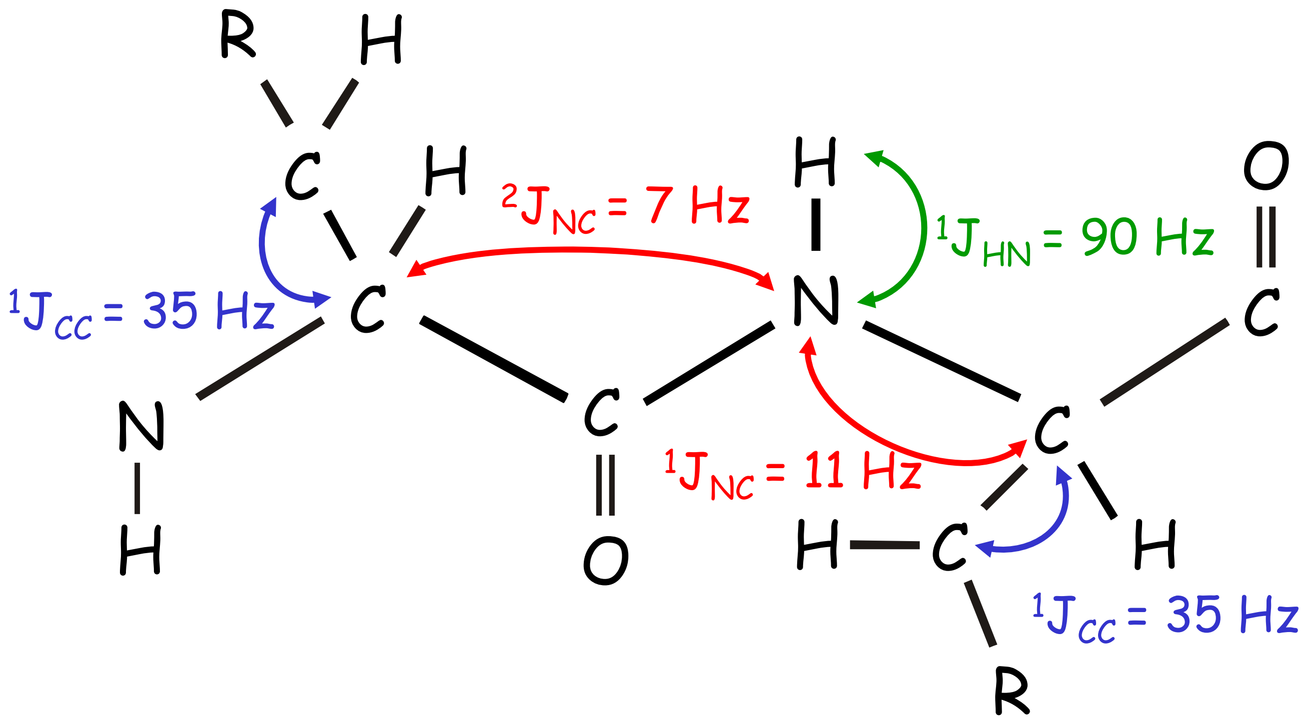
Sequence specific assignment of proteins



