NMR BASED STRUCTURAL BIOLOGY

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Practical information

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The Toolbox

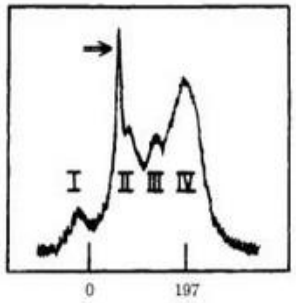
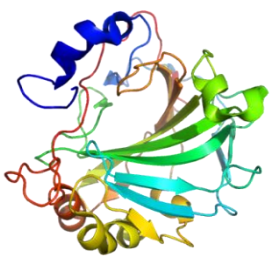
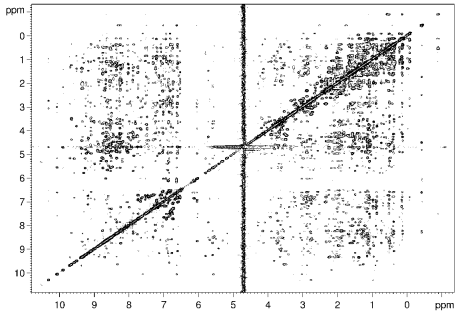


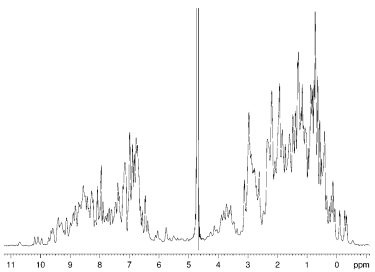
Fig. 1.—Nuclear magnetic resonance spectrum of ribonuclease.

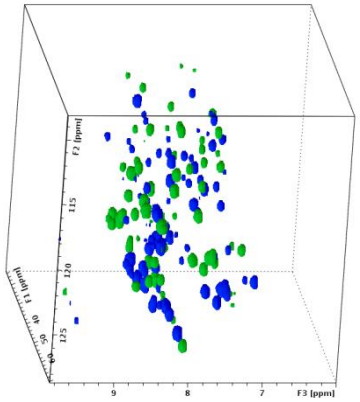
The scale shown was established using the aromatic peak of toluene in an introduced capillary as zero. Since the spectrum range is the range of usual proton chemical shifts and since it does not change noticeably with concentration, it seems that we have an essentially unbroadened spectrum of the non-exchangeable protons of ribonuclease.

Because of their location, peaks I and IV can be assigned tentatively to aromatic hydrogens and to hydrogens on aliphatic carbon atoms attached only to other aliphatic carbons. The relative areas of each peak, after subtracting the estimated contribution of water protons, obtained from several spectra of two independently prepared samples were: I, 9 ● 1%, II, 26 ± 2%, III, 18 ± 3%, IV, 47 ± 3%. From the known composition







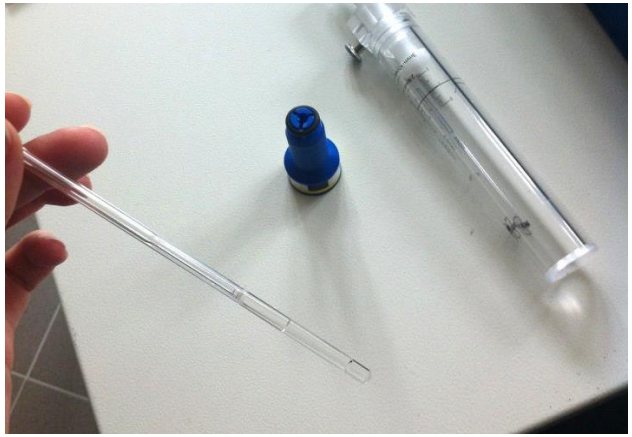


Essential equipment

protein sample concentration ~ 20uM – 200uM – 4mM…

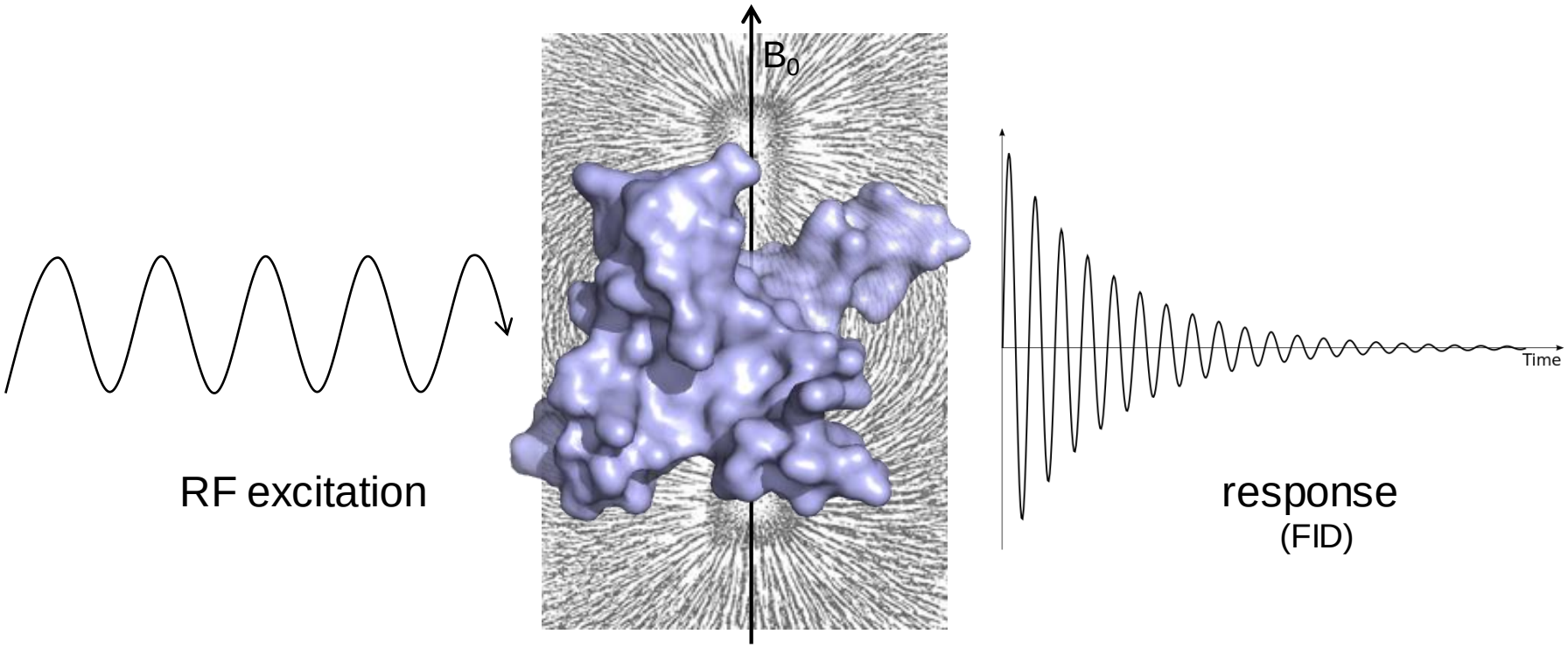
	5mm	3mm
tube:		
sample volume:	350ul	160ul

NMR instrument:



NMR expert:

Principle



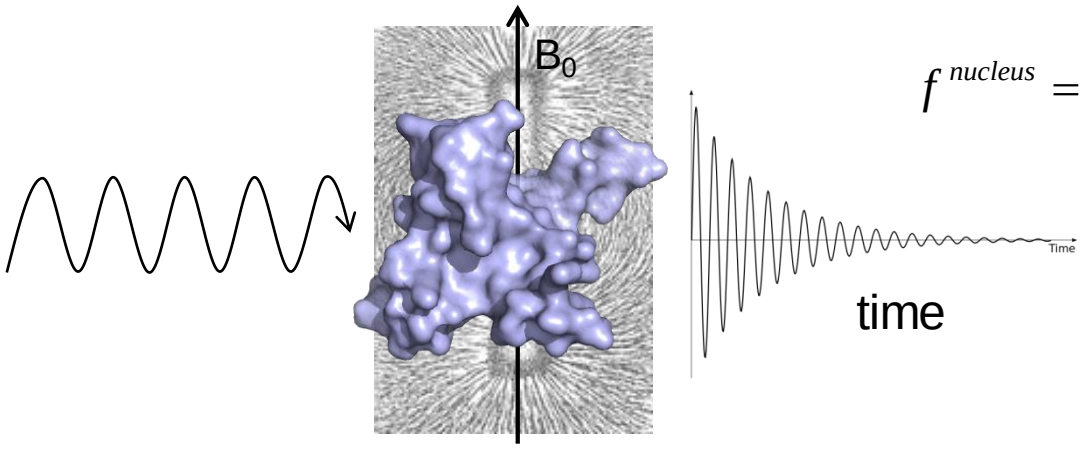
RF excitation

response
(FID)

nuclear **m**agnetic **r**esonance

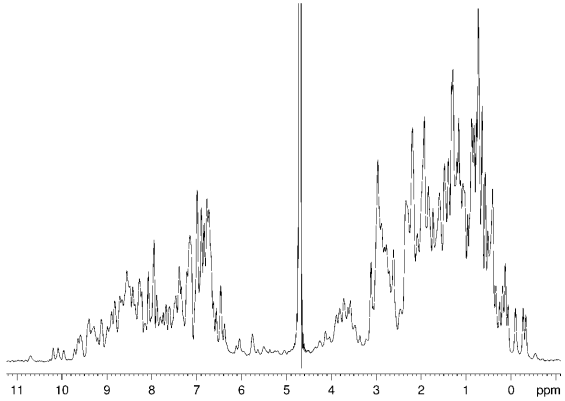
resonance frequency of nuclei in magnetic field

Principle

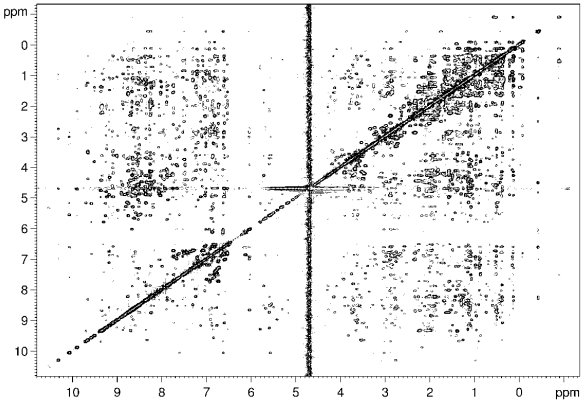


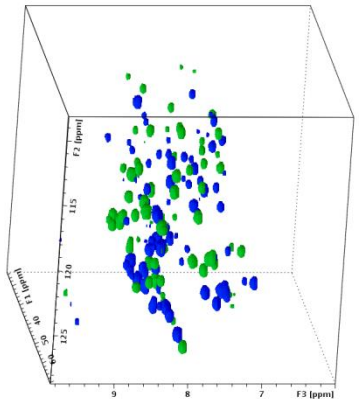
$$f^{\,nucleus} = \frac{\gamma}{2\pi}(1-\sigma)B_0$$

Fourier transformation



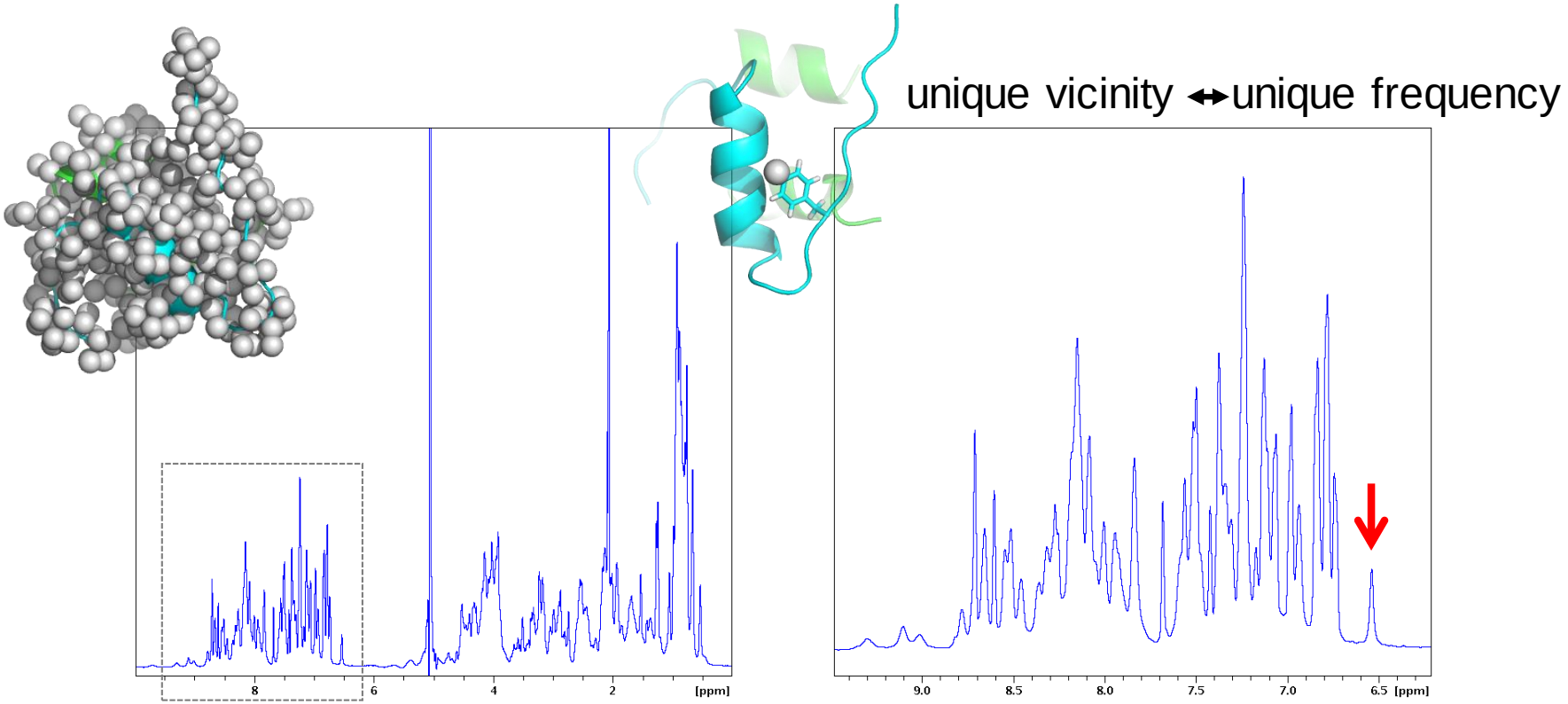
frequency





Protein NMR

- What do we observe?
- NMR signal - the very direct observation of a particular atom/group in the molecule, unlike reflections in the crystal lattice



