Protein NMR spectroscopy

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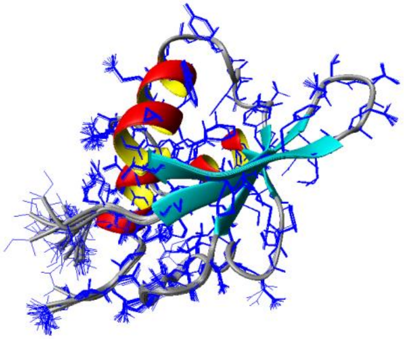
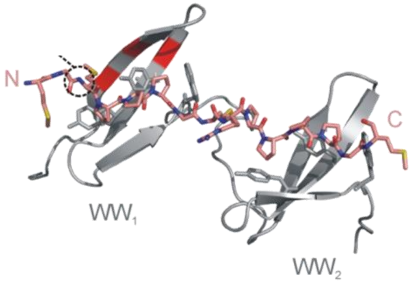
Applications of protein NMR

structural information



protein-ligand interactions

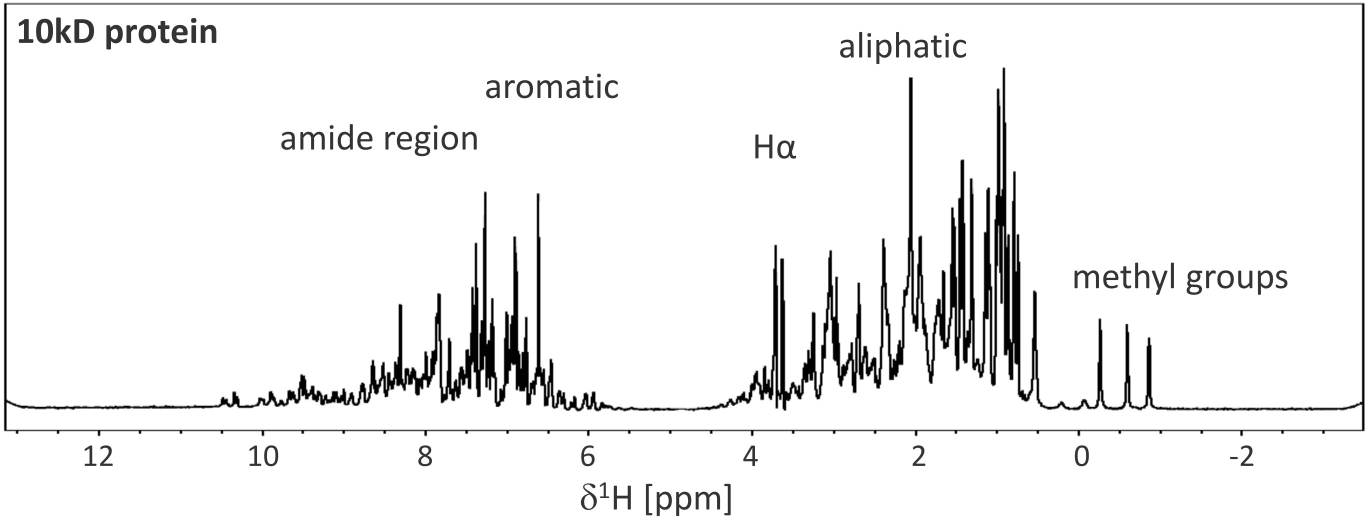
NMR of proteins in solution



dynamic information

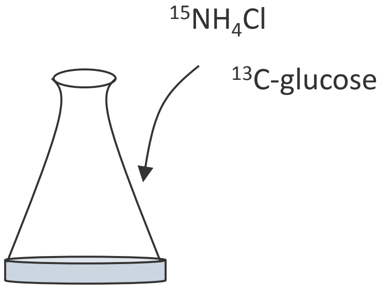
What is special about proteins?

- they are big – often too big for standard NMR approaches



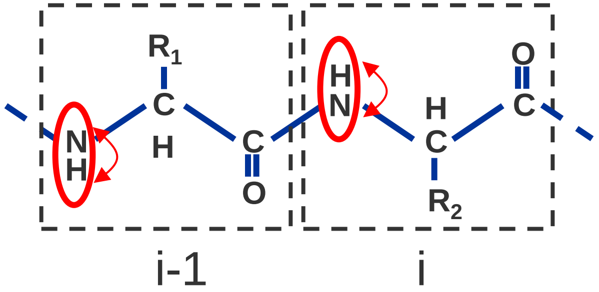
atoms in proteins	**<u>stable</u> Isotope composition (natural abundance)**
Hydrogen (H)	**^{1}H (99.9885%)** ^{2}H (0.0115%)
Carbon (C)	^{12}C (98.93%) **^{13}C (1.07%)**
Nitrogen (N)	^{14}N (99.632%) **^{15}N (0.368%)**

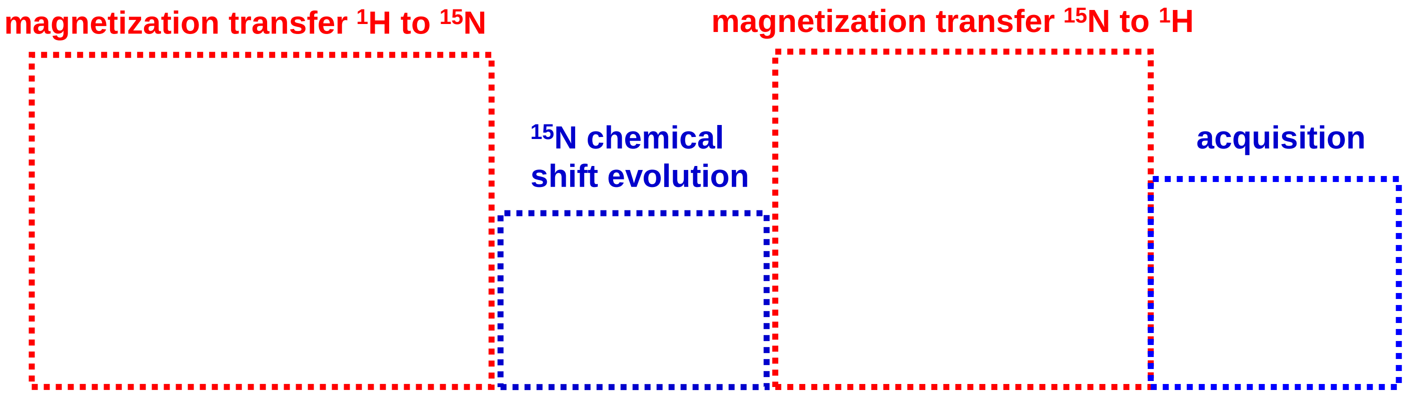
- problem: signal overlap
- ➢ overcome by isotope labeling of proteins expressed in bacteria in combination with more dimensional spectra (nobel prize for Kurt Wüthrich 2002)



^{1}H-^{15}N-HSQC spectrum – the “protein fingerprint”

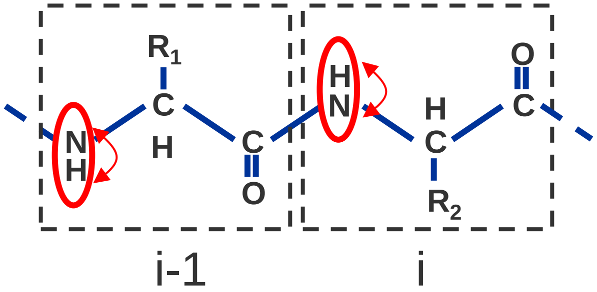
the HSQC (heteronuclear single quantum coherence) spectrum correlates covalently linked spins by magnetization transfer via J-coupling (J_{NH}=92Hz)