○ = sequential signals

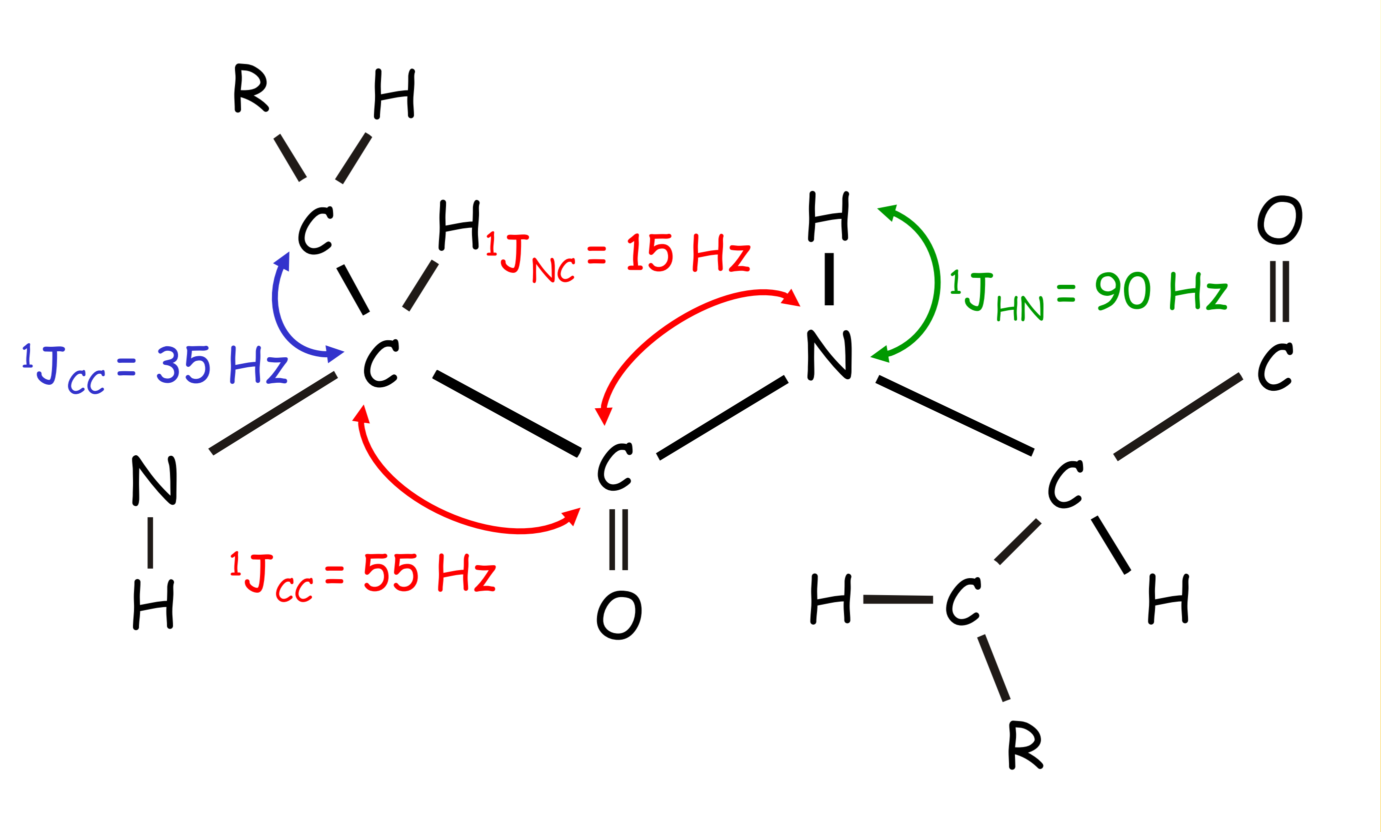
Sequence specific assignment of proteins