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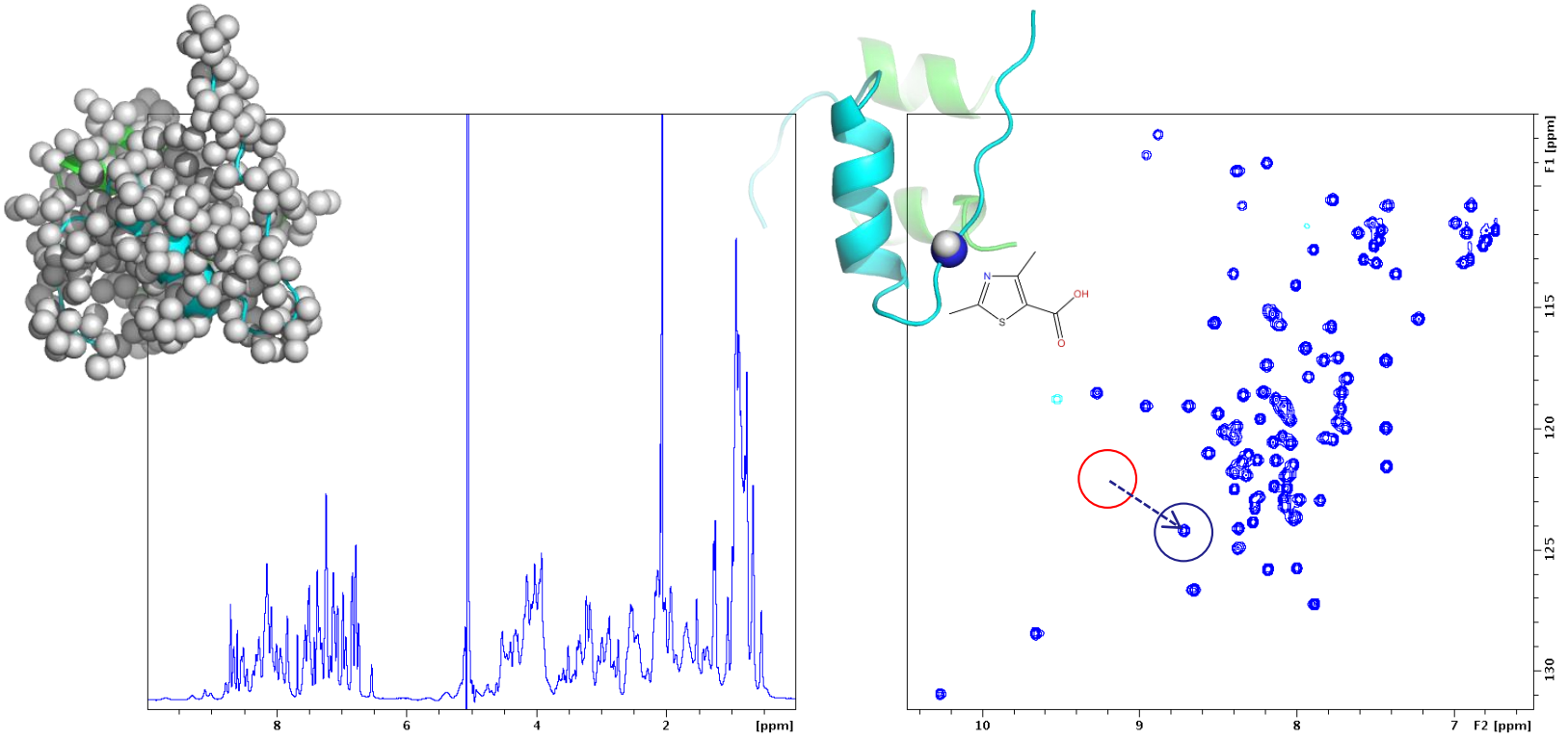
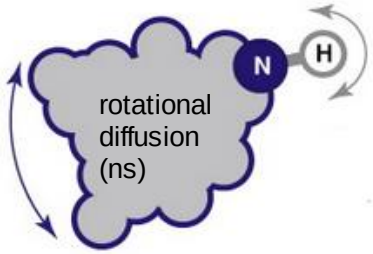
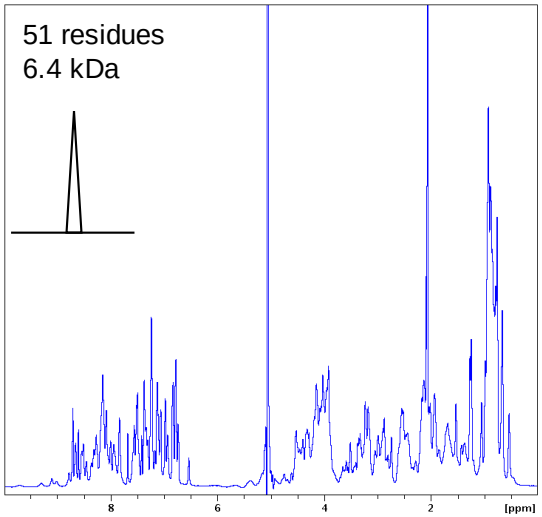


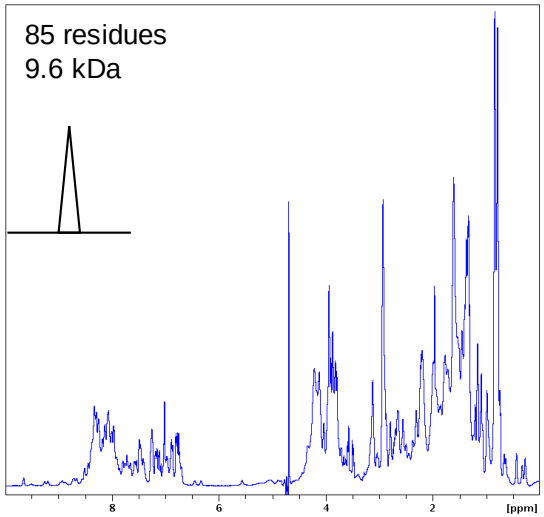
Isotope labelling (^{15}N)

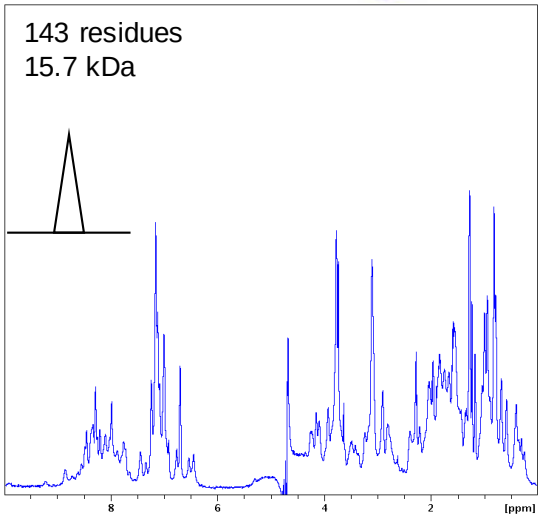
Protein NMR

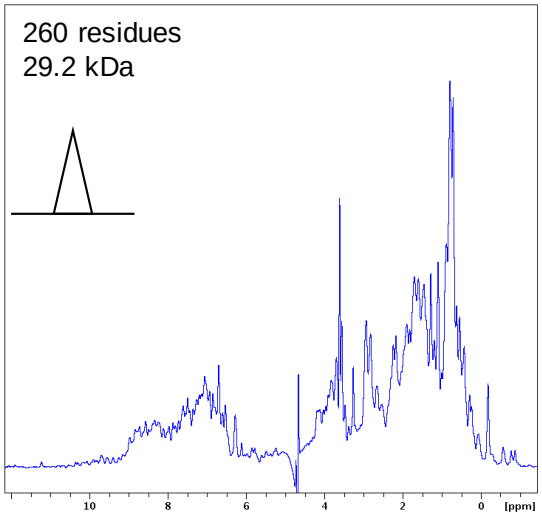


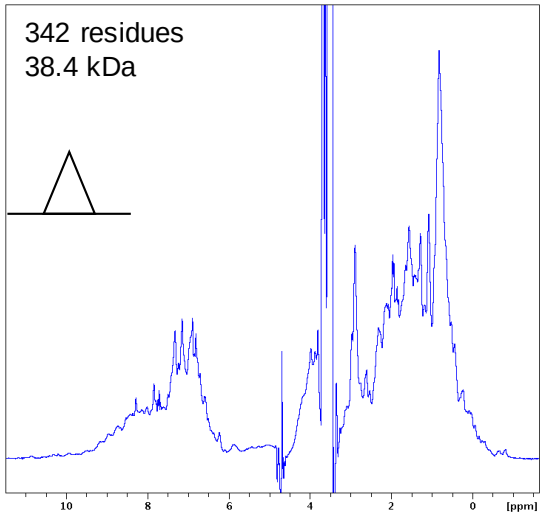
- size of the studied molecules (slower tumbling → line-broadening)

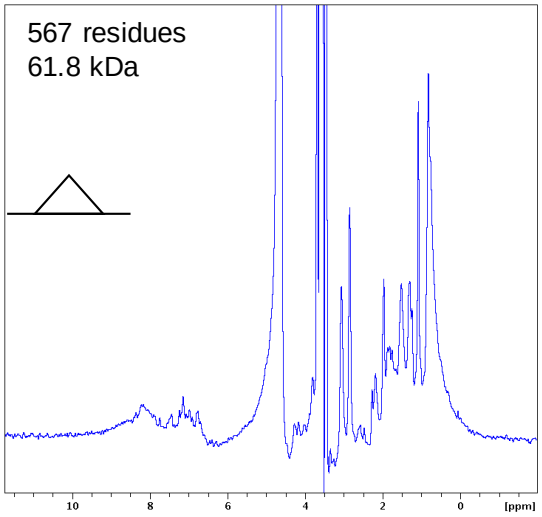




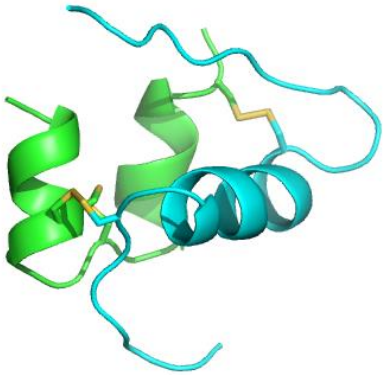




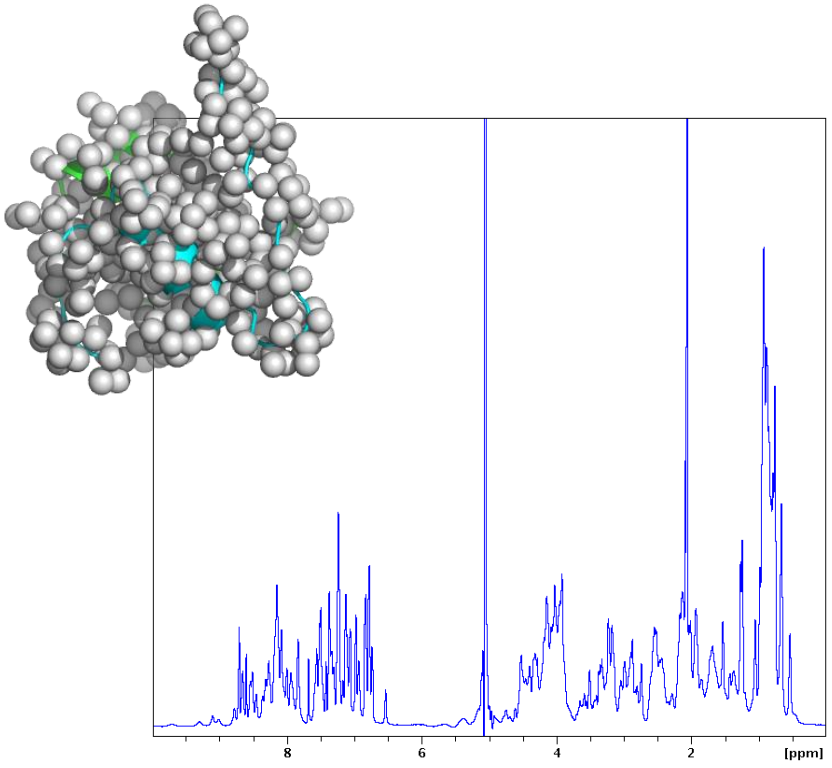


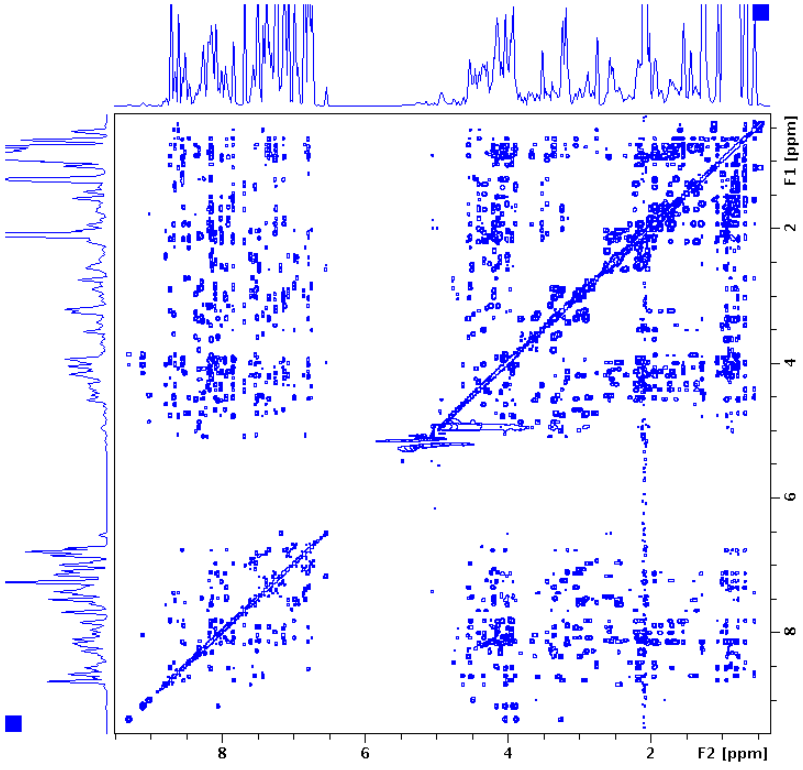


Protein NMR



- size of the studied molecules (slower tumbling → line-broadening)
- number of atoms (complexity + repetitive structures → overlaps)
- sensitivity issues