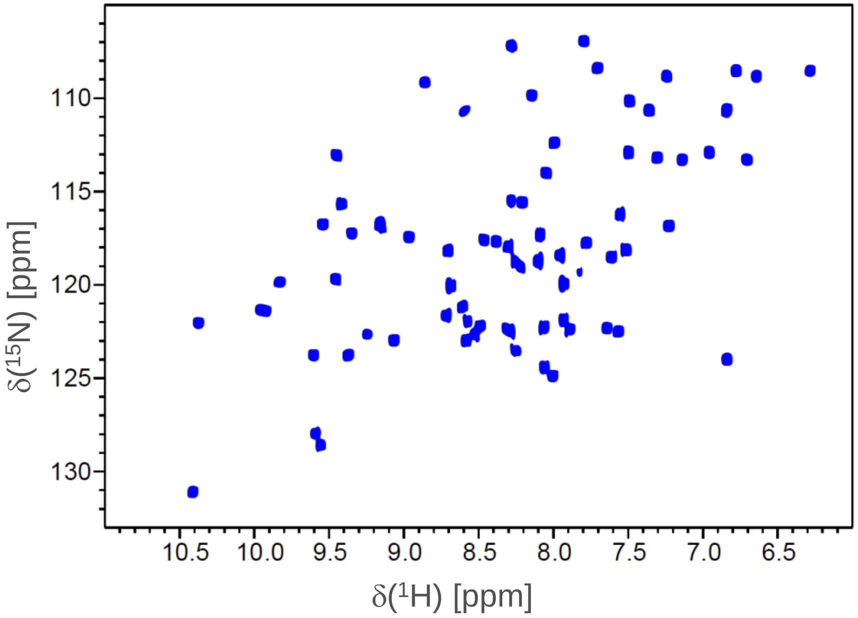


^{1}H-^{15}N-HSQC spectrum – the “protein fingerprint”

- less peaks / better resolution
- one peak per amide-group
- ➢ roughly one peak per residue (fingerprint!!)



^{1}H-^{15}N-HSQC of a 10kD protein



- ➢ peak position (chemical shift) contains structural information
- ➢ peak line-width delivers information on protein dynamics

which peak belongs to which residue?
assignment needed!

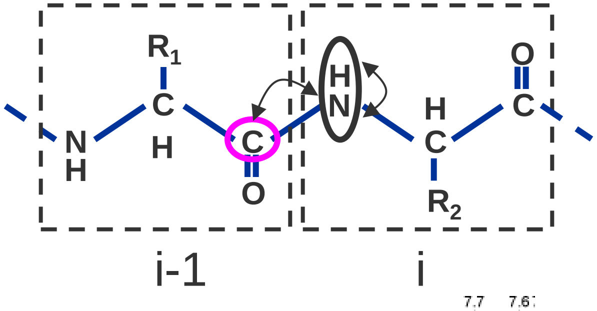
HNCO / HN(CA)CO - sequential assignment

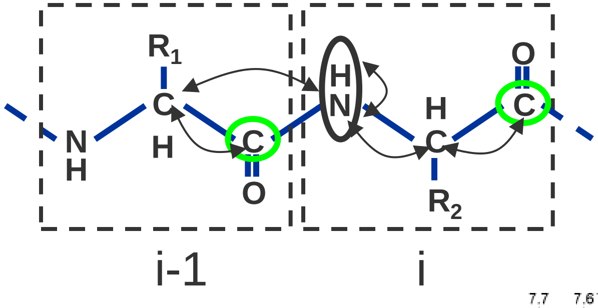
HNCO

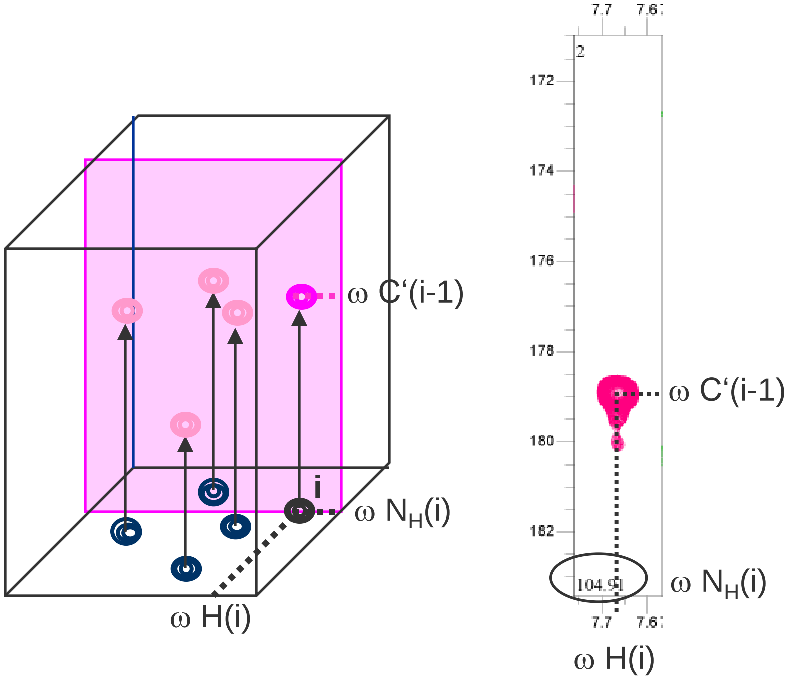
signal for C‘ of NH(i-1)

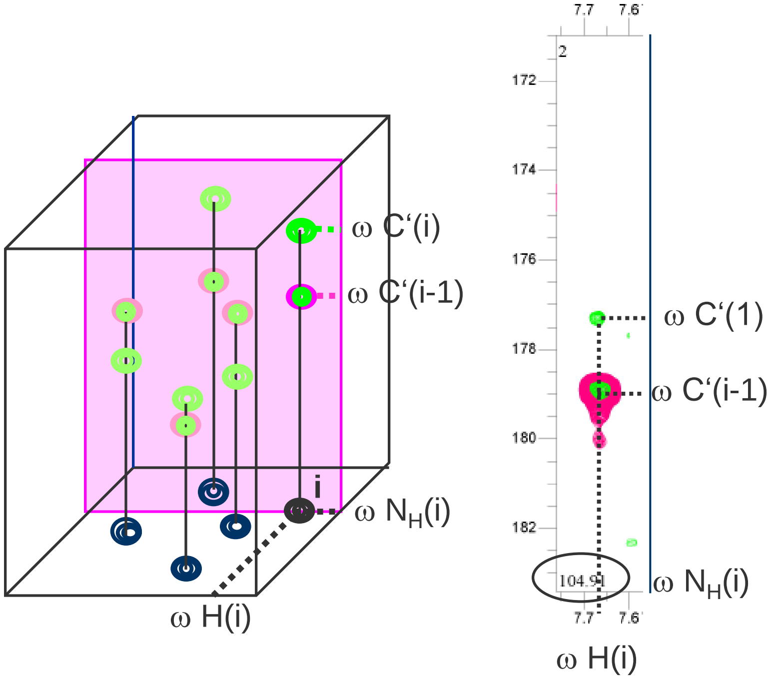
HN(CA)CO

signal for C‘ of NH(i) and NH(i-1)