HNCACB

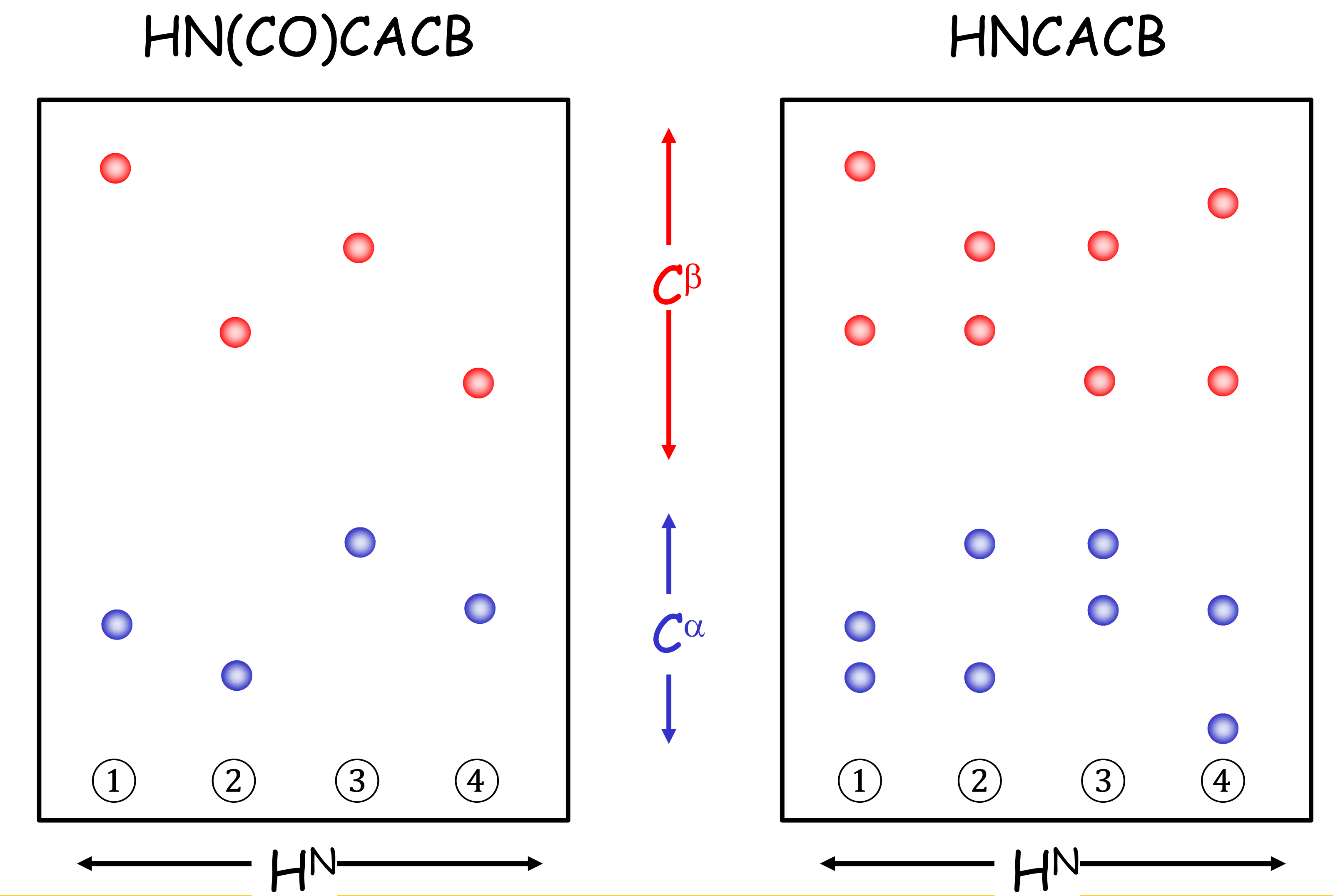


Sequence specific assignment of proteins

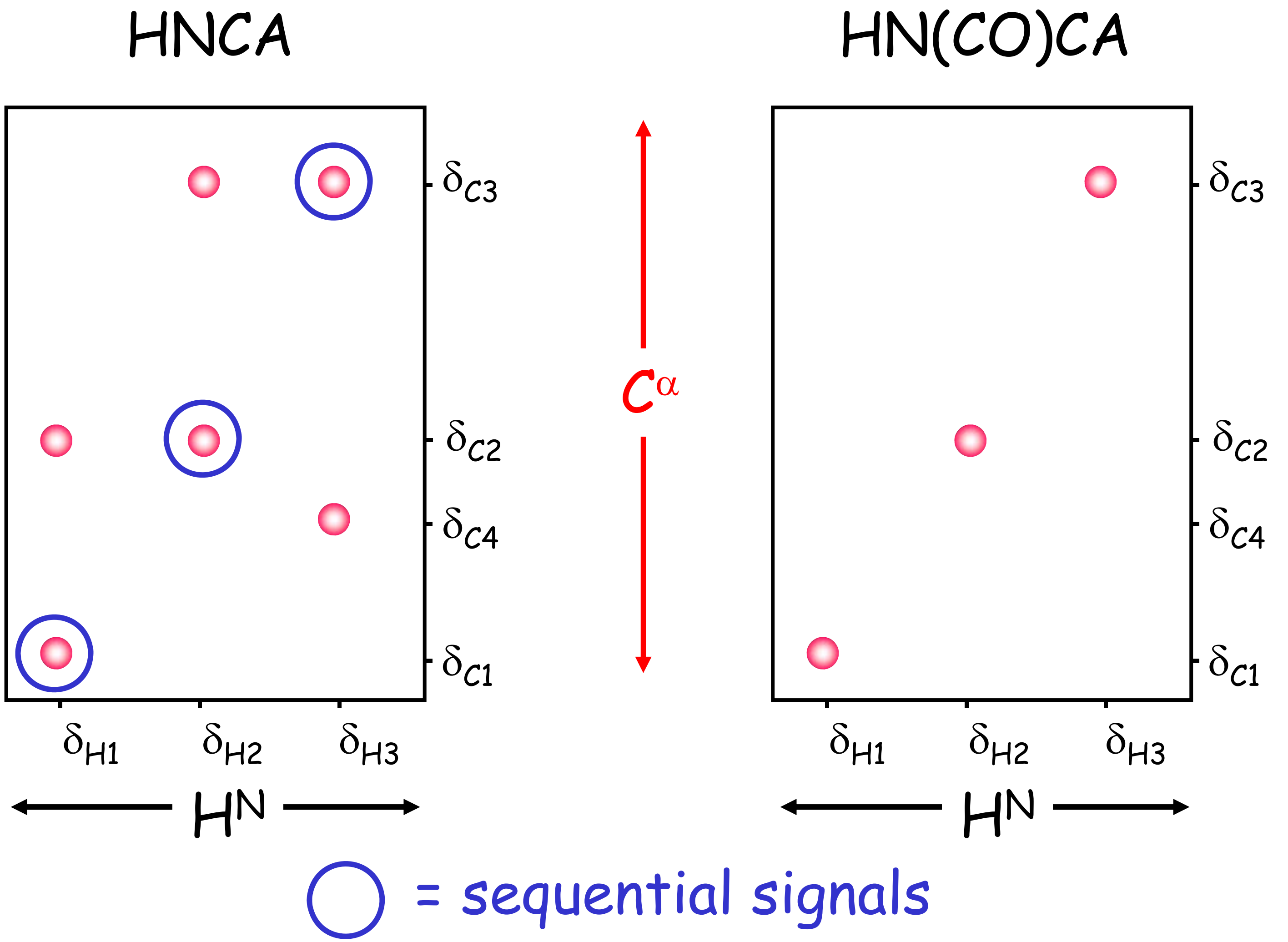
HN(CO)CACB



Sequence specific assignment of proteins

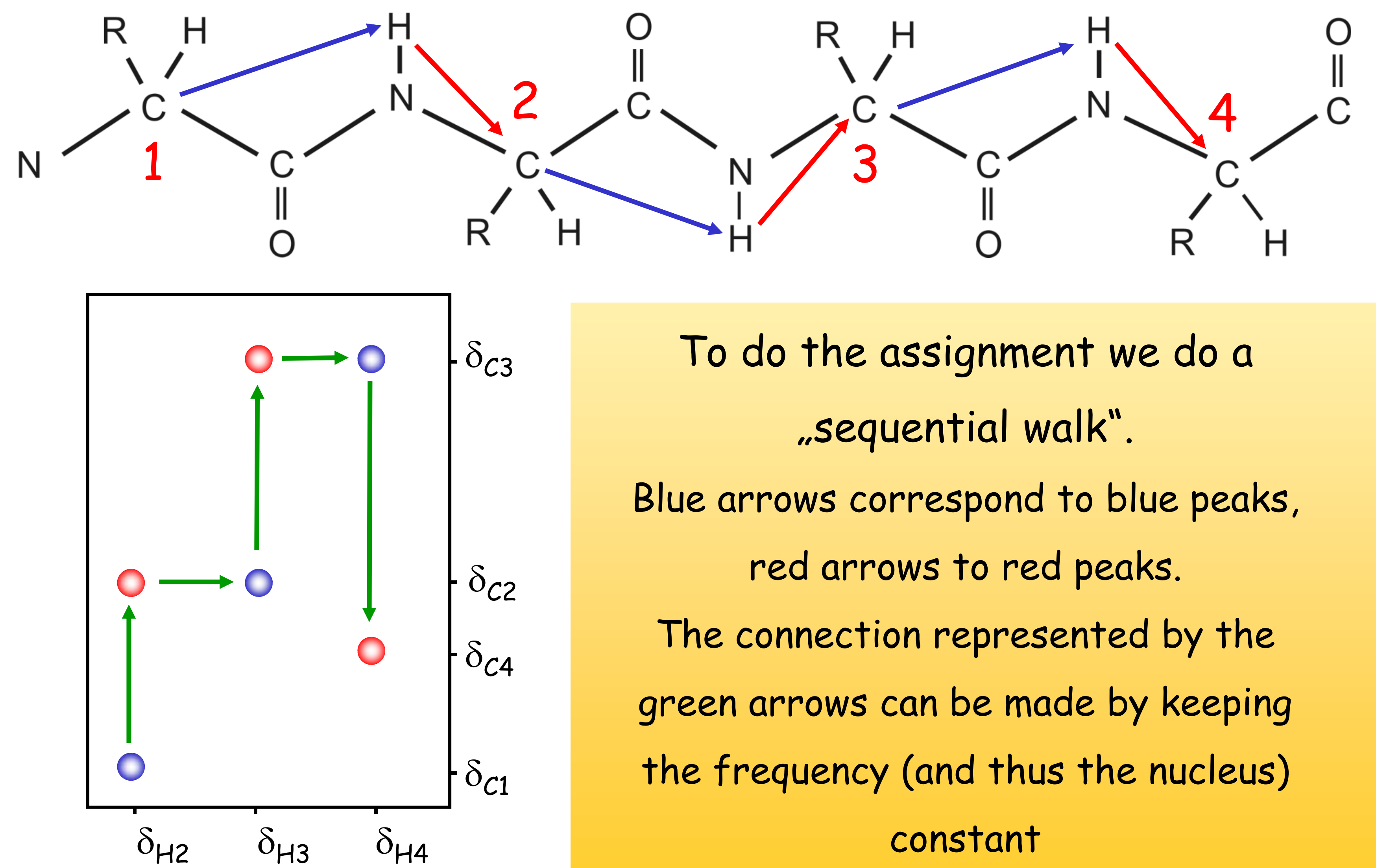