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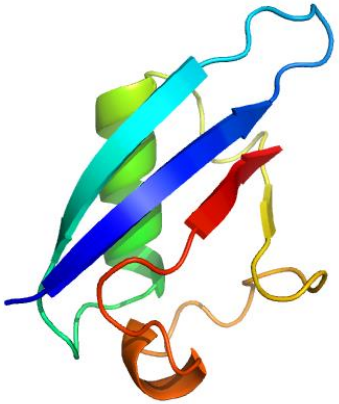
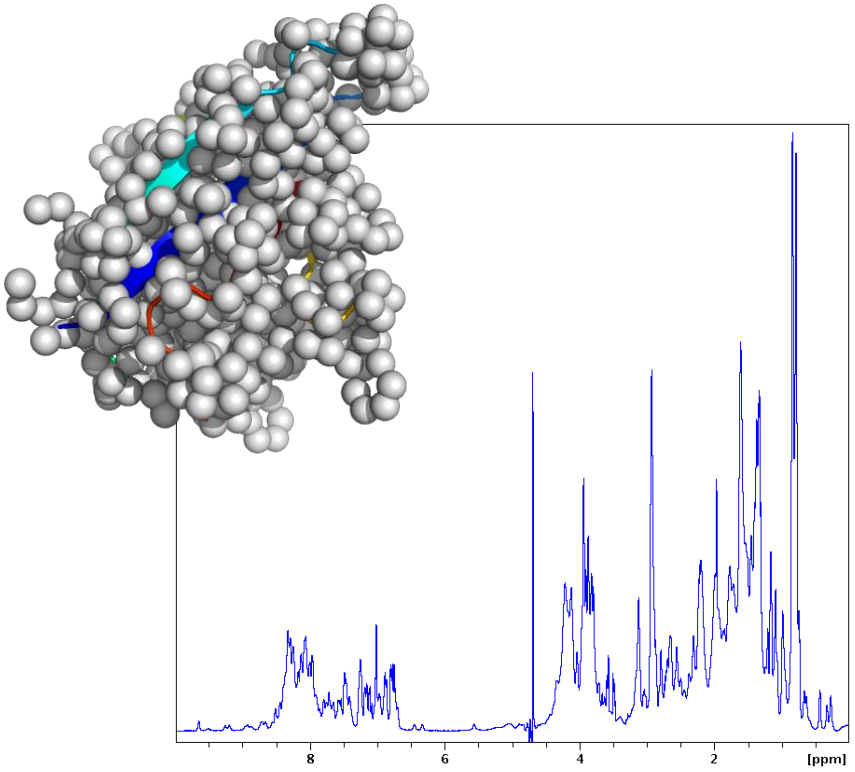
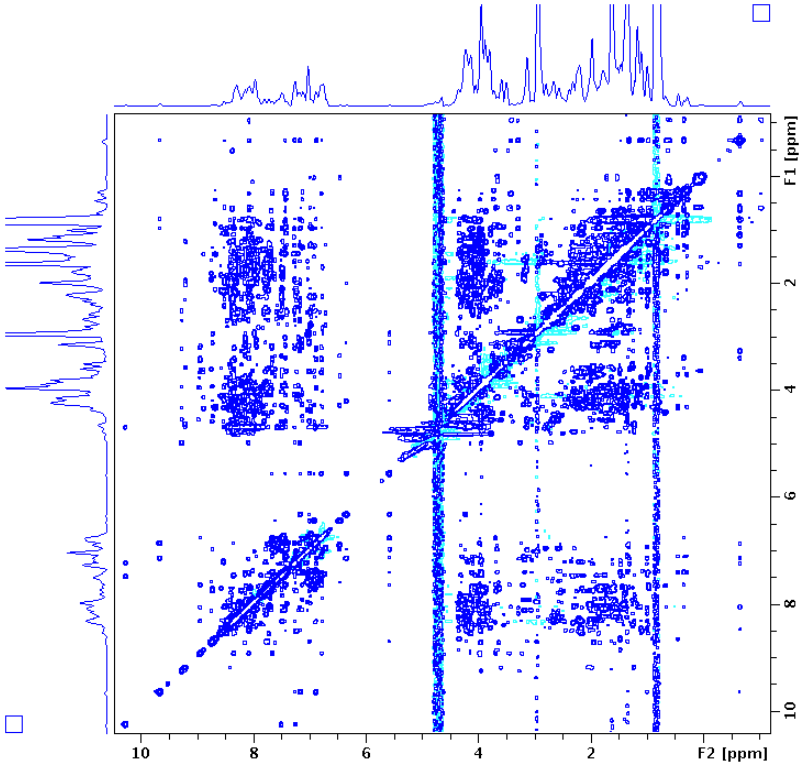
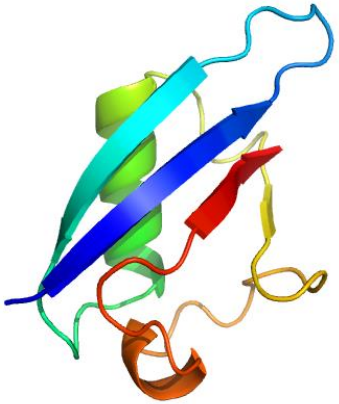






Protein NMR



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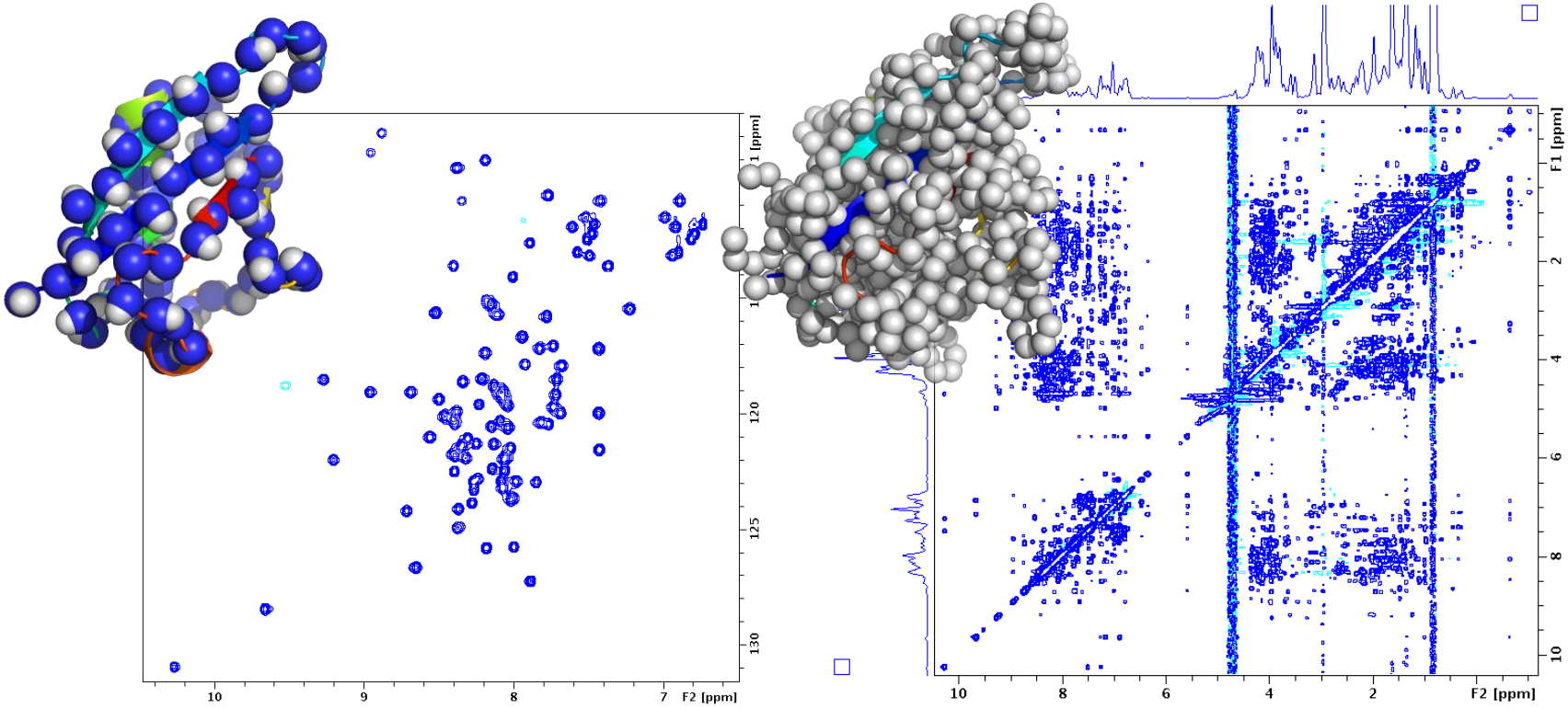

Isotope labelling (^{15}N)

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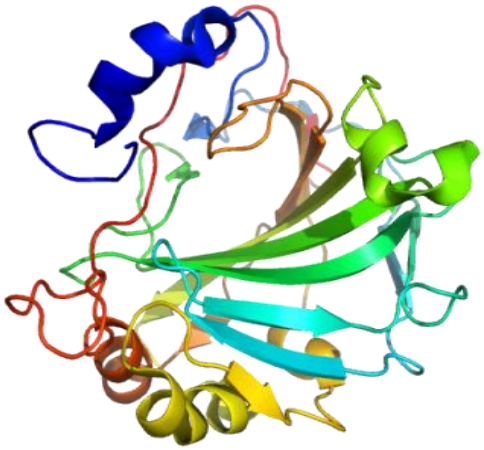

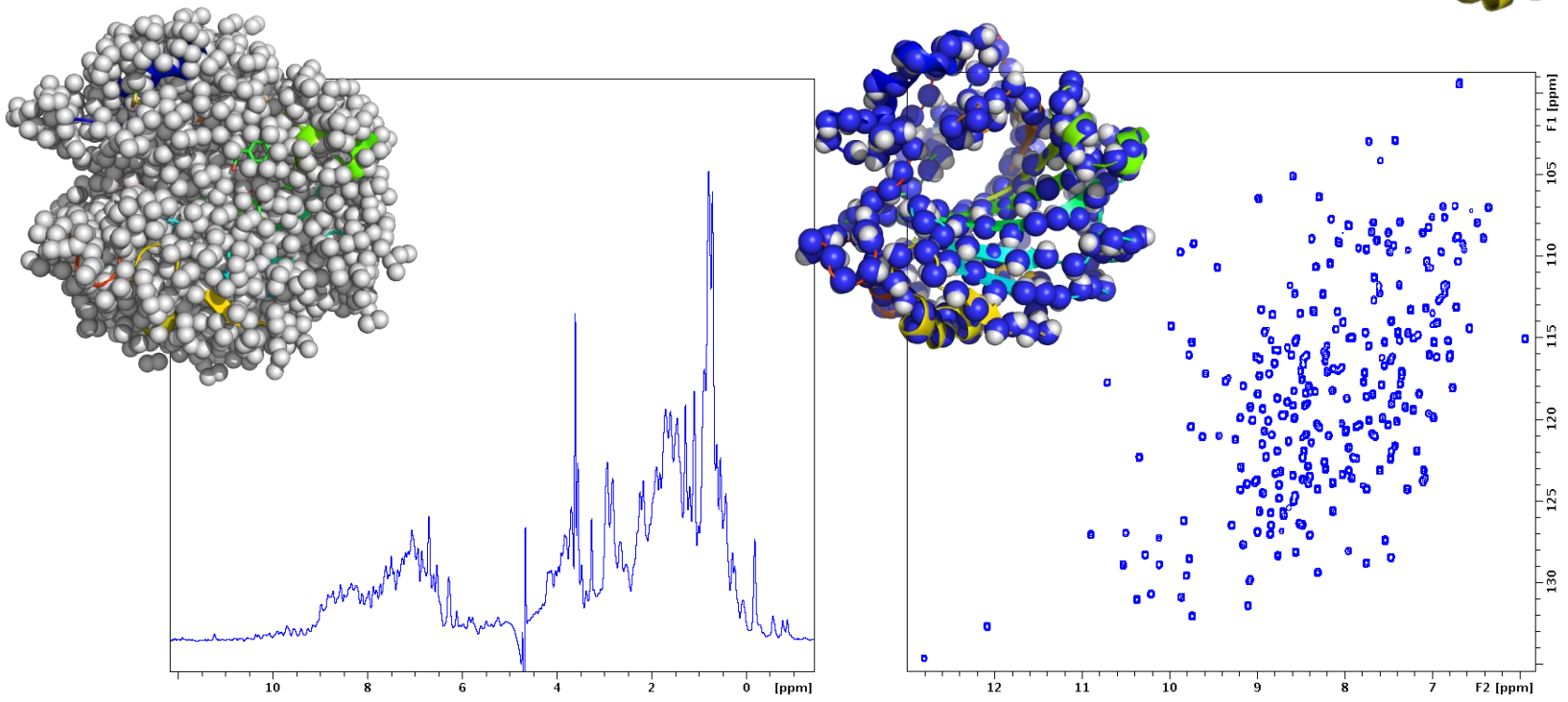

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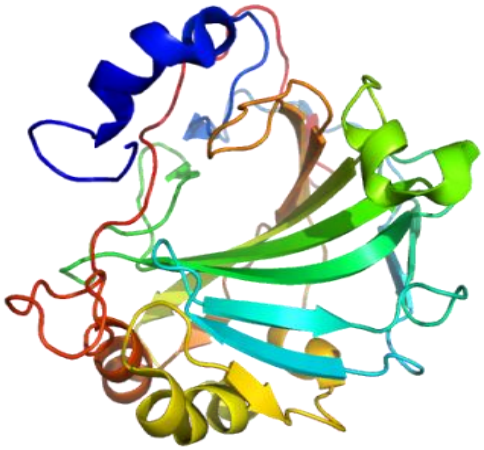
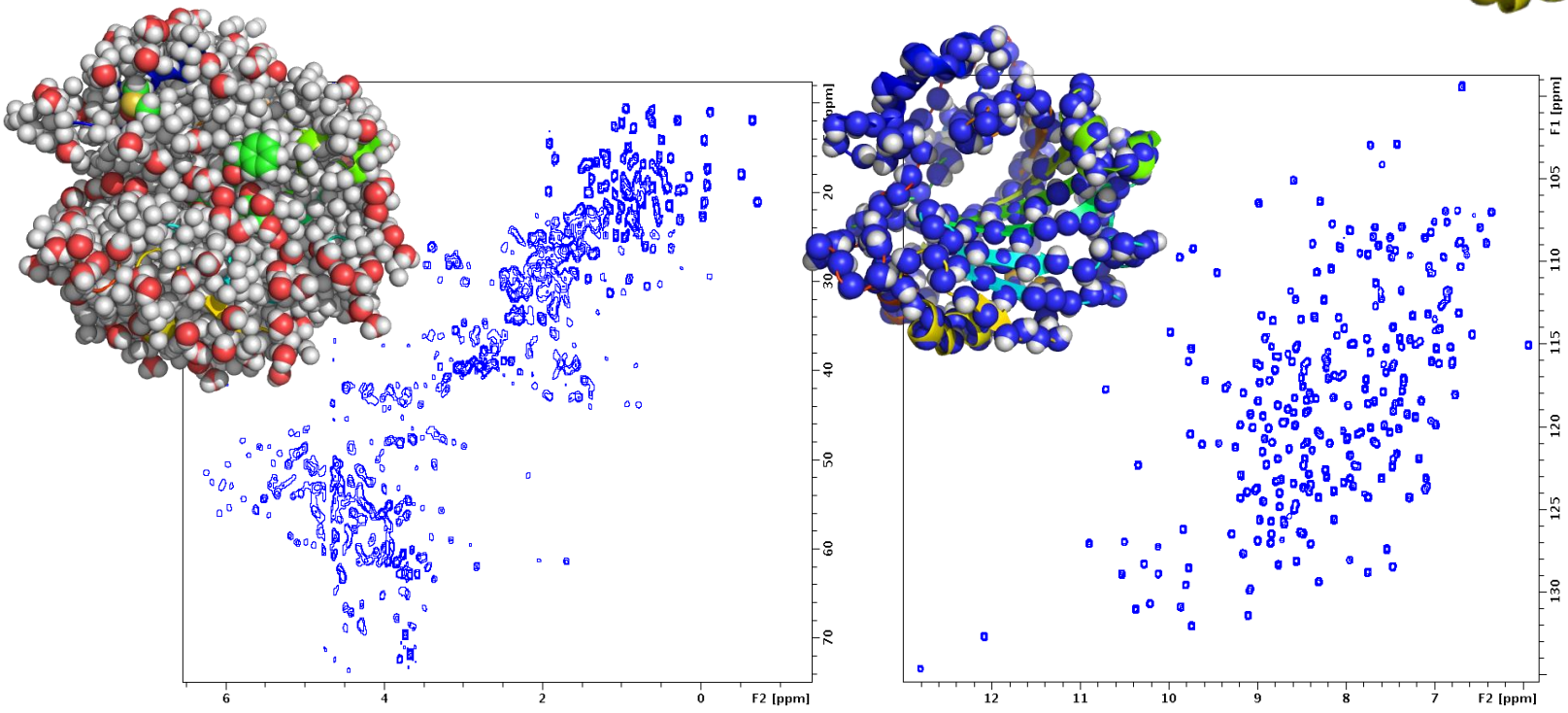