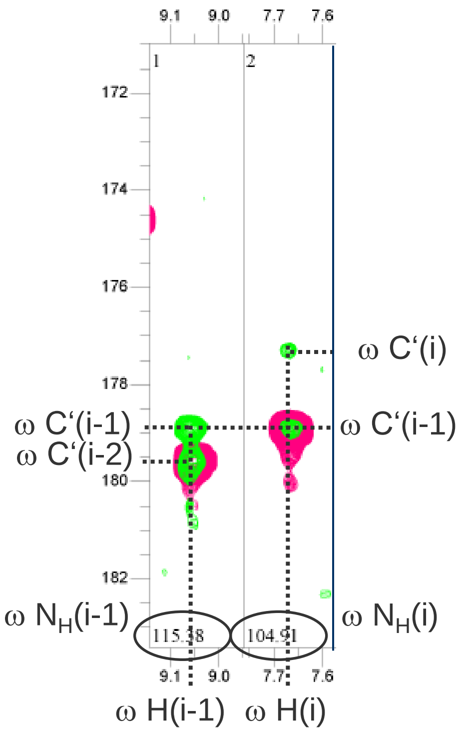






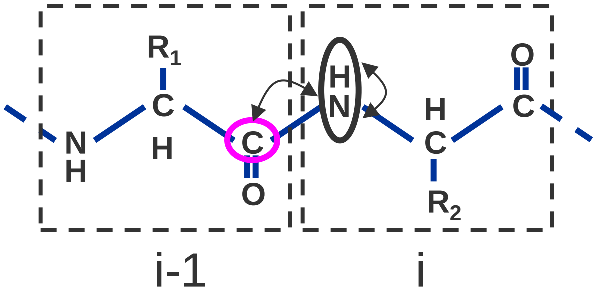
HNCO / HN(CA)CO sequential assignment

HNCO / **HN(CA)CO**



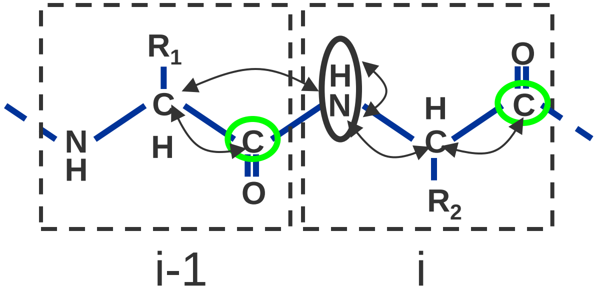
HNCO

signal for C‘ of NH(i-1)



HN(CA)CO

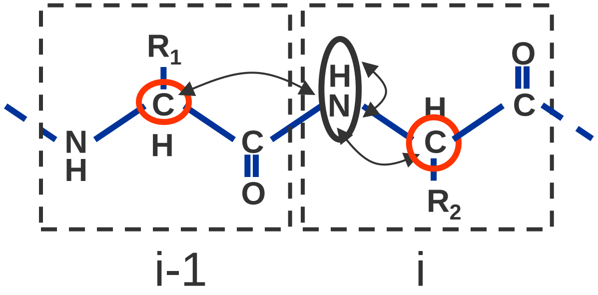
signal for C‘ of NH(i) and NH(i-1)



HNCA / HN(CO)CA sequential assignment

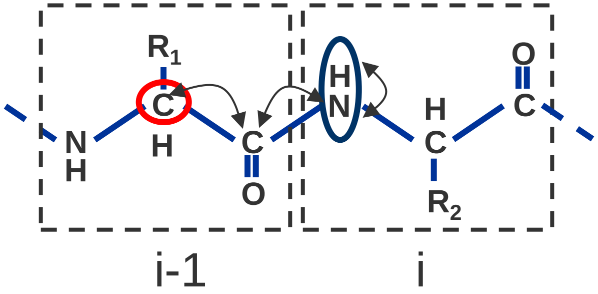
HNCA

signal for Cα of NH(i) and NH(i-1)

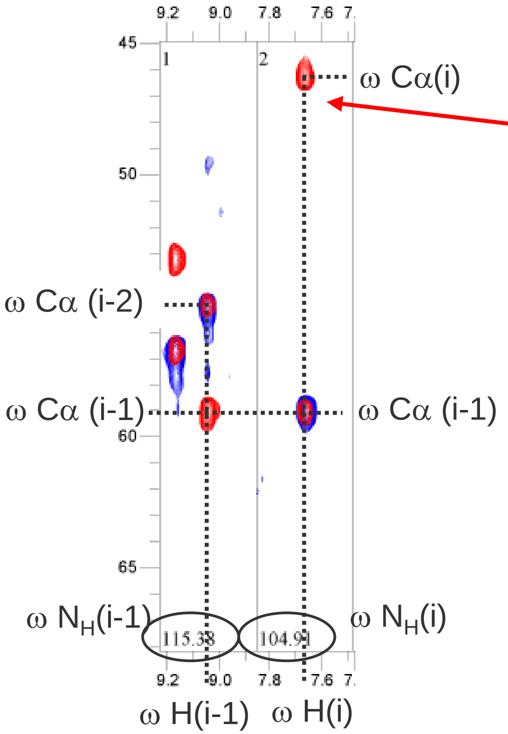


HN(CO)CA

signal for Cα of NH(i-1)



HNCA / **HN(CO)CA**



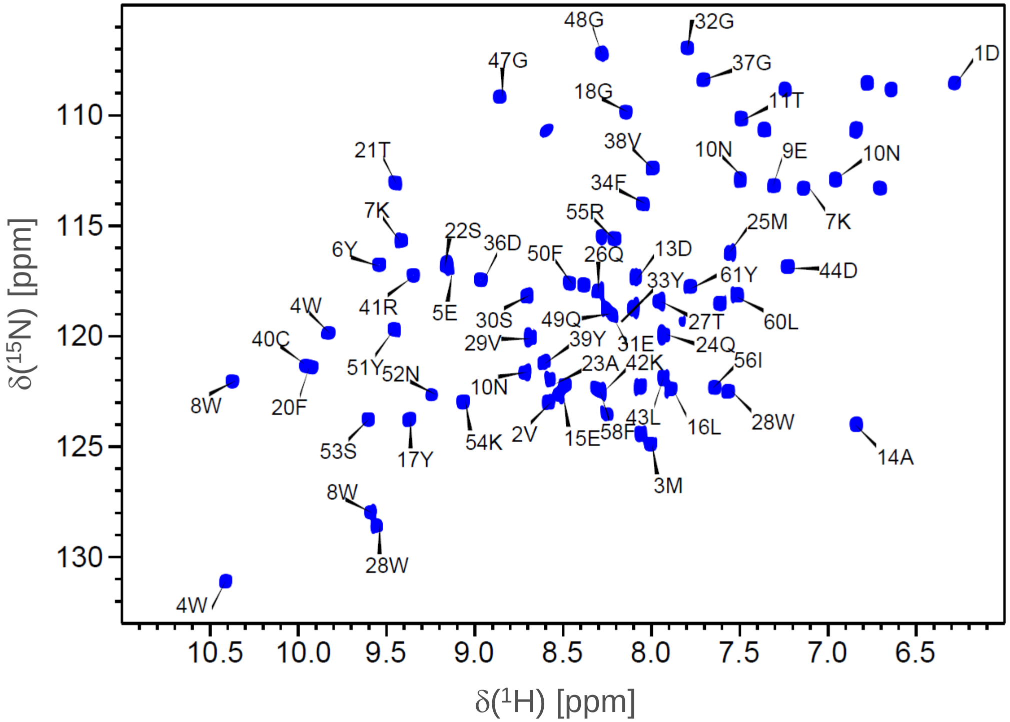
very likely a glycine!

-x-**Gly**-

-x-Val/Ile/Thr-x-x-**Gly**-

-14Leu-15Thr-16Ala-17Ser-**18Gly**-

assigned HSQC spectrum

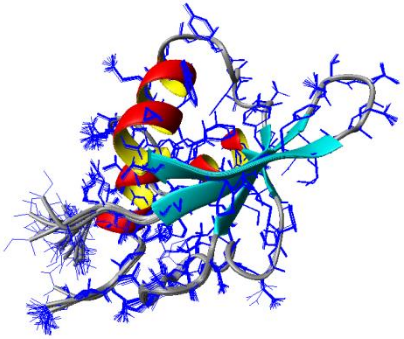


available now:

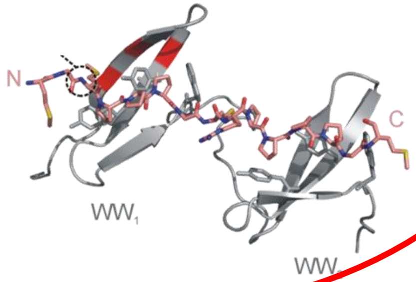
- assignment of NH signals in HSQC
- backbone Cα and CO assignments

how to access this information

structural information



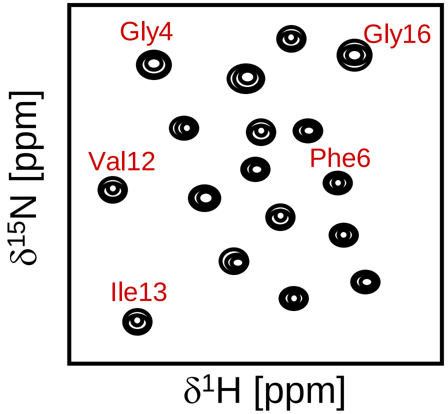
protein-ligand interactions

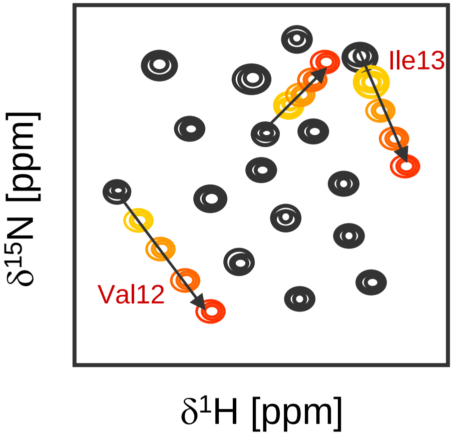


dynamic information

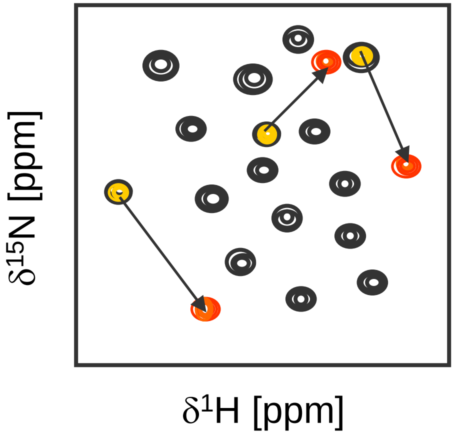
Mapping protein-ligand interactions

^{1}H-^{15}N-HSQC spectra:
one signal per NH-group



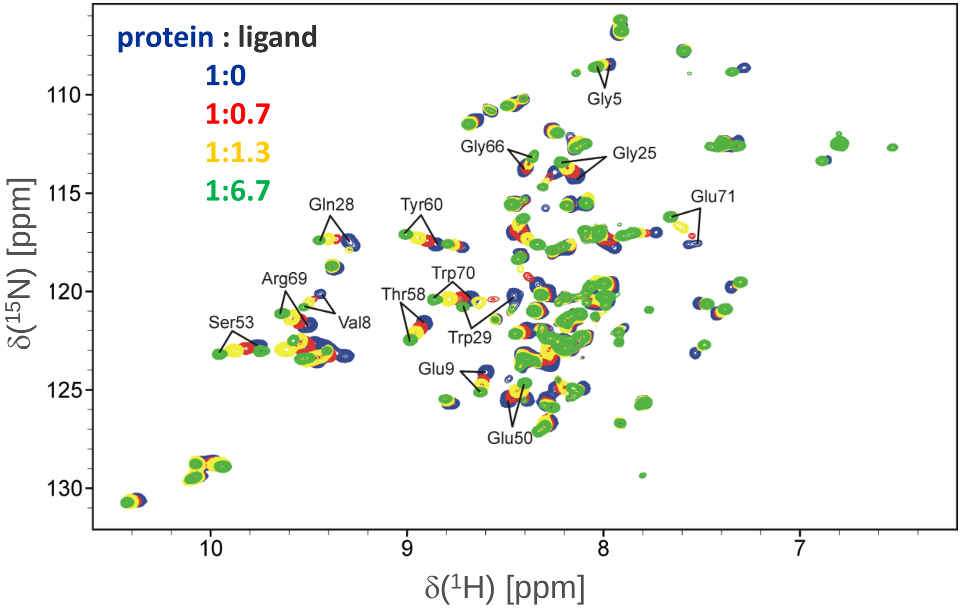


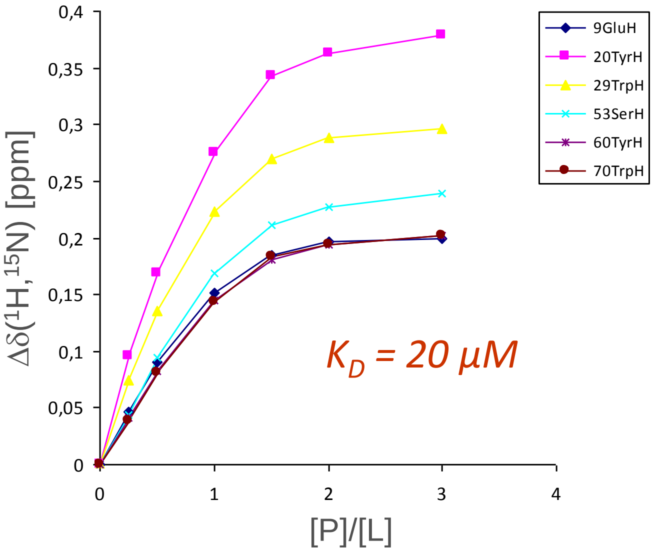
“fast exchange”
one sharp average resonance



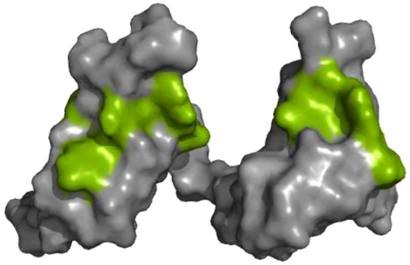
“slow exchange”
two distinct resonances

protein-ligand interactions



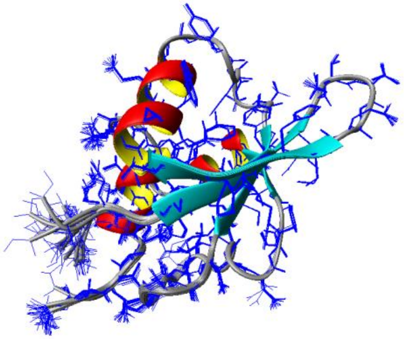


HSQC titrations allow to define binding sites and K_D values of protein-ligand interactions