Sequence specific assignment of proteins



○ = sequential signals

Sequence specific assignment of proteins



To do the assignment we do a „sequential walk“.

Blue arrows correspond to blue peaks, red arrows to red peaks.

The connection represented by the green arrows can be made by keeping the frequency (and thus the nucleus) constant

Sequence specific assignment of proteins

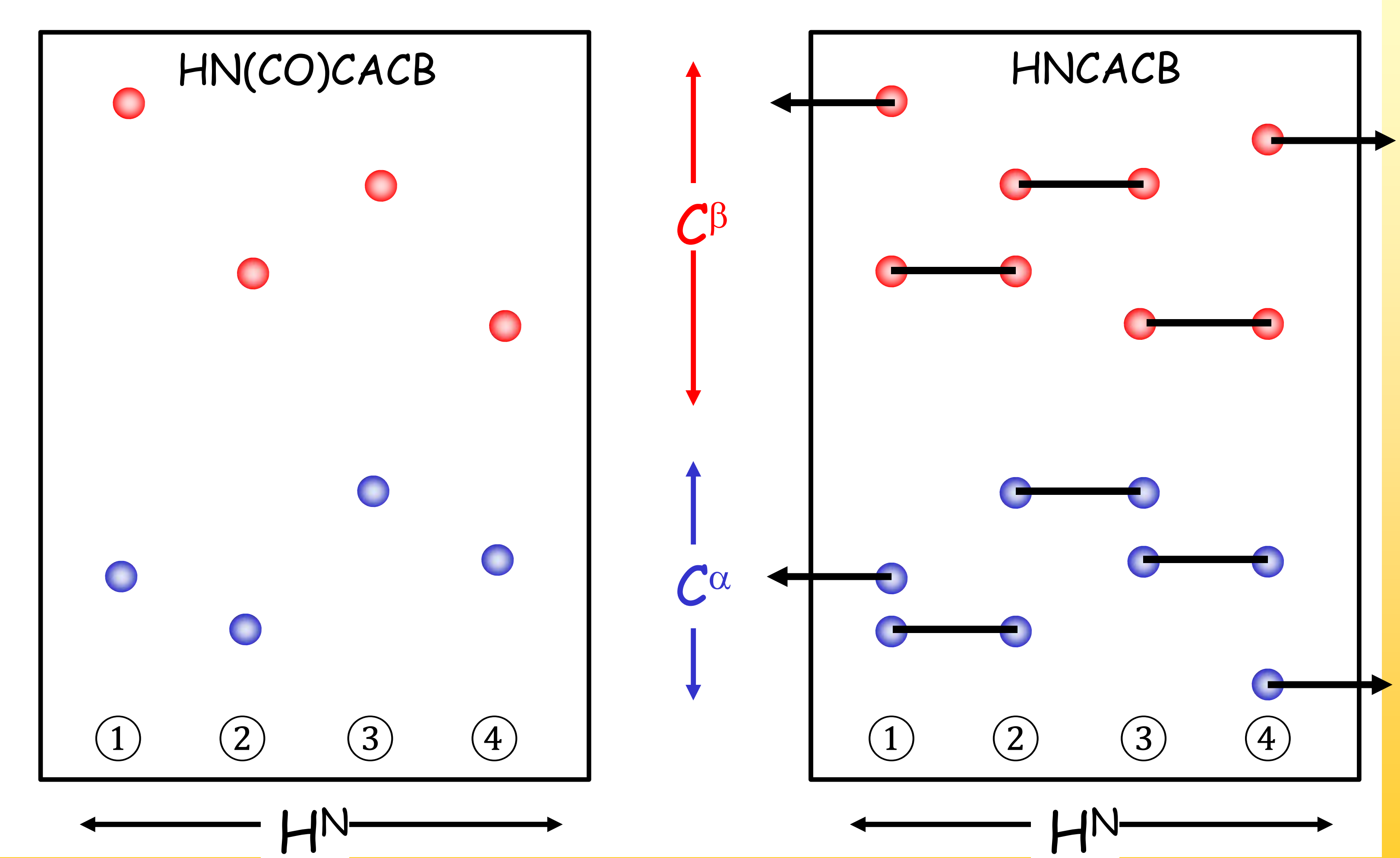
Using the HNCA/HN(CO)CA there can be the problem that two C^{α} shifts are identical.