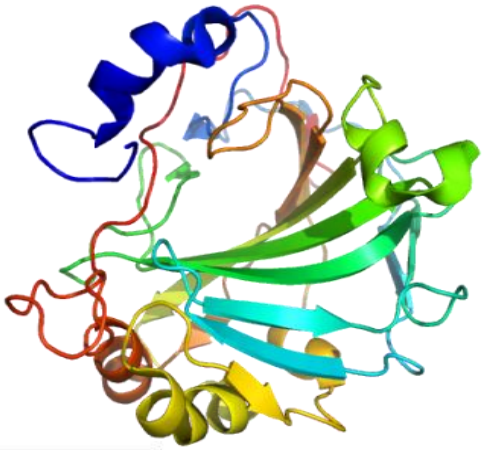


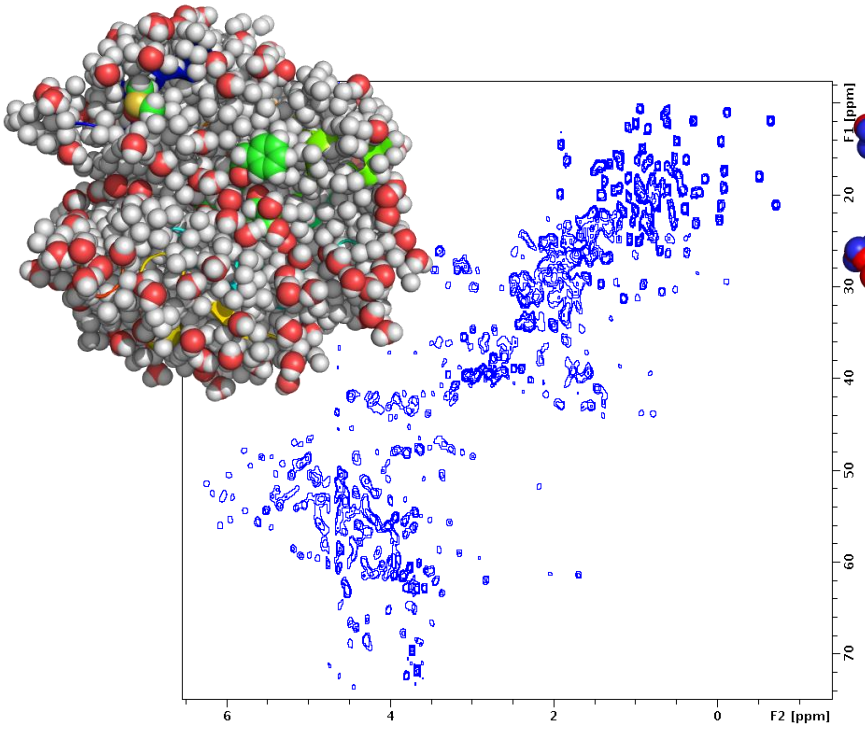
Isotope labelling (^{13}C)

Isotope labelling (^{15}N)

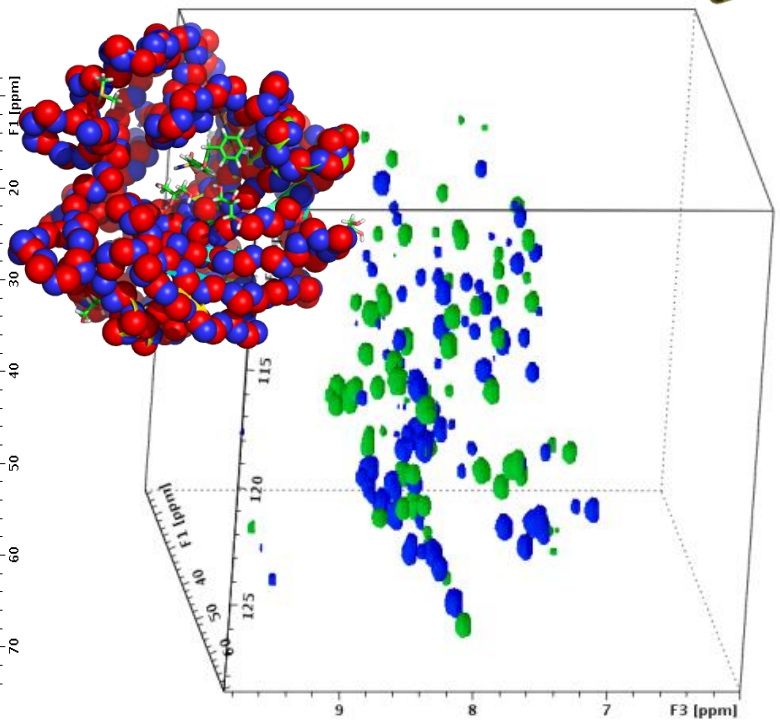
Protein NMR

- size of the studied molecules (slower tumbling → line-broadening)
- number of atoms (complexity + repetitive structures → overlaps)
- sensitivity issues





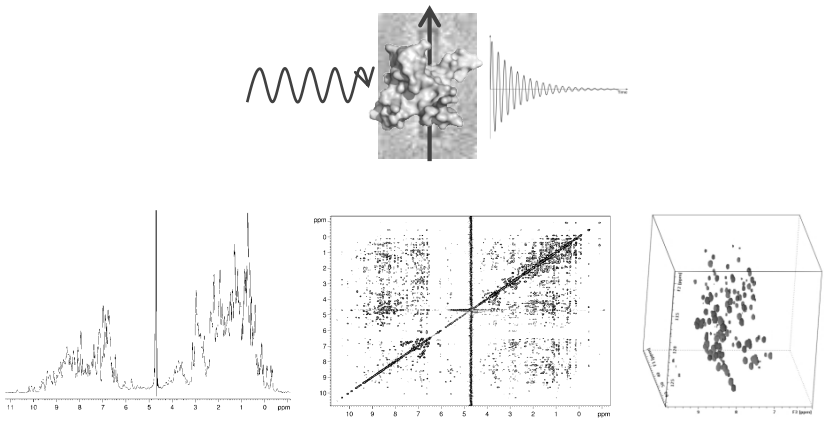
Isotope labelling (^{13}C)

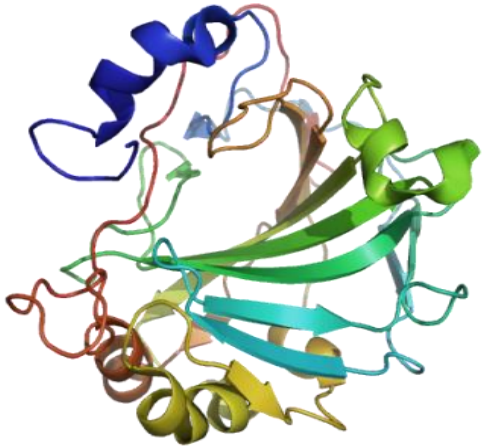


Isotope labelling ($^{13}C/^{15}N$)

Three example cases of biopolymer studies, when a 3D structure is in question

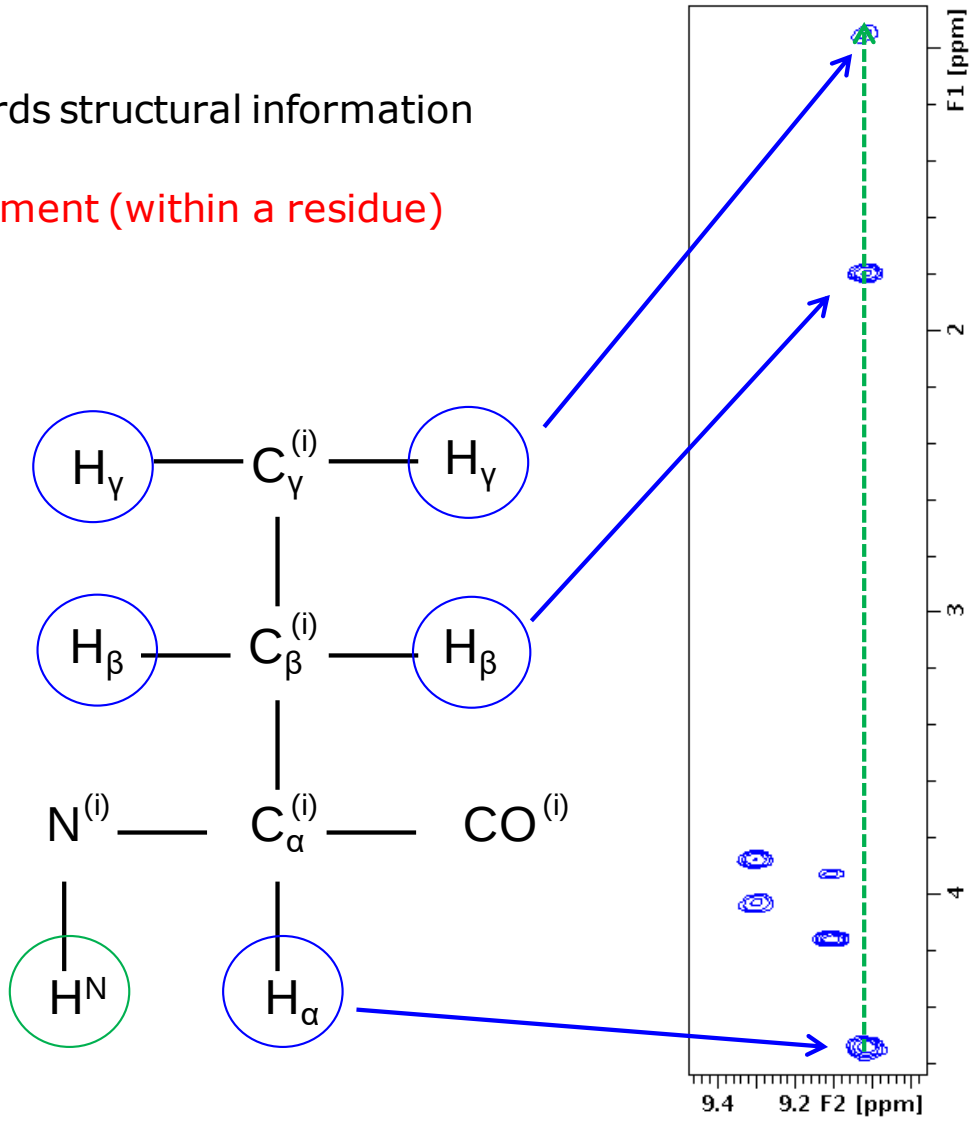
- steps towards structural information
  - ✓ Sample preparation
  - ✓ Signal assignment
  - ✓ Obtaining structural information
  - ✓ Structural calculation





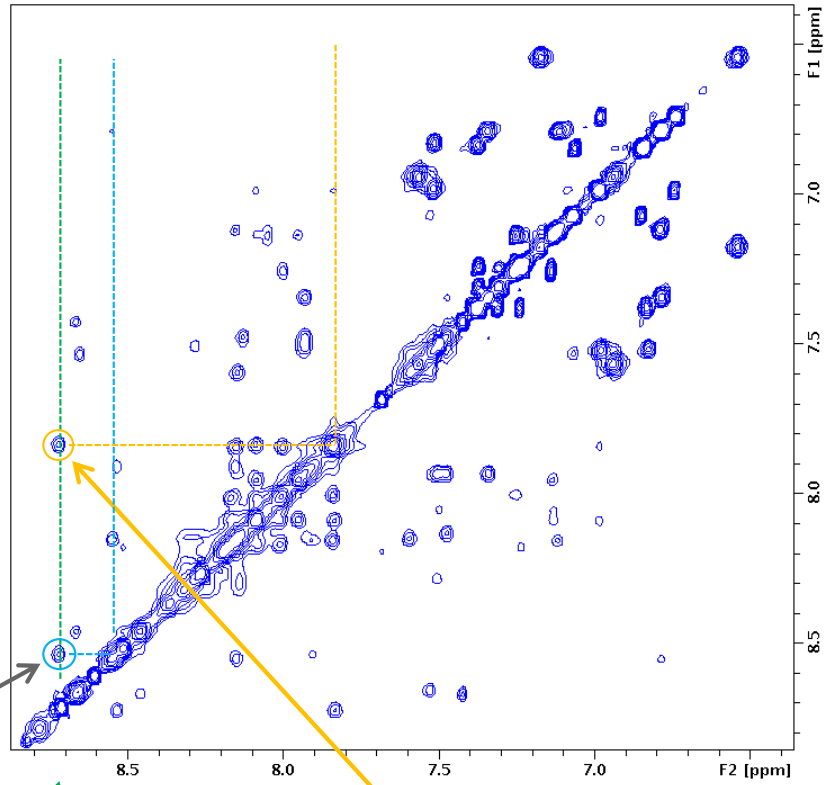
Protein NMR

- Three steps towards structural information
  - ✓ Signal assignment (within a residue)

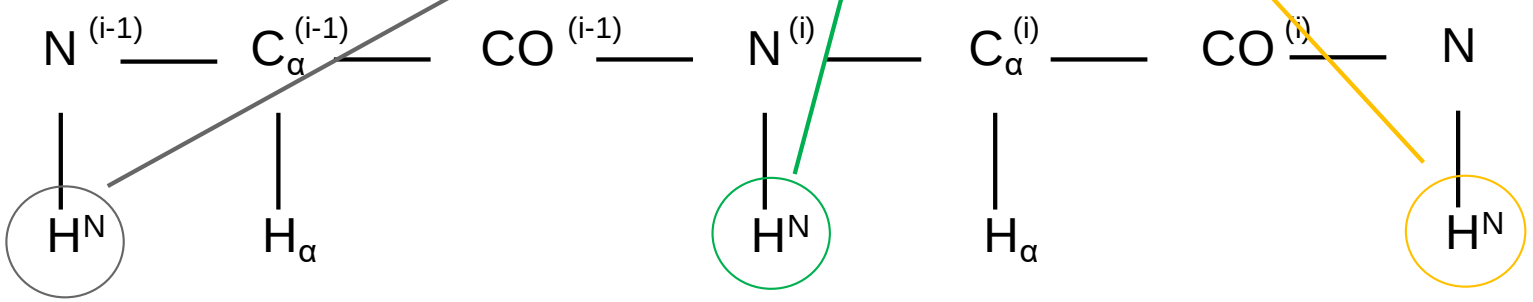


Protein NMR

- Three steps towards structural information
  - ✓ Signal assignment (sequential)