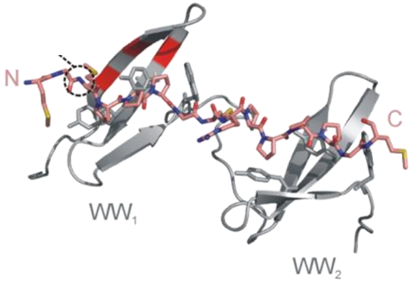


how to access this information

structural information



protein-ligand interactions

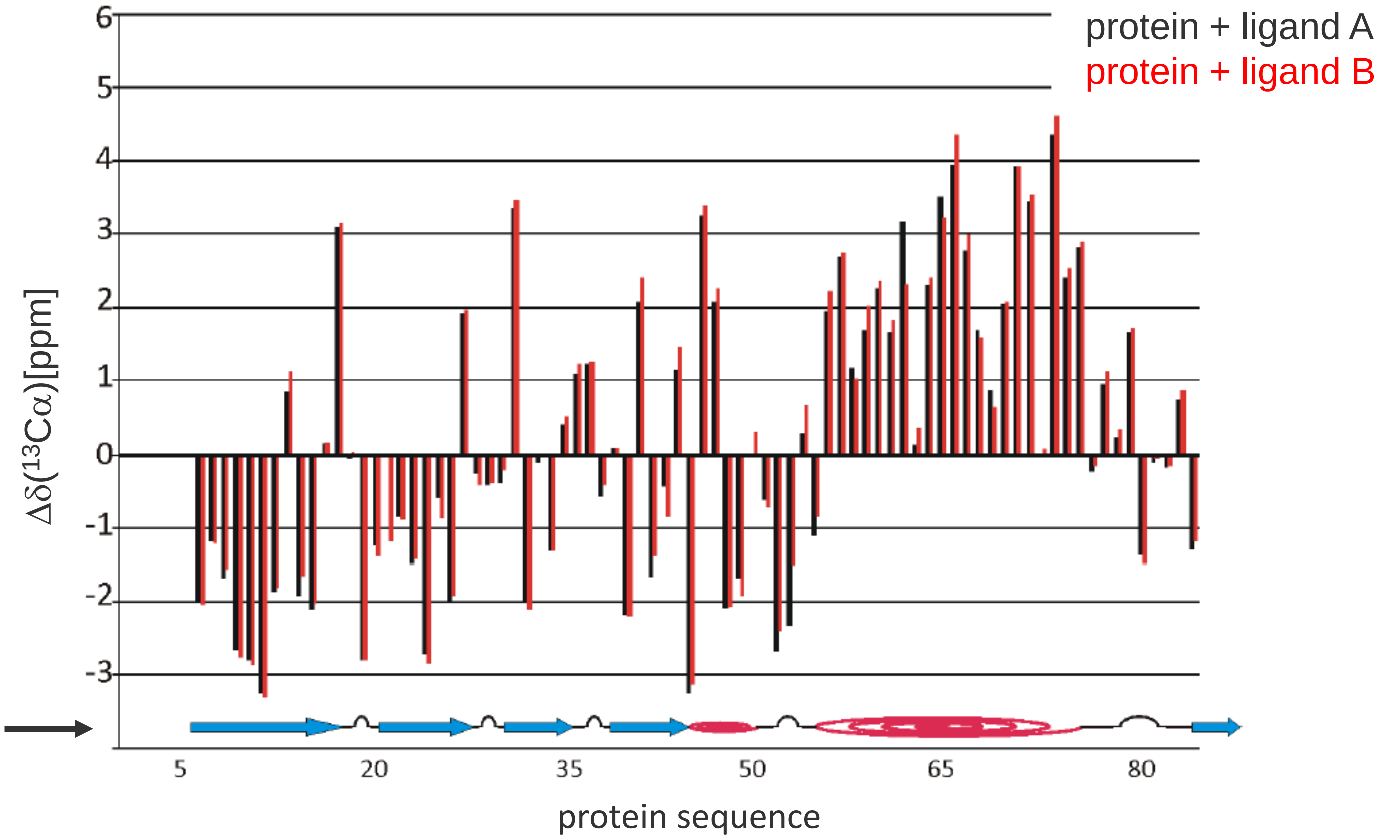


dynamic information

collect structural information: secondary structure from backbone shifts

- $C\alpha$, $C\beta$, CO assignments already contain secondary structure information
- $C\alpha$ chemical shifts of α-helical or β-sheet regions differ from random coil values

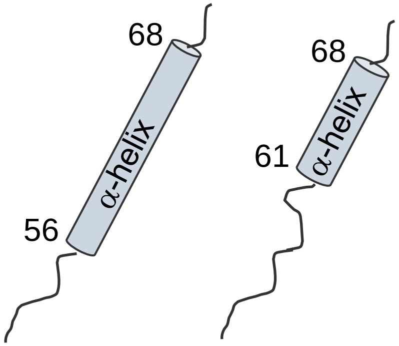
$$\Delta\delta C_{\alpha} = \delta C_{\alpha}(measured) - \delta C_{\alpha}(random\ coil)$$

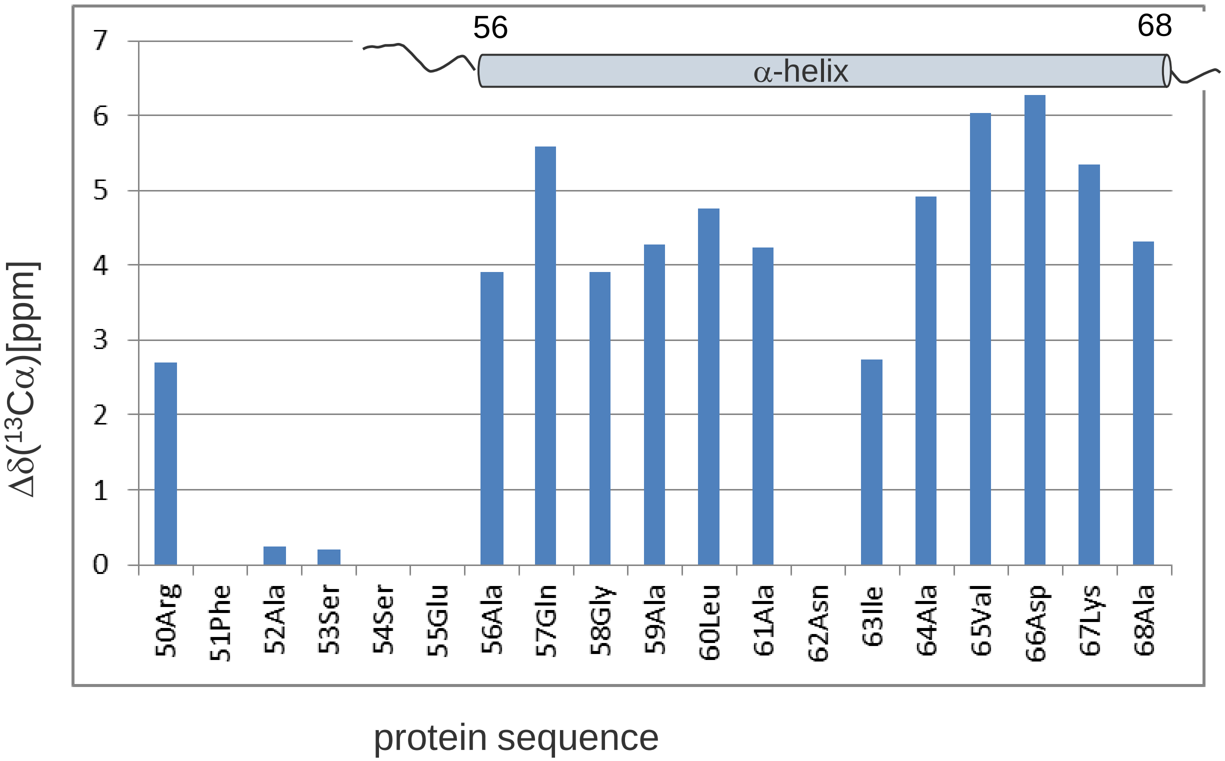


secondary structure derived from crystal structure

collect structural information: secondary structure from backbone shifts

two published crystal structures – which is present in solution?





collect structural restraints: torsion angle

Karplus correlation: $^{3}J\,(\varphi) = A\;cos^{2}(\varphi - 60) - B\cos(\varphi - 60) + C$

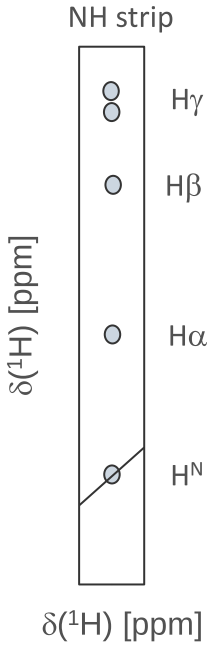
- quantitative J-correlation methods allow to determine $^{3}J(H^{N}\text{-}H^{\alpha})$ coupling constants
- cross-peak intensity ratio is correlated with coupling constant

collect structural information: side-chain assignments

for bigger proteins

2D TOCSY
(total correlation spectroscopy)
correlation of entire spin system

3D TOCSY-HSQC



collect structural restraints:
NOE secondary structure and distance information

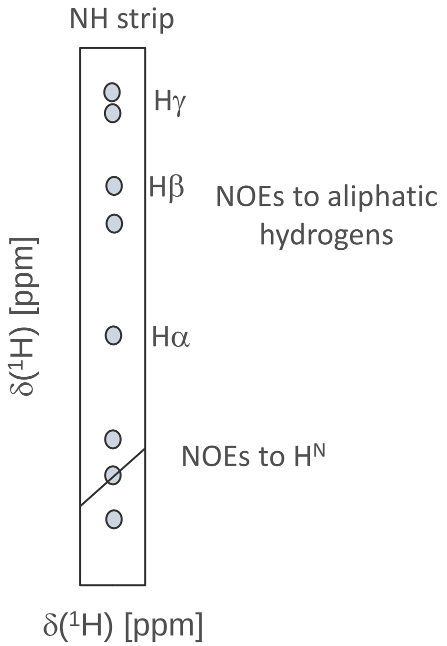
NOE (nuclear Overhauser effect) is due to through-space dipolar interaction between spins in spatial vicinity (<5Å; NOE intensity $\propto \frac{1}{r^6}$)