Then it is possible to use the pair HNCO and HN(CA)CO as well in a similar fashion and it is unlikely that overlap occurs in both.

But it is even better to used C^{α} and C^{β} simultaneously in the HNCACB and HN(CO)CACB pair.

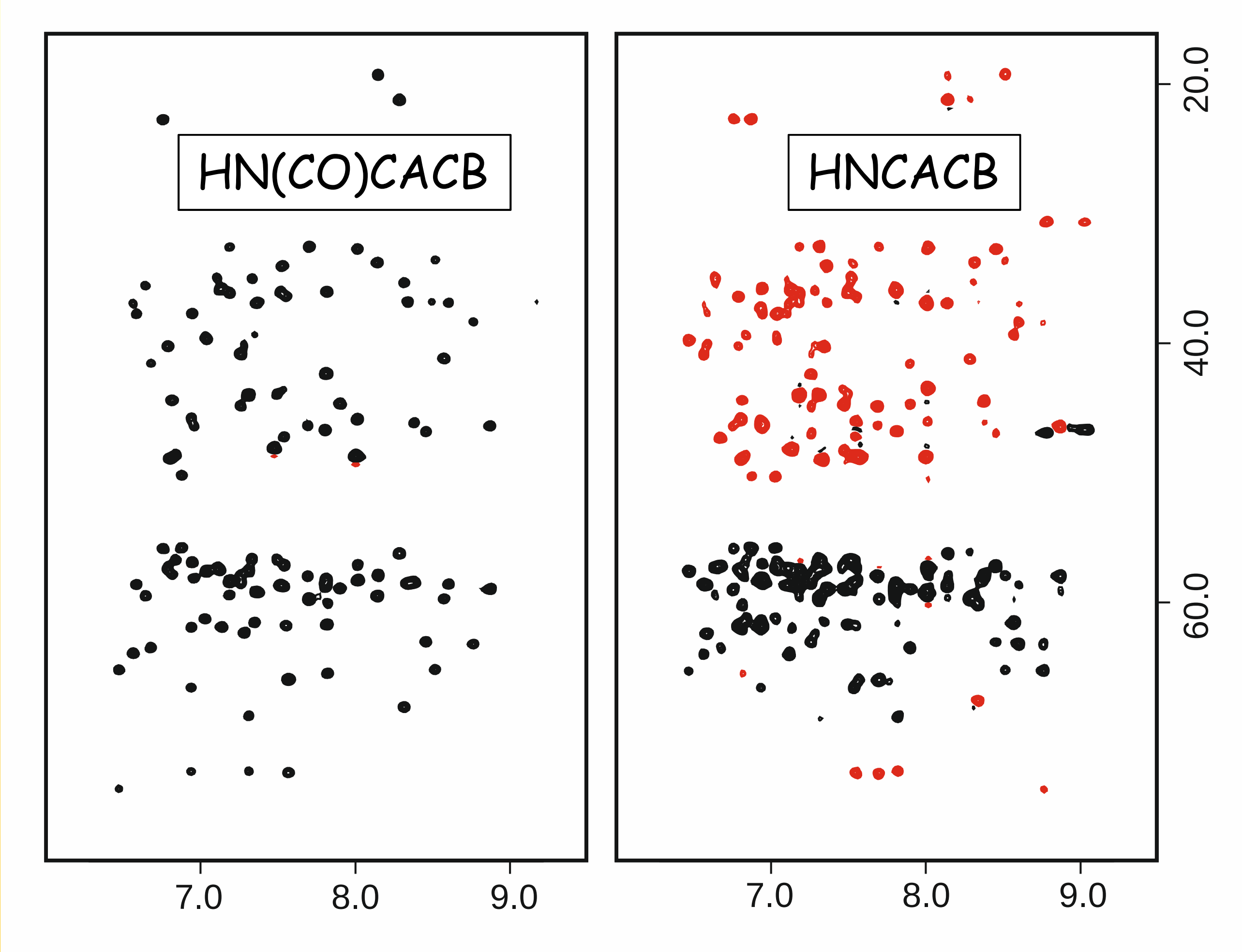
Sequence specific assignment of proteins