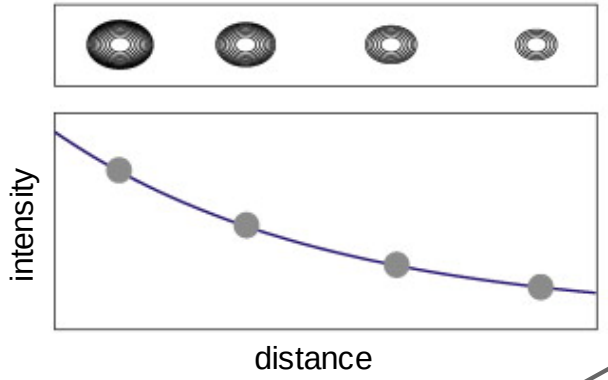


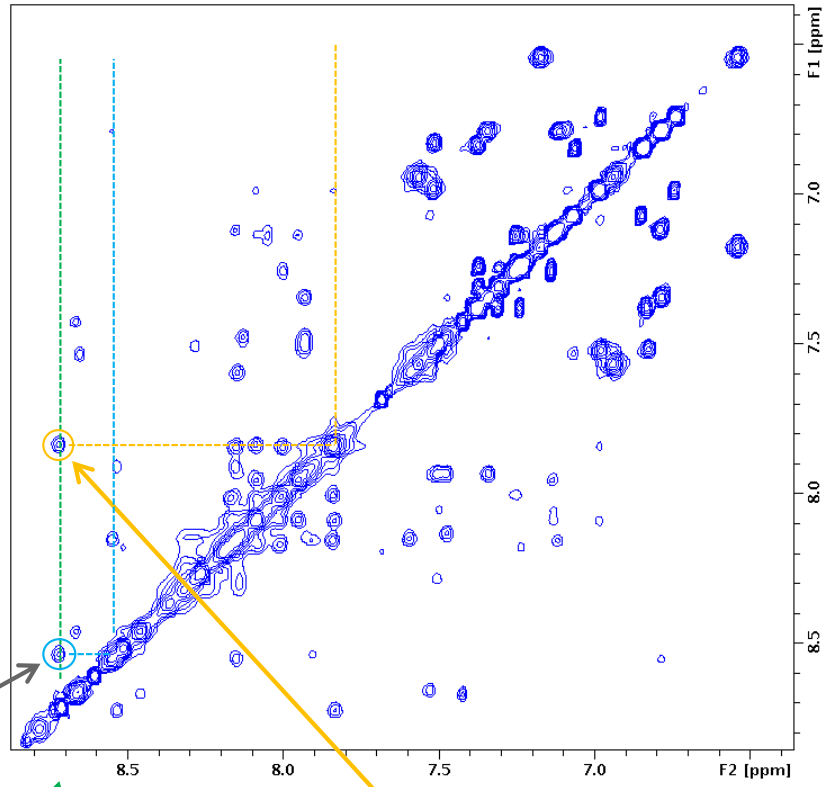
Connecting neighbouring residues

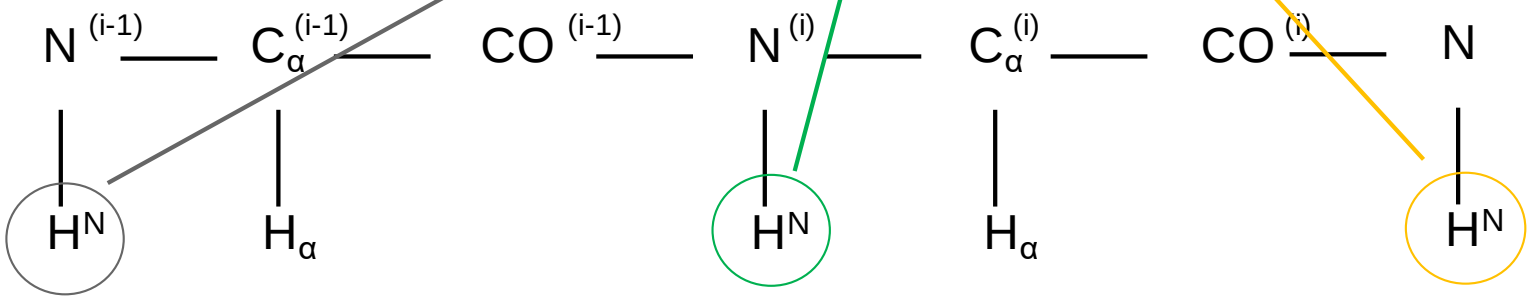


Protein NMR

- Three steps towards structural information
  - ✓ Signal assignment
  - ✓ Obtaining structural information

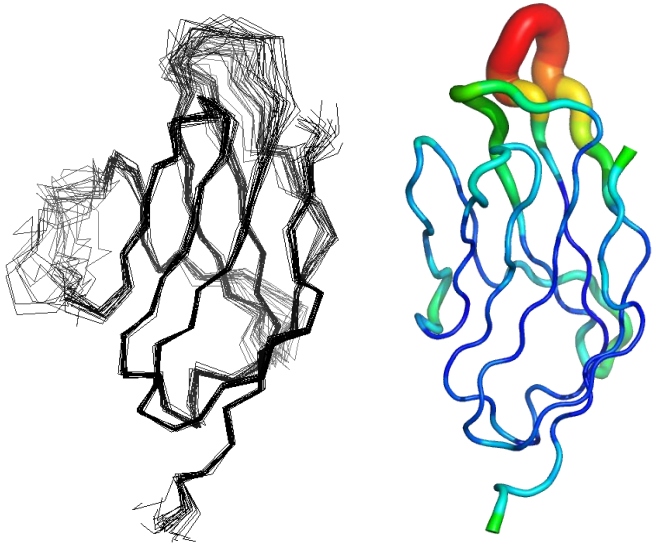




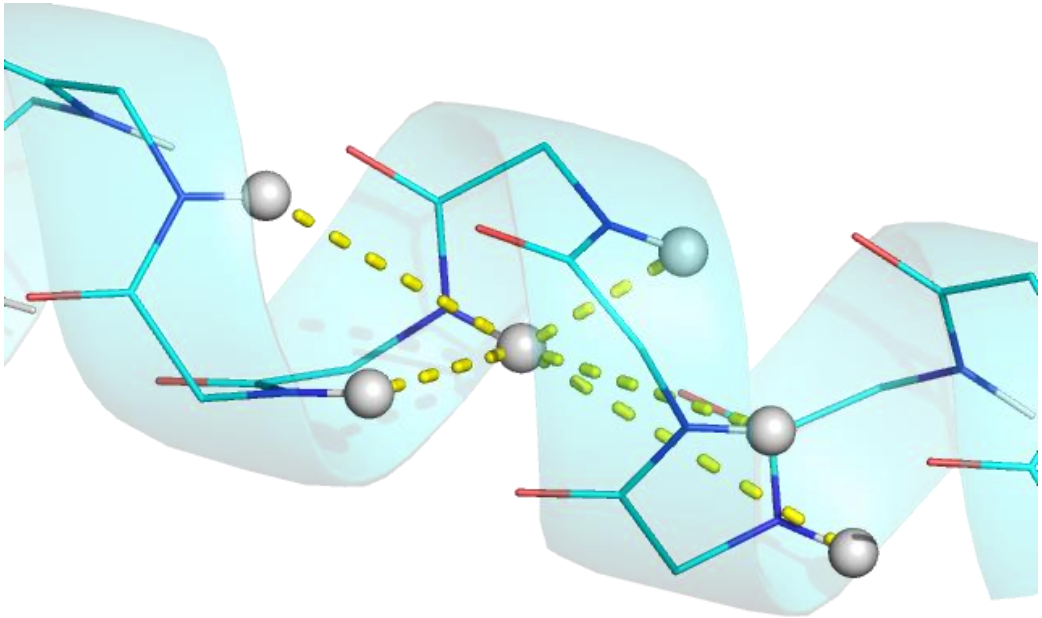


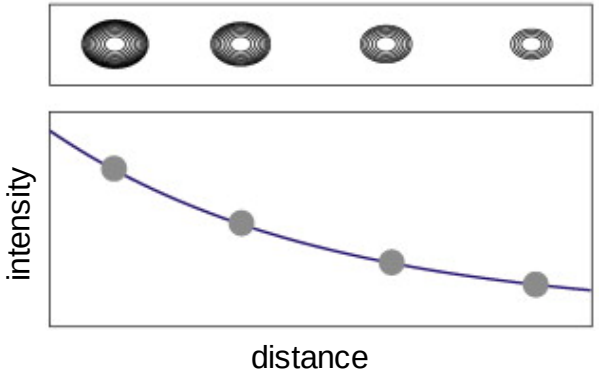
Protein NMR

- Three steps towards structural information
  - ✓ Signal assignment
  - ✓ Obtaining structural information
  - ✓ Structural calculation



models fully satisfying experimental data

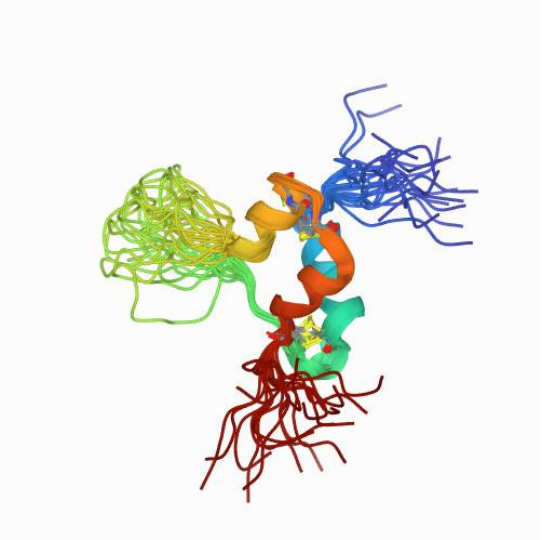




Case 1: a small peptide ~20 AA, 2 kDa

- Material:
  - 1-5mg synthetized
  - make 350 ul of 1-4 mM solution
  - buffer, pH, salts of choice, 90% H_2O/10% D_2O
- Measurement
  - 1D 1H ~ 1min
  - 2D HC ~ 2-6 hrs
  - 2D HN ~ 1-6 hrs
  - 2D NOESY ~ 12hrs
  - 2D TOCSY ~ 12hrs

  } 1.5 day
- Analysis
  - signal assignment ~ 1day (expert mode {VV,MB}) or more (me)
  - data for structure calculations ~ hours
  - structure calculation, validation ….



Hexnerová et al. JBC 2016

Case 2: protein ~200 AA, 21 kDa

- Material:
  - recombinant protein expression in *E.coli* 1-5mg 15N from $(NH_4)_2SO_4$;^{13}C from $C_6H_{12}O_6$
  - make 350 ul of 200-500 uM solution ~ 1.5 mg
  - buffer, pH, salts of choice, 90% H_2O/10% D_2O
- Measurement
  - Basic set
    - 1D 1H ~ 1min
    - 2D HC ~ 1 hrs
    - 2D HN ~ 10 m

    } 1.5 hour
  - Backbone assignment
    - 3D HNCO ~6h
    - 3D HNCACB ~12-24h
    - 3D CBCACONH ~12 h
    - 3D HNCACO ~ 12-24h

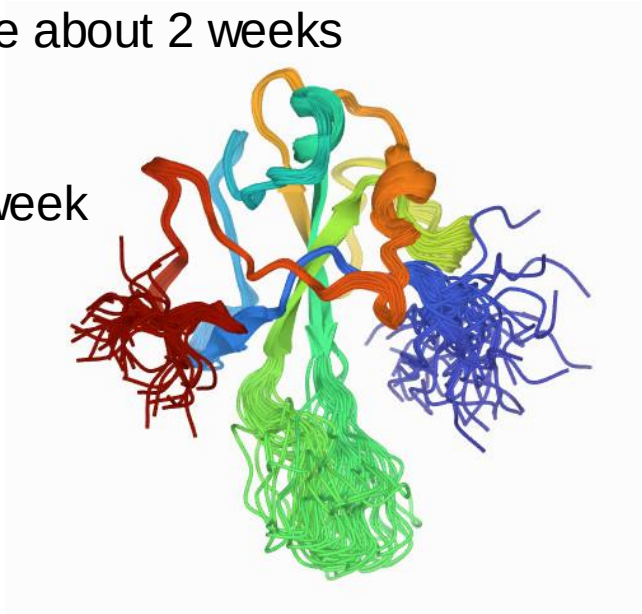
    } weekend measurement

Case 2: protein ~200 AA, 21 kDa

- Measurement
  - sidechain assignment
    - 3-5 more 3D experiments ~ 3-4 days
  - NOESY for structure determination
    - 3D ^{13}C edited NOESY-HMQC ~ 3-5 days
    - 3D ^{15}N edited NOESY-HSQC ~ 1-2 days

total experimental time about 2 weeks

- Analysis
  - backbone signal assignment ~ 1day – 1 week
  - sidechain assignment few days
  - data for structure calculations ~ hours
  - structure calculation, validation ….

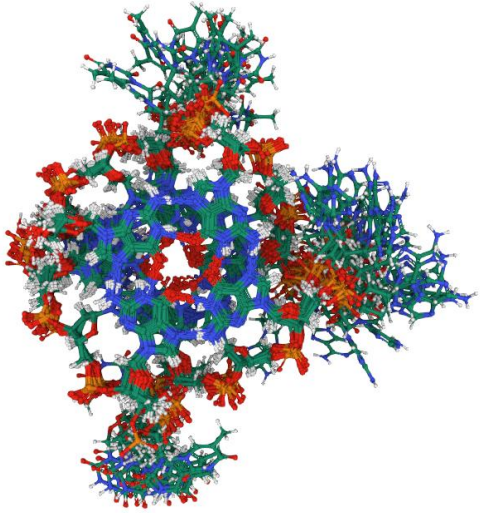


Veverka, Bouřa PlosPat 2020

Case 3: nucleic acid ~20-30 nucleosides, 7-10 kDa

- Material:
  - 4-7mg synthetized DNA, ion exchange chromatography recommended
  - make 350 ul of 2 mM solution
  - buffer, pH, salts of choice, 90% H_2O/10% D_2O
  - selectively labelled sample might be necessary at extra cost
- Measurement
  - 1D 1H ~ 1min
  - 2D HC ~ 12 hrs
  - 2D NOESY ~ 12 - 24 hrs
  - 2D TOCSY ~ 12 - 18 hrs

2.5 day + variable temperatures



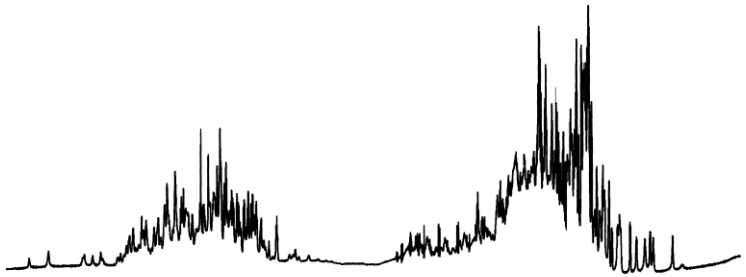
Volek et al, NAR 2021

- Analysis
  - signal assignment ~ days, with selective labeling
  - data for structure calculations ~ hours
  - structure calculation, validation ….