2D NOESY

for bigger proteins

3D ^{1}H-^{15}N-NOESY-HSQC

intra- or intermolecular cross-peaks

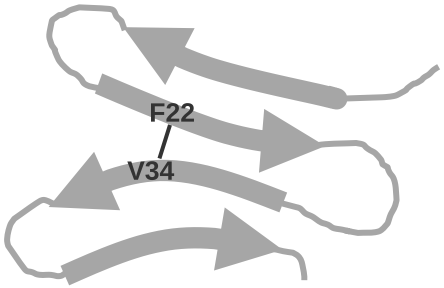


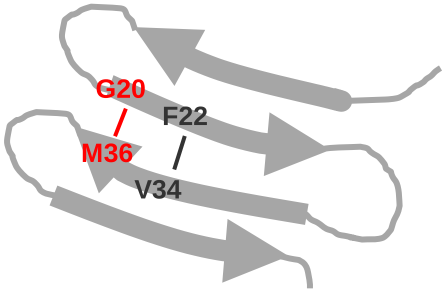
collect structural restraints:
NOE secondary structure and distance information

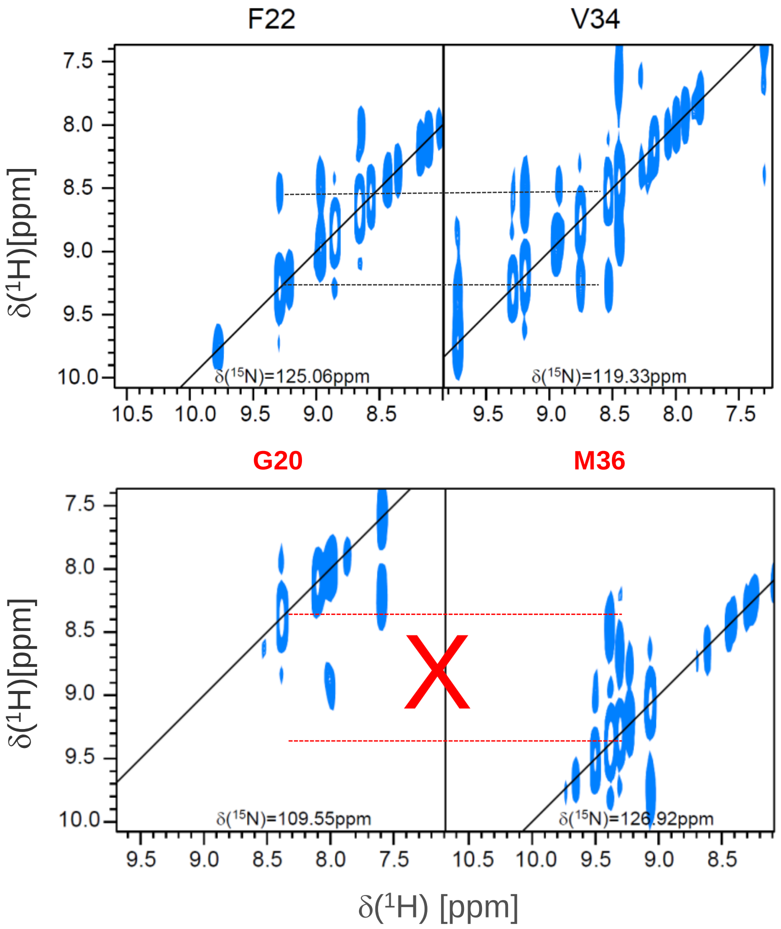
NOE cross-peak intensity correlates with distance between atoms

strong	1.8-2.7 Å
medium	1.8-3.3 Å
weak	1.8-5.0 Å

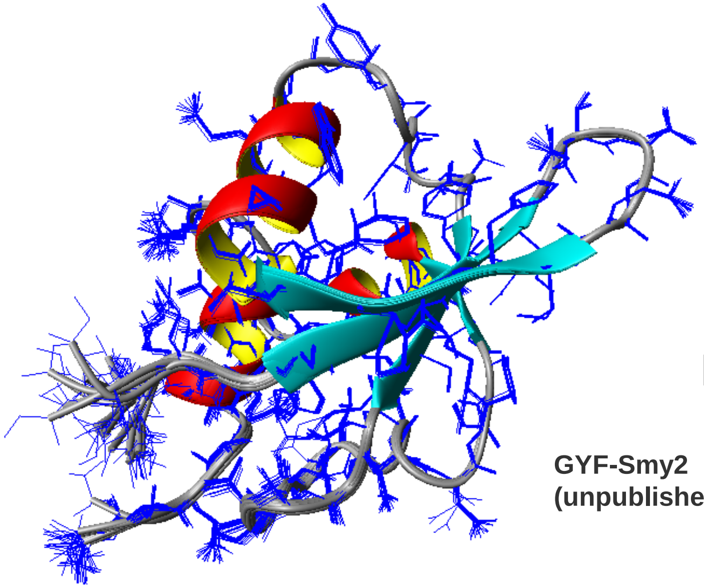
collect structural restraints:
NOE secondary structure and distance information



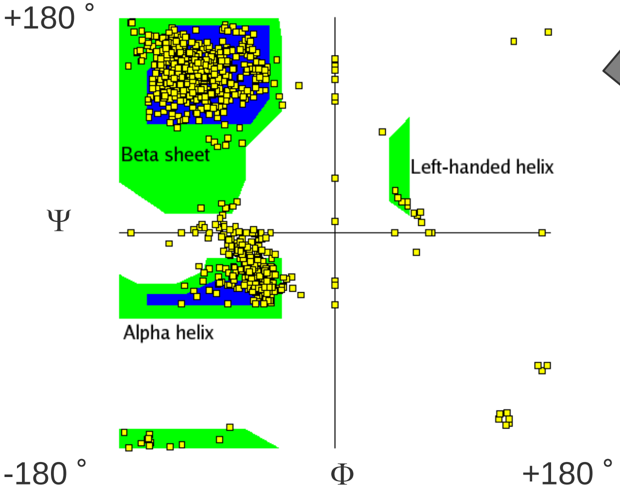




Structure determination



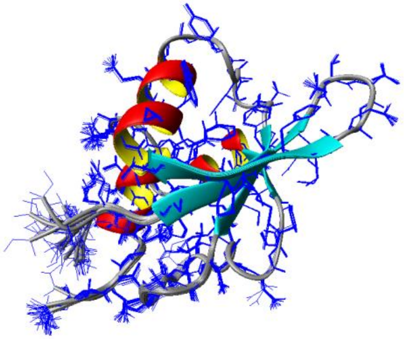
GYF-Smy2
(unpublished)