Sequential assignment



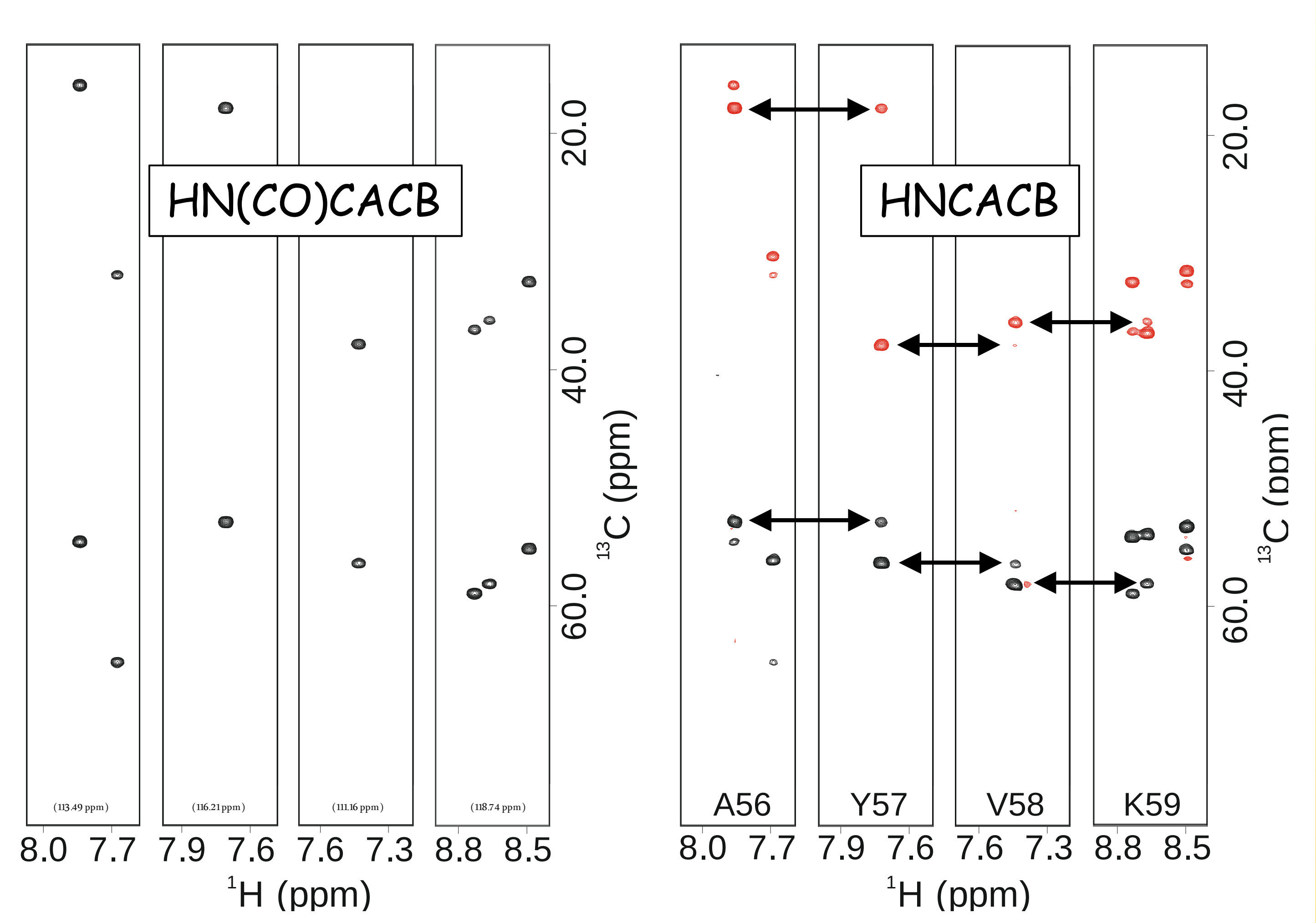
Sequence specific assignment of proteins