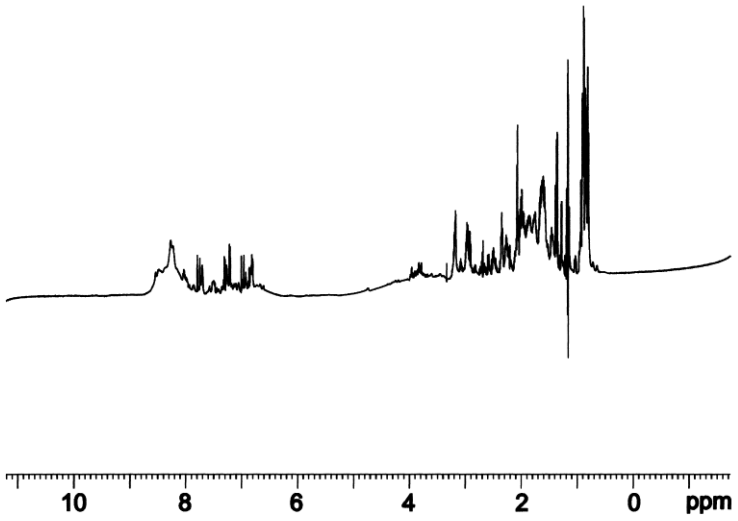
Question: Are there unstructured regions in my protein?

Question: Are there unstructured regions in my protein?

globular



instrinsically disordered
or misfolded



~1 minute measurement, 30 uM 160 uL

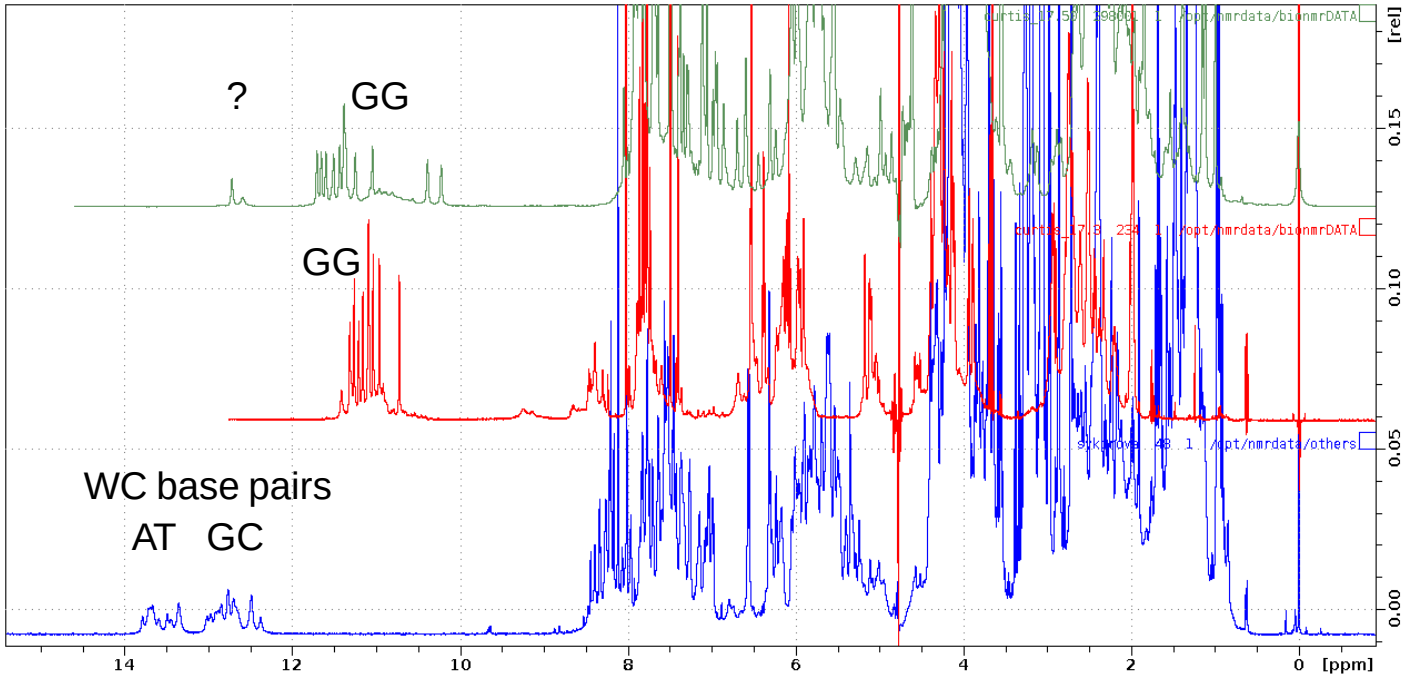
Question: How is my DNA?

Question: How is my DNA?

G-quad DNA
CurtisLab

G-quad DNA
CurtisLab

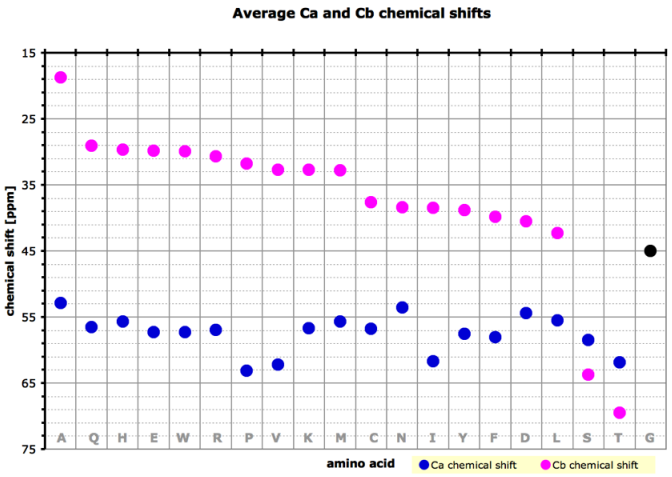
DS DNA
HocekLab

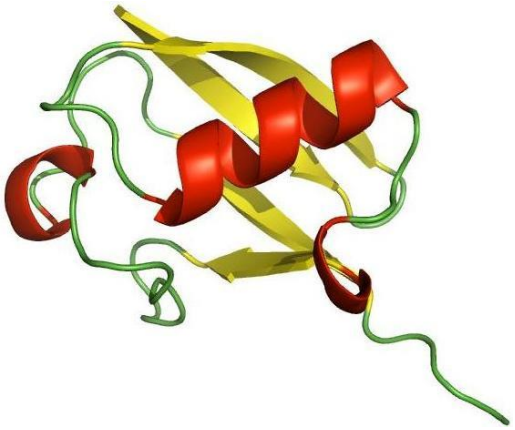


~10 minute measurement, >30 uM 160 uL

Question: What is the secondary structure of my protein?

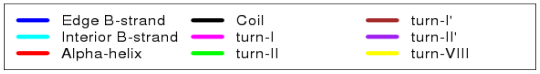
Question: What is the secondary structure of my protein?

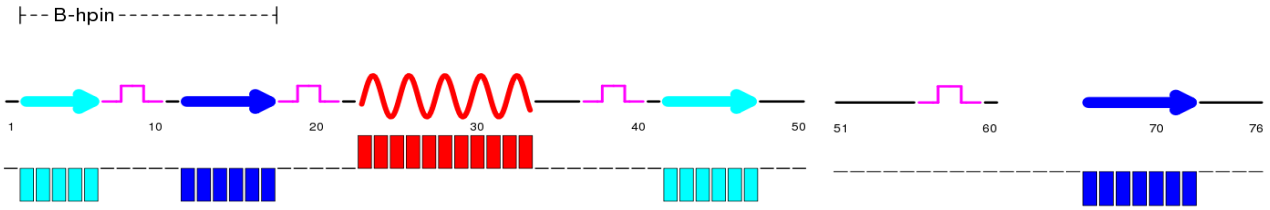




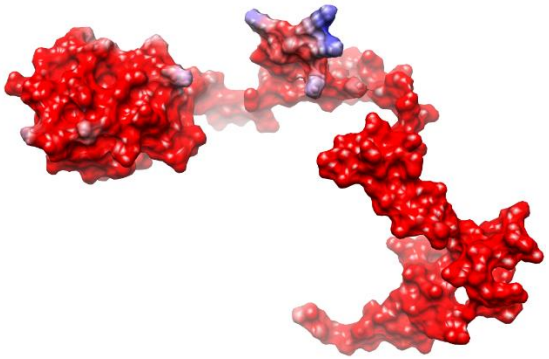
secondary chemical shift:

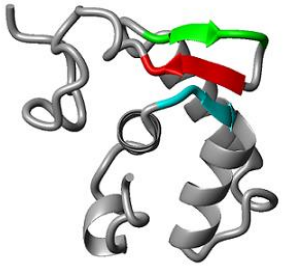
$$\Delta\delta = \delta_{observed} - \delta_{random\ coil}$$

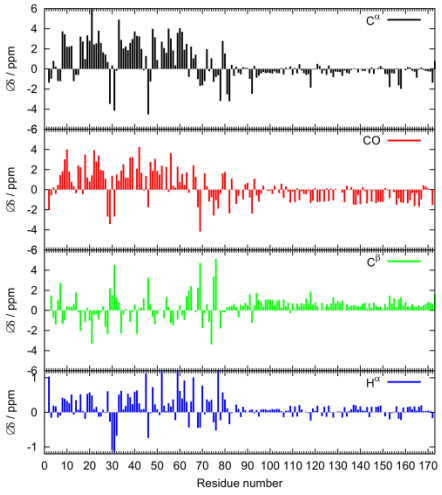


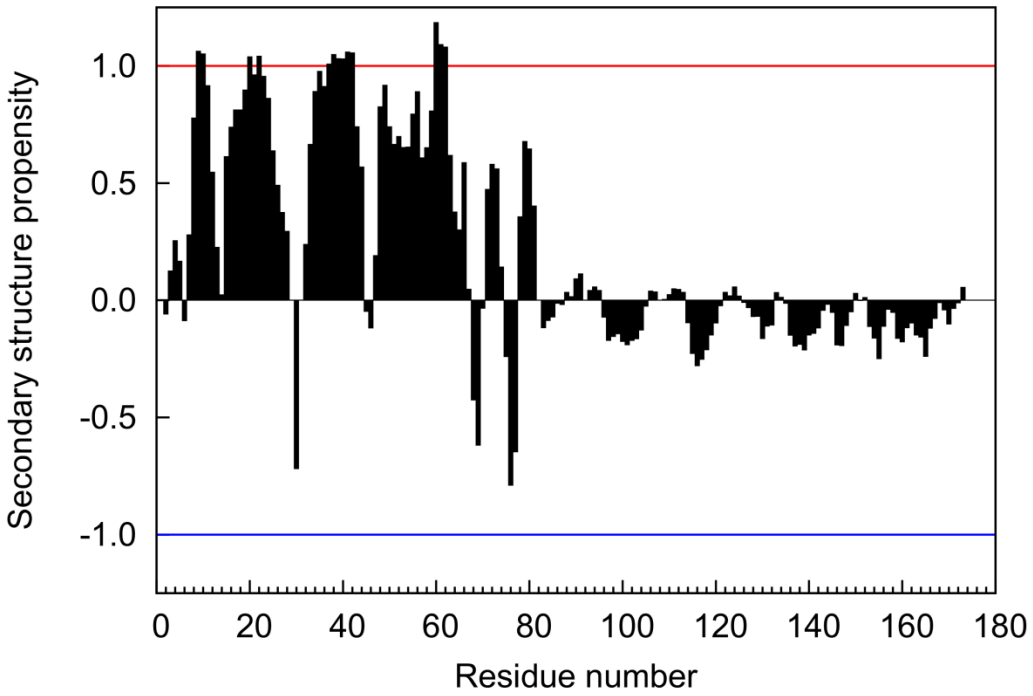


Question: What is the secondary structure of my **partially unstructured** protein?