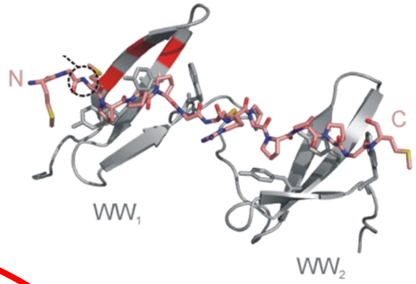
Ramachandran Plot

how to access this information

structural information



protein-ligand interactions

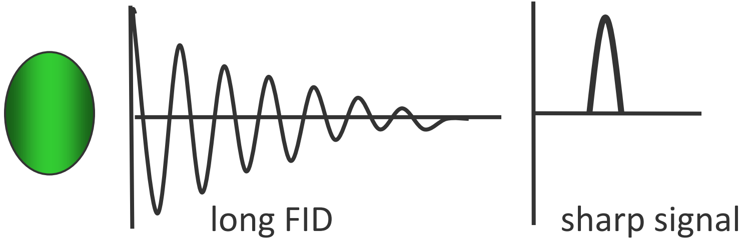


dynamic information

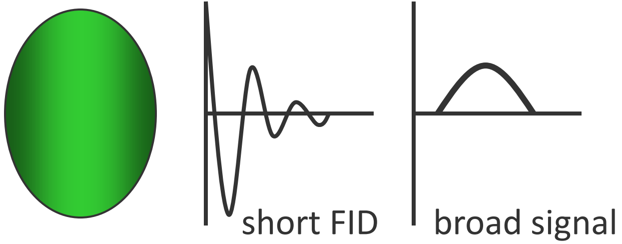
protein dynamics:
peak line-width and relaxation

relaxation is dominated by rotational motion of the molecule:

small protein:



big protein:



$$I(t) = I_0 e^{i(\omega t)} e^{-Rt}$$

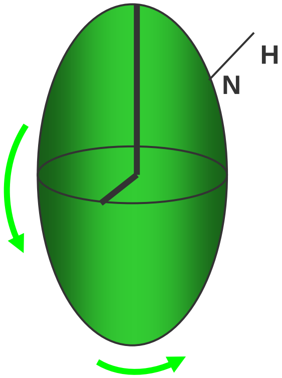
signal intensity (I) oscillating
with Larmor frequency

exponential decay
of the signal dominated
by relaxation rate R_2

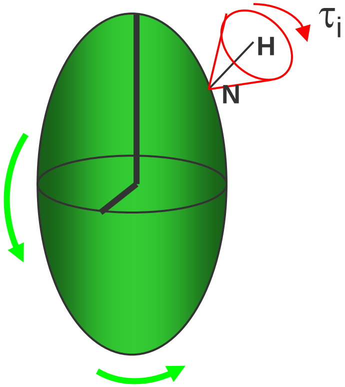
➔ the peak line-width carries information on rotational motion of the molecule and internal dynamics

dynamics: quantitative information

➔ qualitative information:
broad signals indicate oligomerization,
protein-protein interaction, conformational exchange

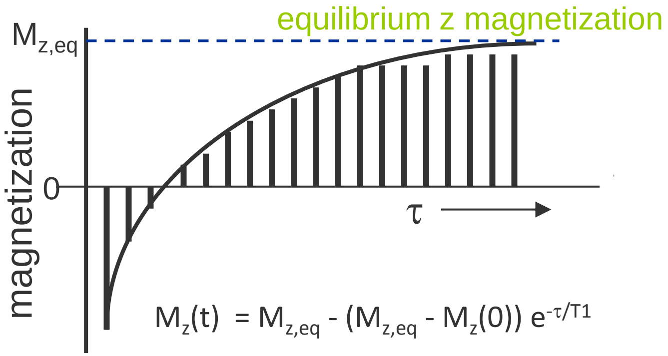


➔ quantitative information:
regions of the protein displaying additional fast internal motion (τ_i, ps-ns) or conformational exchange (R_{ex}, µs-ms) can be identified from relaxation rate measurements

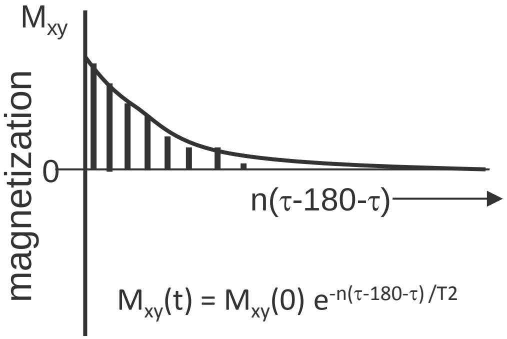


relaxation time measurements

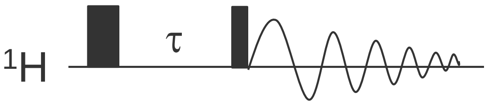
longitudinal (T_1) relaxation time



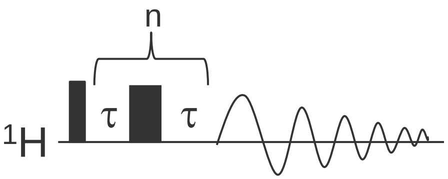
transverse (T_2) relaxation time



Inversion recovery to measure T_1

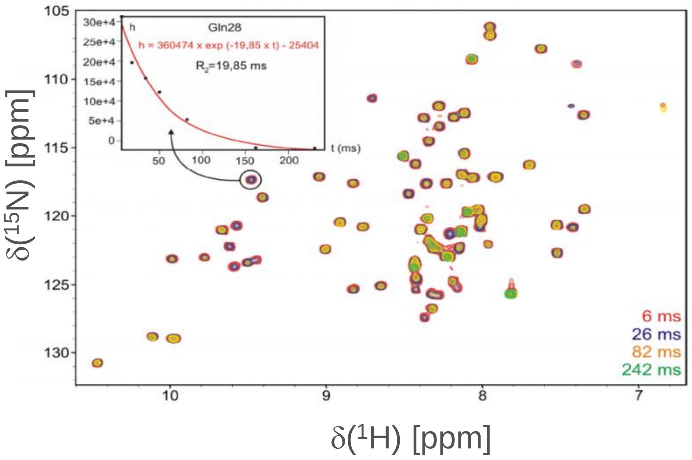


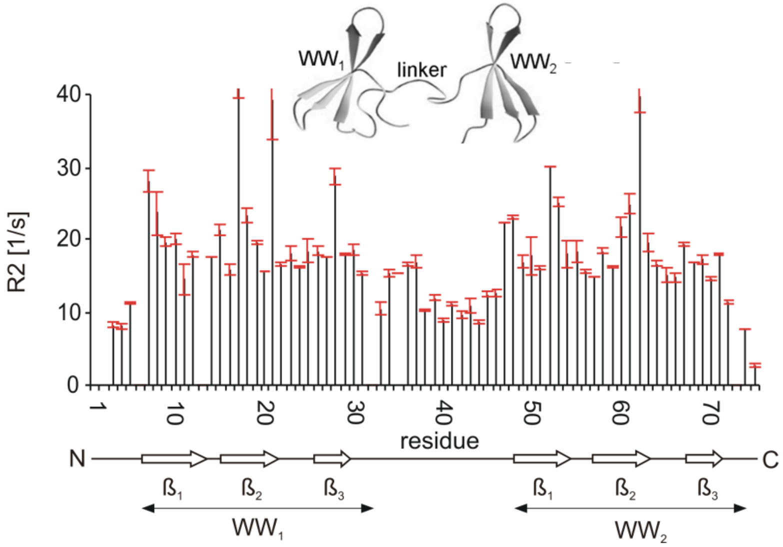
CPMG spin-echo to measure T_2



dynamics: quantitative information

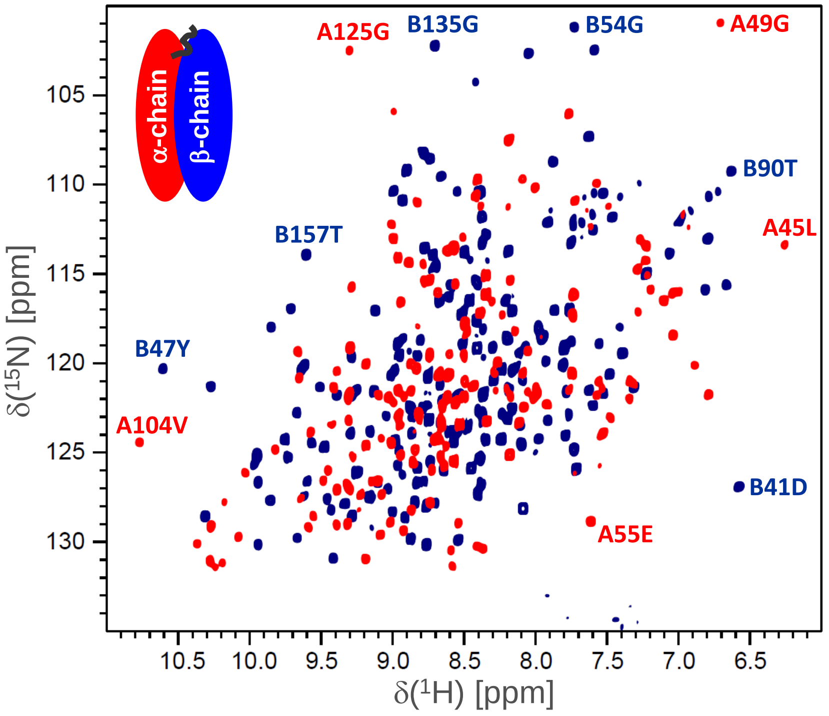
^{1}H-^{15}N T_1, T_2, and hetNOE measurements allow to deduce protein backbone flexibility