Sequentielle Zuordnung

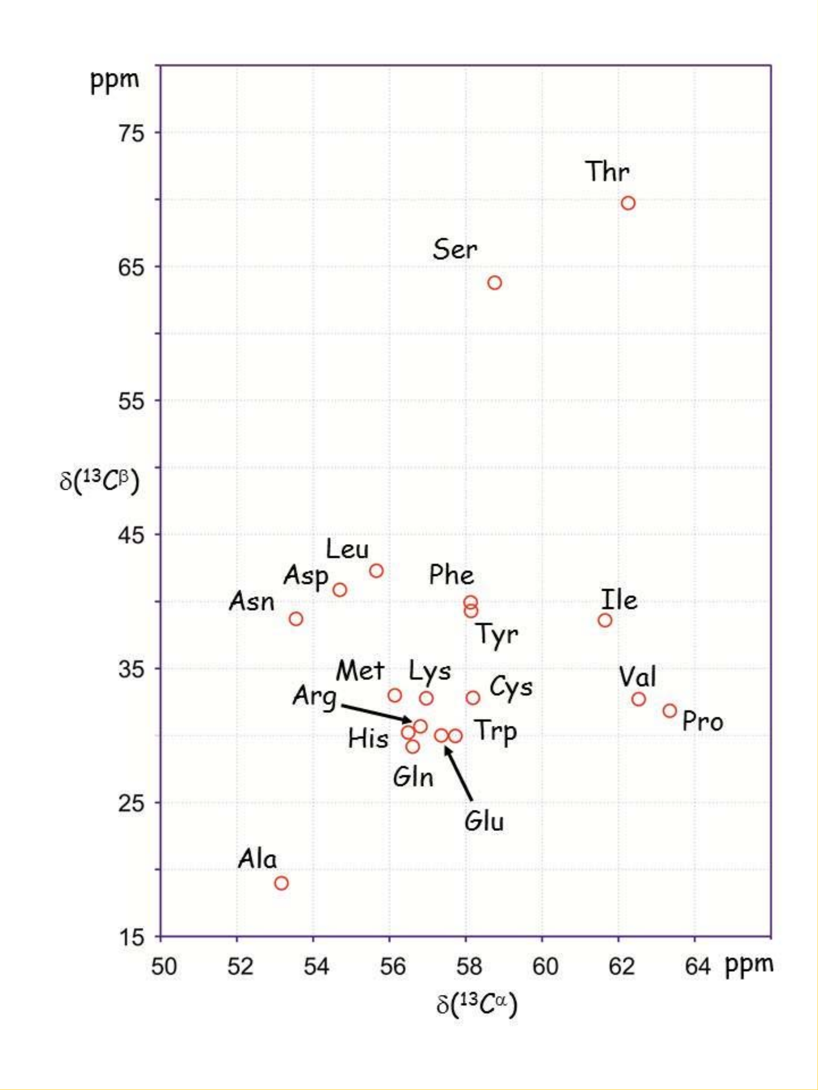


Sequence specific assignment of proteins

Sequentielle Zuordnung



Sequence specific assignment of proteins



Amino acid „typing“

Ala	53,170	18,990
Arg	56,810	30,680
Asn	53,550	38,710
Asp	54,700	40,880
Cys	58,190	32,810
Gln	56,610	29,180
Glu	57,360	30,000
Gly	45,370	
His	56,490	30,220
Ile	61,650	38,610
Leu	55,660	42,300
Lys	56,970	32,790
Met	56,140	33,000
Phe	58,130	39,950
Pro	63,350	31,850
Ser	58,760	63,800
Thr	62,260	69,730
Trp	57,730	29,970
Tyr	58,140	39,300
Val	62,530	32,720

That's it

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