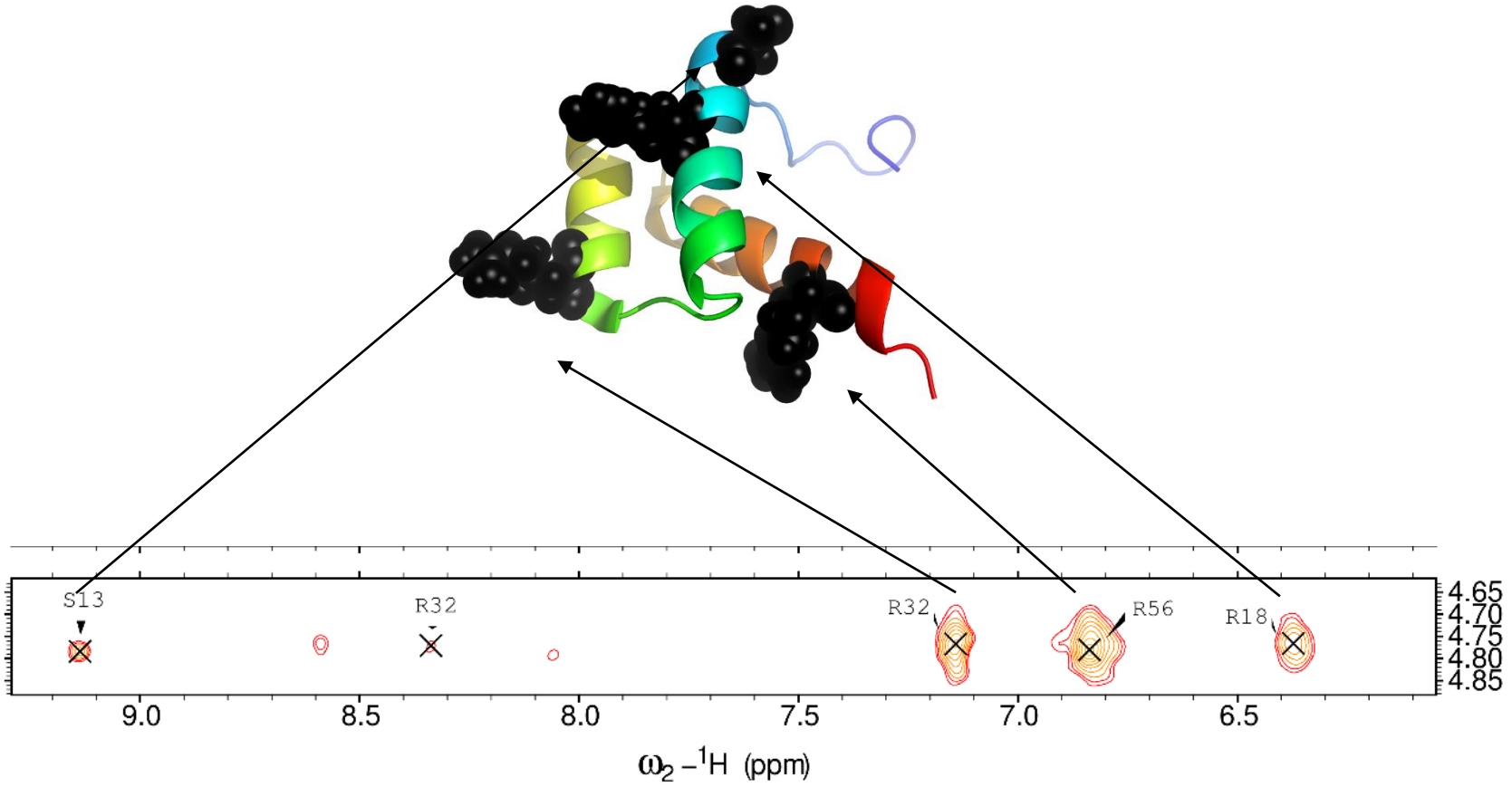






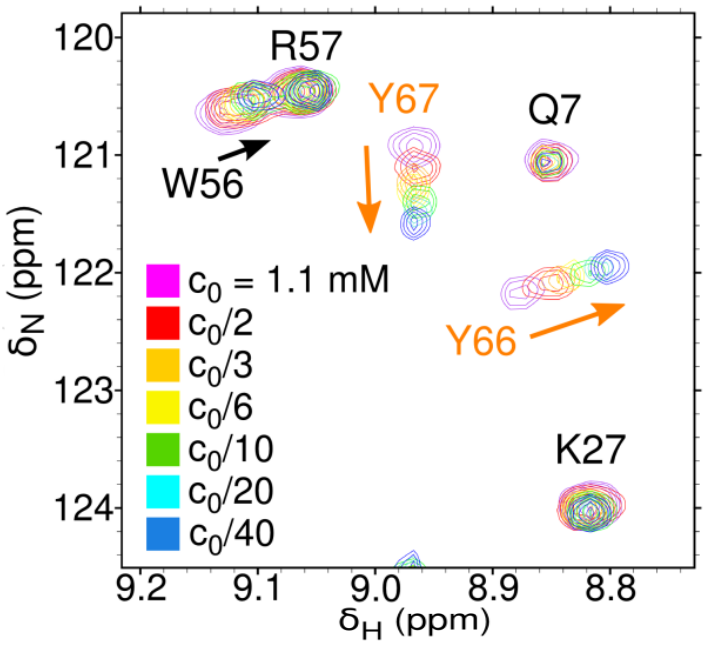
Question: Are there bound waters in my protein?

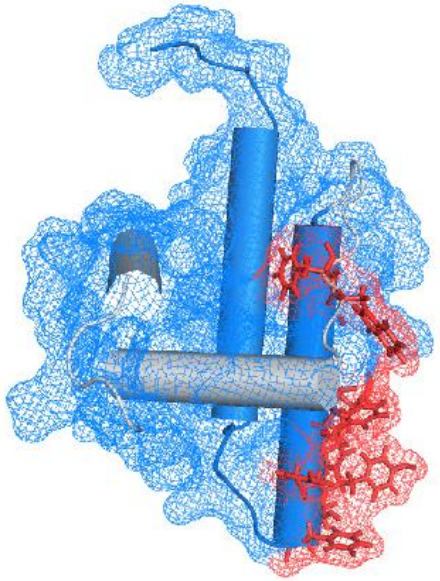
Question: Are there bound waters in my protein?

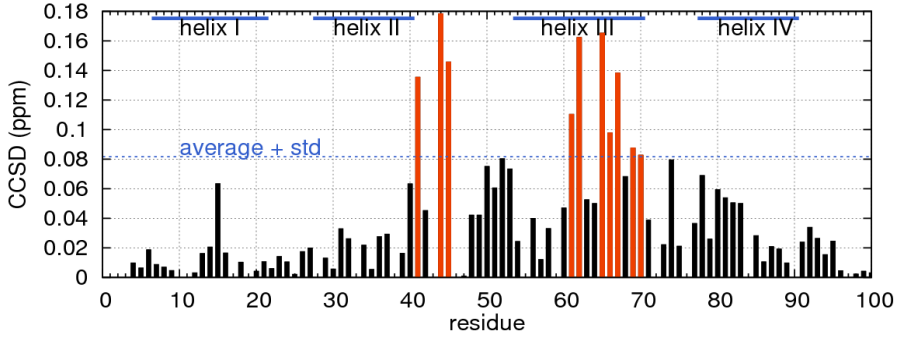


Question: Does my protein oligomerize?

Question: Does my protein oligomerize?

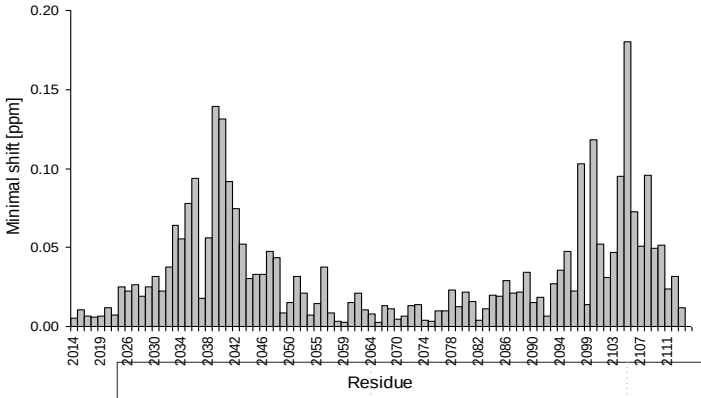


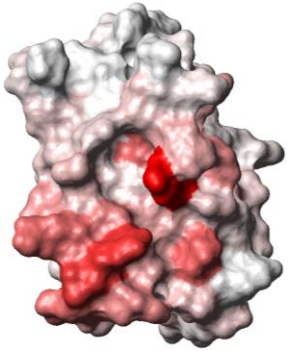


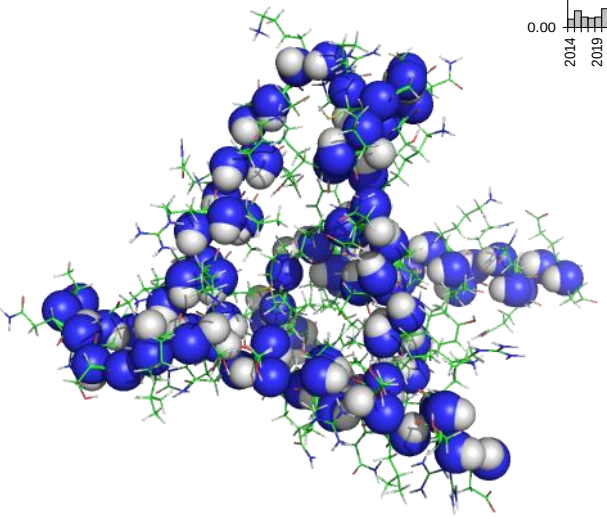


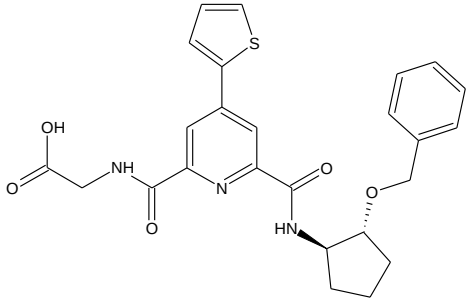
Question: Does my protein bind a small molecule?

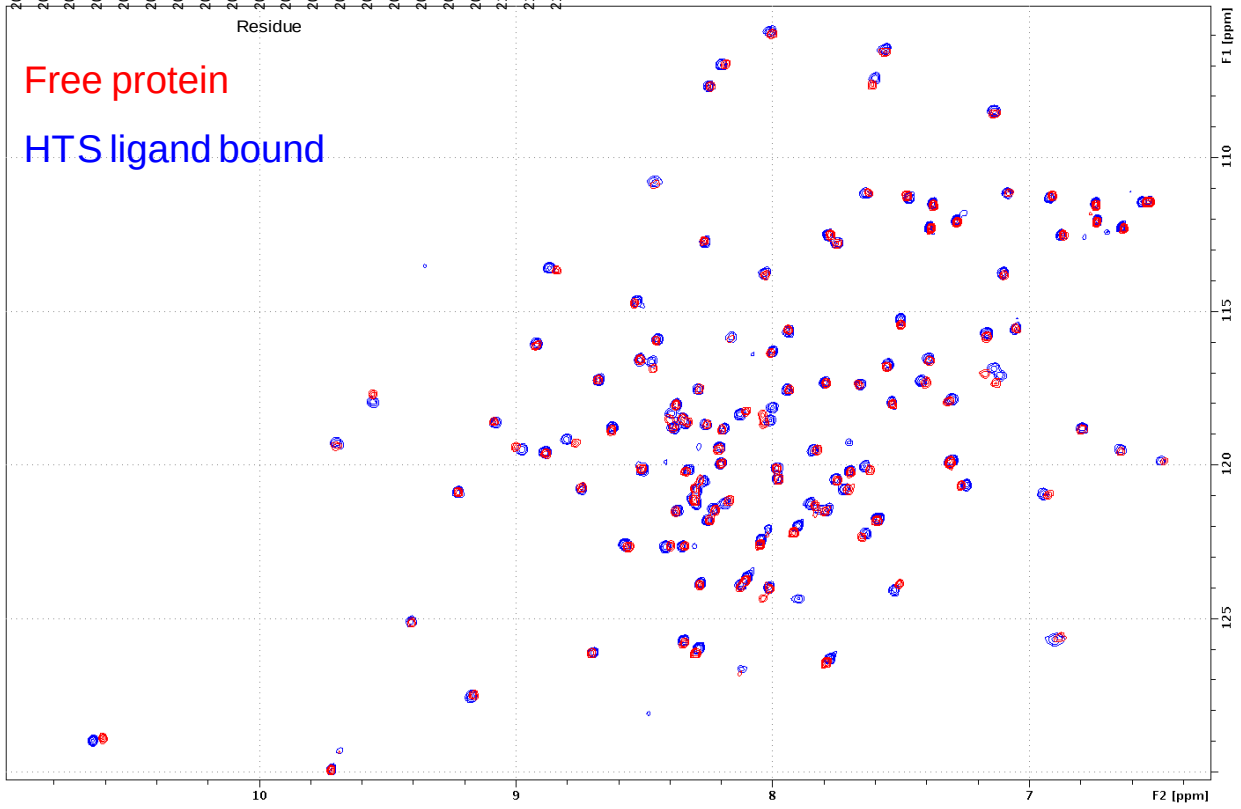
Question: Does my protein bind a small molecule?





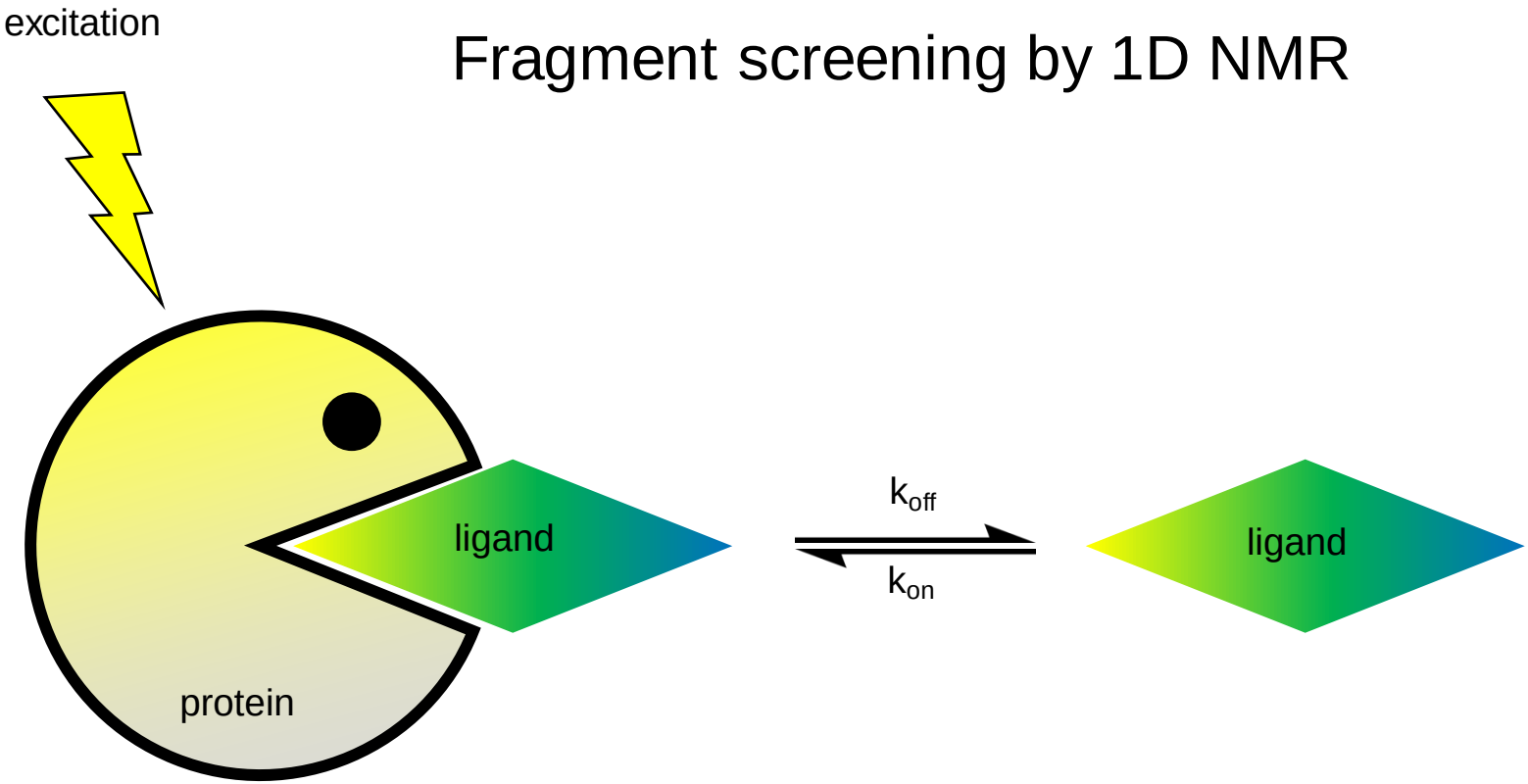




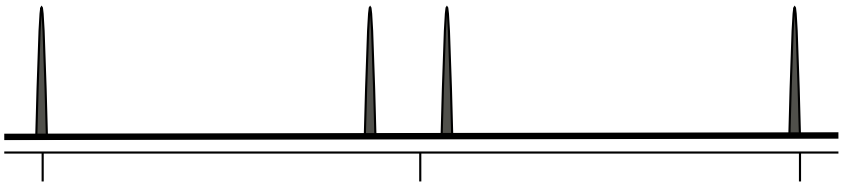


Question: Does a small molecule bind my protein?