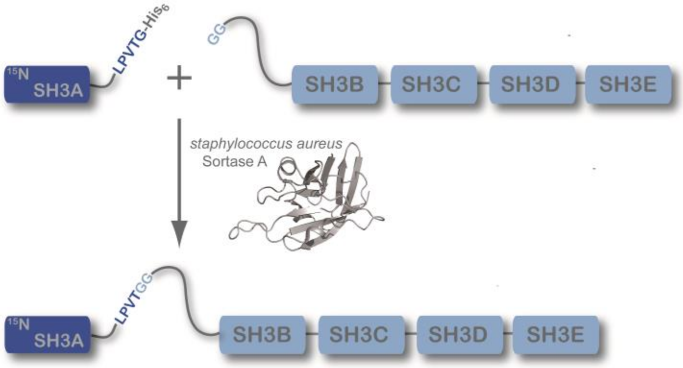


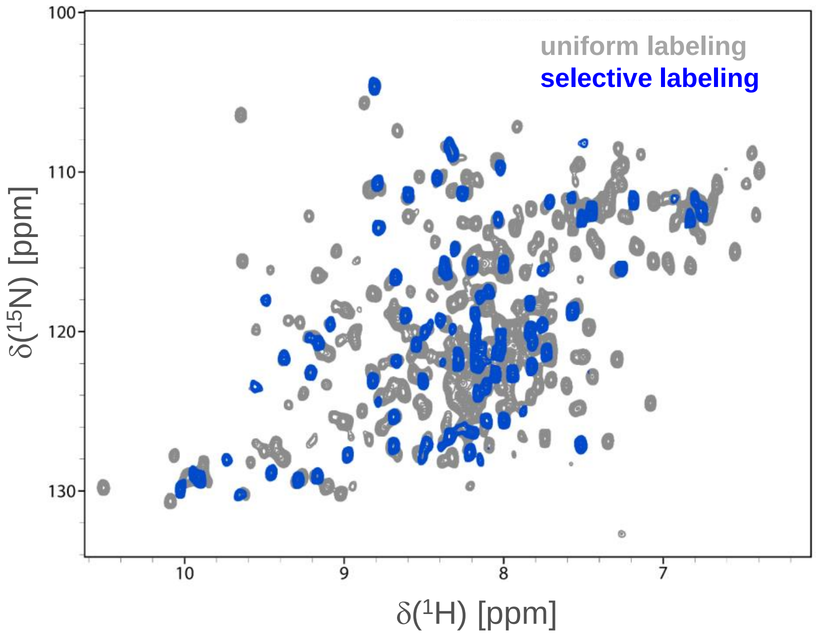


labeling strategies in big proteins

$^1H^{15}N$-TROSY-HSQC of deuterated 46kD protein

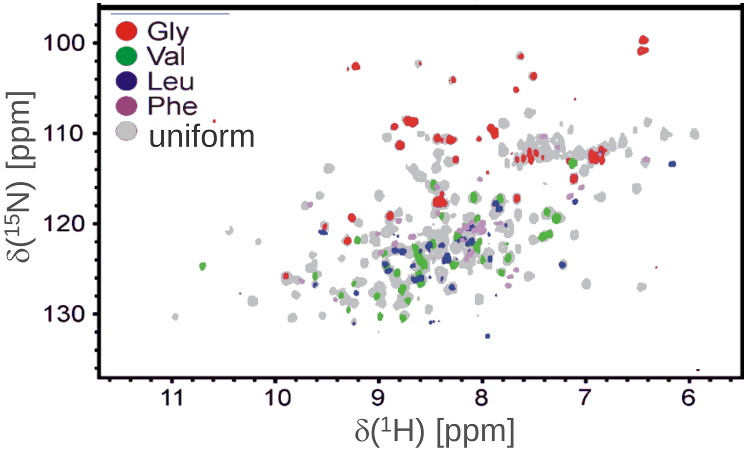




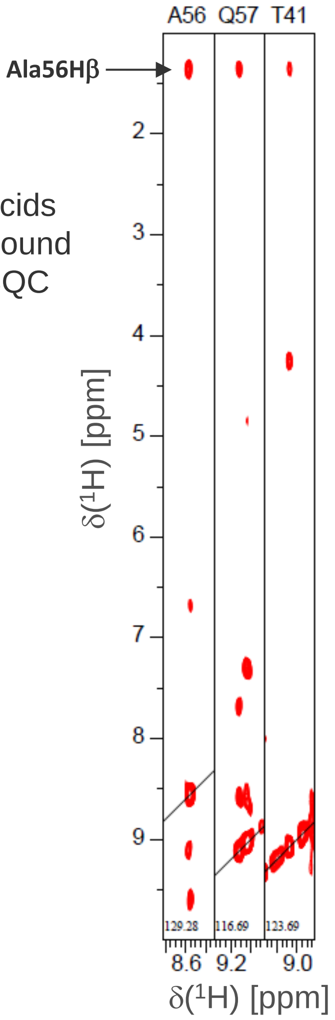


labeling strategies in big proteins

amino-acid type specific ^{15}N labeling
^{1}H-^{15}N-HSQC

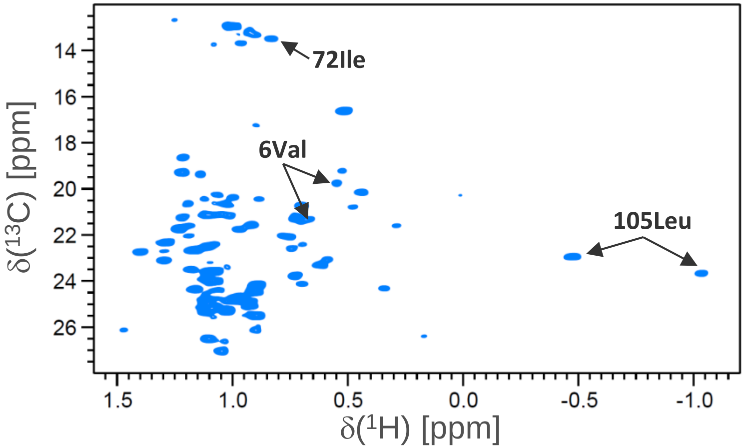


incorporation of
protonated amino acids
in deuterated background
^{1}H-^{15}N-NOESY-HSQC



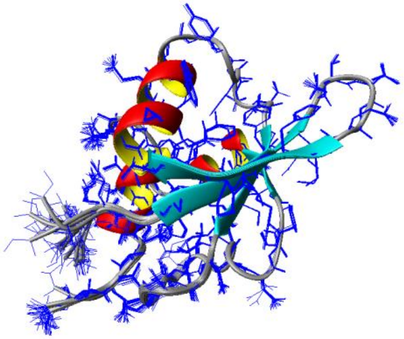
labeling strategies in big proteins

I,L,V-methyl group specific ^{1}H-^{13}C labeling
^{1}H-^{13}C-HSQC

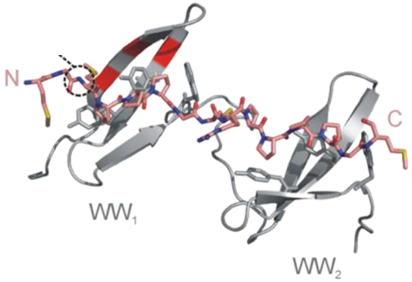


how to access this information

structural information



protein-ligand interactions



dynamic information

Acknowledgements

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NMR facility, FU Berlin

Dr. Peter Schmieder
Monika Beerbaum
NMR facility, FMP Berlin