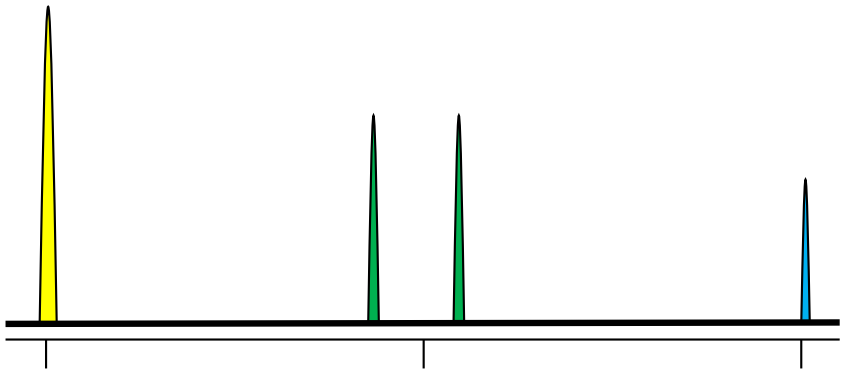
Fragment screening by 1D NMR



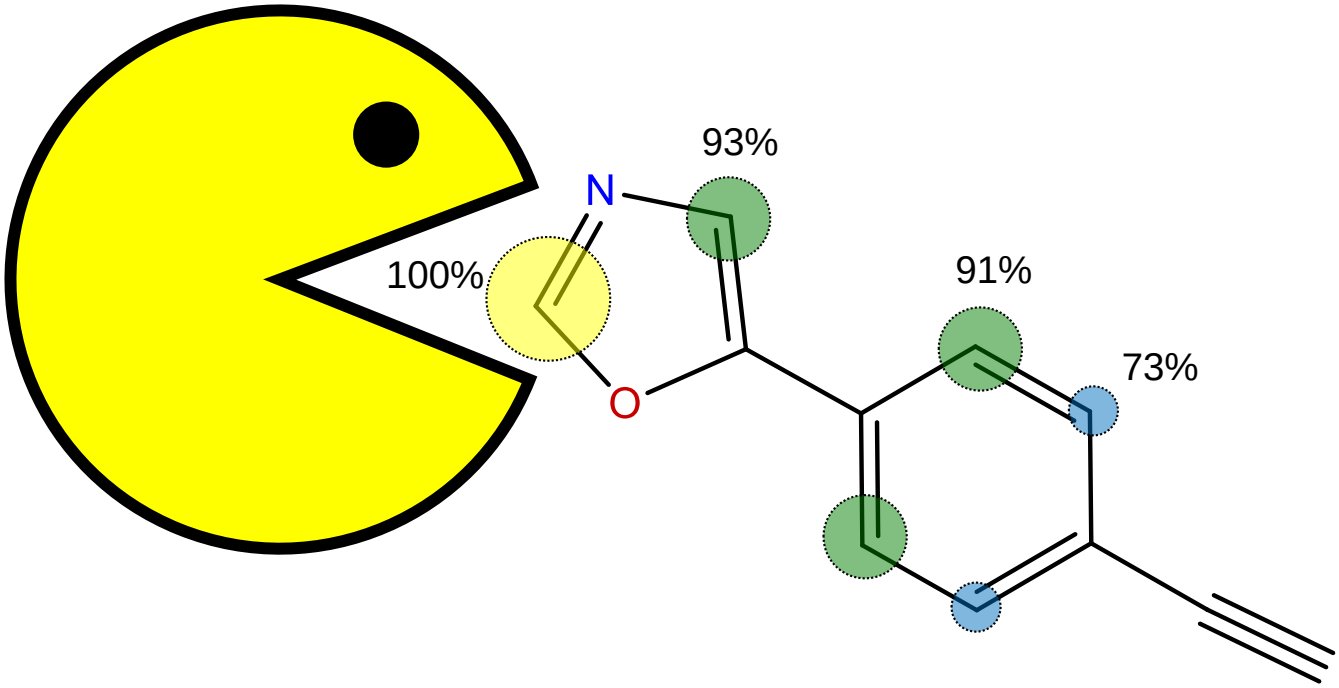
^{1}H NMR (off-resonance)



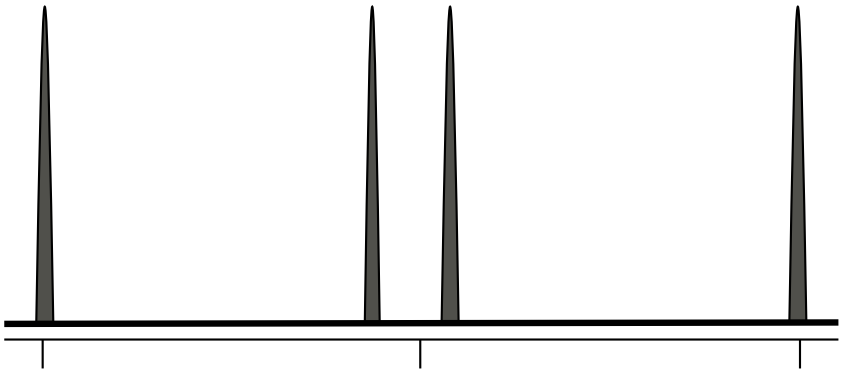
on-resonance



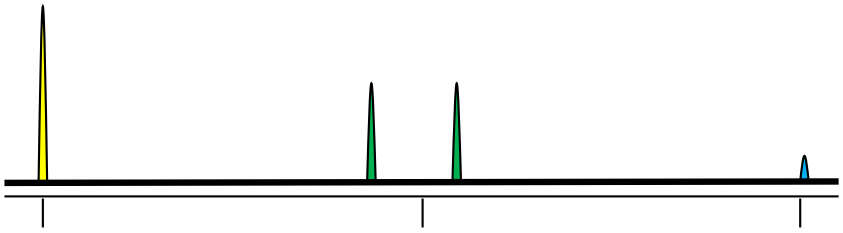
Question: Does a small molecule bind my protein?



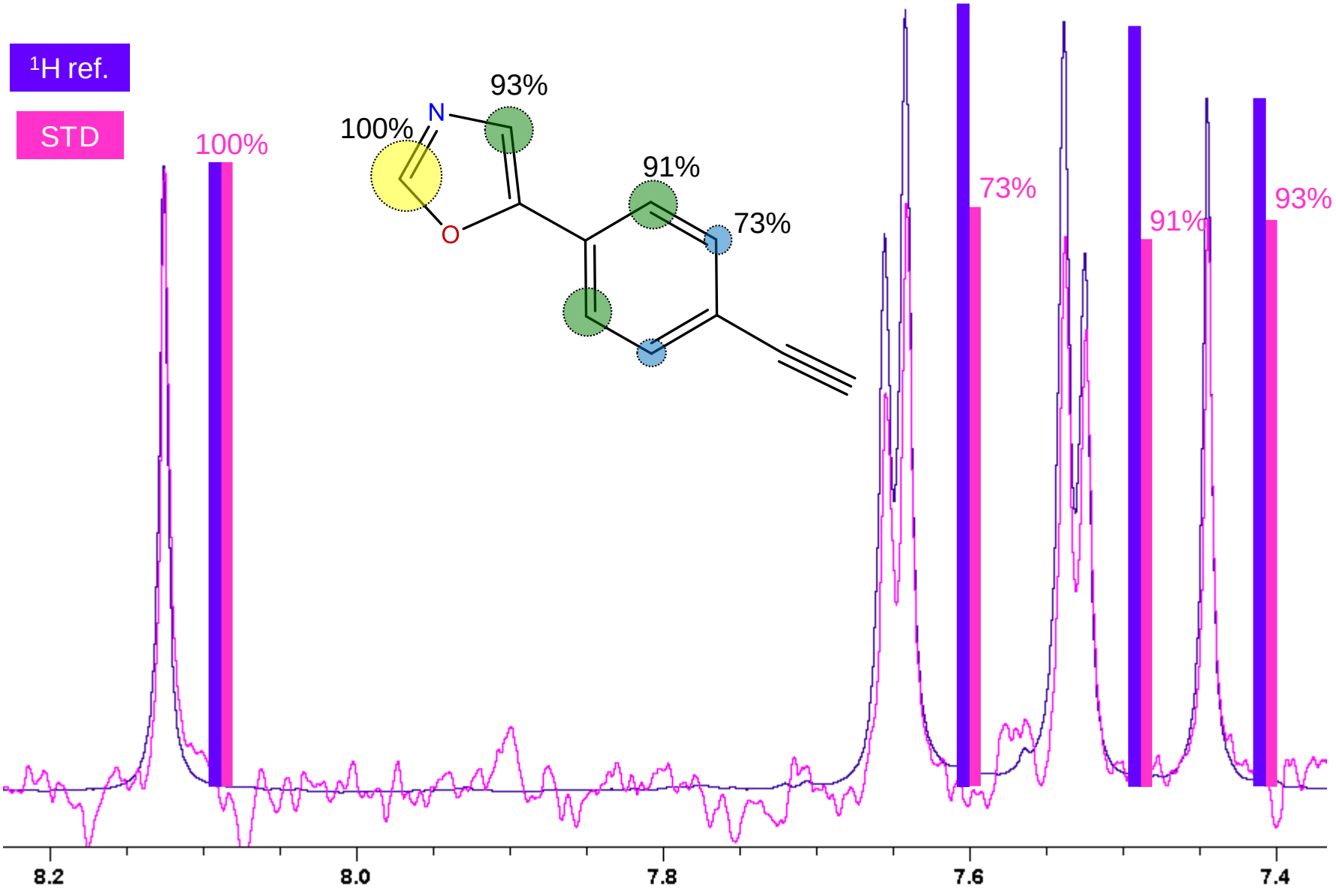
^{1}H NMR



STD-NMR (difference spectrum)

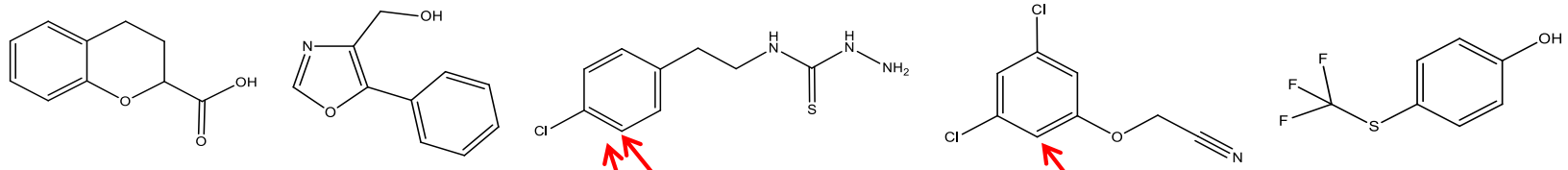


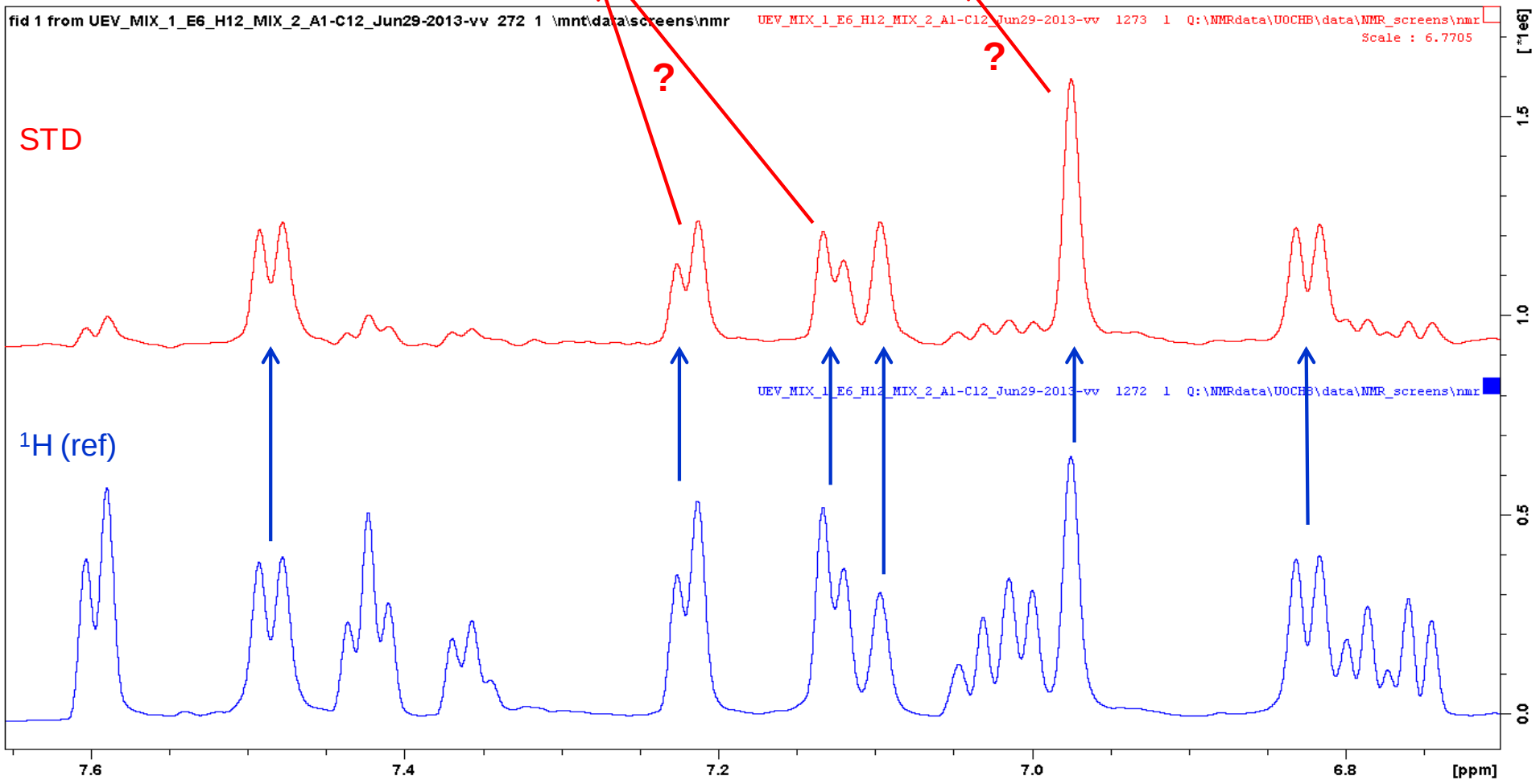
Question: Does a small molecule bind my protein?



Question: Does a small molecule bind my protein?

Mixture of 5-6 compounds = TIME SAVER





Question: and many more…..

translational diffusion
rotational diffusion
^{15}N relaxation local ps-ns dynamics
relaxation dispersion for slower motions
CEST, DEST – detection of invisible states
spin labels for protein complexes, paramagnetic relaxation enhancement
residual dipolar couplings
selective labelling for huge systems
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acknowledgement

Structural Biology

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