1 Basic principles

1. What particles are magnetic? May a magnetic particle have a zero spin number? May a magnetic particle have a zero electric charge? Give examples of nuclei which are magnetic. What nuclei are found in biomacromolecules? Which of them are magnetic? Which of them can be routinely studied by NMR?

2. What is the energy of a magnetic moment in a magnetic field. How is the frequency of precession related to the external magnetic field? What determines distribution of magnetic moments?

3. The precession frequency of protons in different chemical groups differ. Why? Give a relative size of these deviations (in orders of magnitude).

4. Which of the following interactions change the average precession frequency of nuclei in molecules dissolved in an isotropic solvents and which of them significantly contribute to relaxation: direct interaction of magnetic dipolar moments, shielding by electrons, interaction of magnetic dipolar moments mediated by electrons of chemical bonds connecting the interacting atoms.

5. Is the presence of a static homogeneous external magnetic field necessary to create the macroscopic magnetization? Is it sufficient to have the static homogeneous external magnetic field in order to create the macroscopic magnetization? Is it sufficient to have the static homogeneous external magnetic field in order to observe signal in an NMR spectrometer? If not, what else is needed?

6. Explain the following terms: ninety-degree pulse, carrier frequency, frequency offset.

7. What is the Fourier transform good for in NMR spectroscopy?

8. Explain the idea of a two dimensional experiment.

9. Describe evolution of chemical shift and scalar coupling during various echoes.

10. Describe INEPT and HSQC as an example of a heteronuclear correlation experiment.

2 NMR structure determination

Question for everybody

- Describe the process of protein structure determination based on NMR data

3 NMR relaxation

21. Describe two most important physical mechanisms of NMR relaxation (for spin-1/2 nuclei). Describe their contributions to the R_1 and R_2 relaxation rates.

22. Describe the terms "correlation function" and "correlation time" for a rigid spherical molecule in the absence of chemical/conformational changes modifying the chemical shift. Use the loss of coherence as an example.

23. How is NMR relaxation related to molecular motions? Describe the term "spectral density function". Describe the effect of fluctuating fields on the loss of coherence and on the return of the total magnetization to its equilibrium value.

24. What is the effect of internal motions of biomolecules on relaxation of their NMR signals? Use an example of relaxation of ^{15}N in amide groups in the protein backbone and assume that the internal motions and the overall tumbling are independent.

25. Describe the principles of measuring relaxation rates in biomolecules. What relaxation parameters are usually measured in practice?

26. What methods can be used to interpret NMR relaxation parameters in the terms of intramolecular motions? Explain the basic principles.

27. Describe the procedure for the model-free analysis of relaxation data.

28. What is the effect of slow (slower than the correlation time of the overall tumbling) chemical/conformational changes modifying the chemical shift on NMR relaxation?

29. How do the spectra change during titration if the dissociation and association of a ligand is slow/fast? What information can be obtained by analysing the spectra?

30. Describe saturation transfer difference and isotope edited/filtered NOE experiments.

4 NMR spectroscopy of nucleic acids

11. Explain the base-pairing in nucleic acids. Which of the atoms are involved in forming the hydrogen bonds? What can you tell about the base pairing from spectra in Fig. 1?

12. Explain the numbering of atoms in bases and sugars of nuclei acids. Using Fig. 1 and 2, show where signals from hydrogens atoms in different positions appear in NMR spectra.

13. Draw the structure formulas of Adenine, Cytosine, Guanine, Thymine and Uracil. Which of the hydrogen atoms are in the fast exchange regime with solvent water? How does the exchange affect the measured NMR spectra?

14. Which quick NMR experiment would you use for a newly prepared oligonucleotide sample to check whether the molecule forms a single stable structure? Which of the following solvents would you use to prepare the sample and why? (H2O pure, D_2O pure, mixture 90 % H_2O and 10 % D_2O).

15. Explain the syn- and anti conformations around the glycosidic bond. How would you use NMR spectroscopy to distinguish between these two conformations?

16. What is sugar puckering? Which sugar conformations are prevalent in DNA and RNA? How can you study the sugar conformation by NMR spectroscopy?

17. What kind of interaction gives rise to the cross-peaks in COSY and TOCSY spectra? Can you tell from the spectrum in Fig. 3, whether the sample was a DNA or RNA and how many nucleosides of each kind does the molecule include provided the sample is a symmetric duplex and all the bases form Watson-Crick base pairs?

18. Explain the principle of sequential assignment in nucleic acids using NOESY spectra. Use Fig. 4.

19. What information, additional to proton spectra, can you get from heteronuclear spectra, such as 13C-HSQC and 15N-HSQC?

20. Name the most important heteronuclear experiments used for the assignment of nucleic acids and explain their use. What kind of sample do you need for triple-resonance experiments?

Extra questions for those who did not attend/pass the practical course C6775:

5 Assignment of protein NMR spectra

21. Draw examples of individual ^{1}H, ^{15}N, and ^{13}C spin systems in a protein chain.

22. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MTHLNKWPEQ?

23. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MYPCTGQNLE?

24. How many peaks should appear in a well-resolved ^{1}H-^{15}N HSQC spectrum of a peptide MIGPWLKNVD?

25. With the help of the table of typical carbon chemical shifts, assign the following chemical shifts (obtained from an HNCACB experiment) to alanine, tyrosine, tryptophan, glycine, and threonine: (62.3 ppm and 68.4 ppm), (50.3 ppm and 18.4 ppm), (56.3 ppm and 39.4 ppm), (42.3 ppm), and (60.3 ppm and 29.4 ppm).

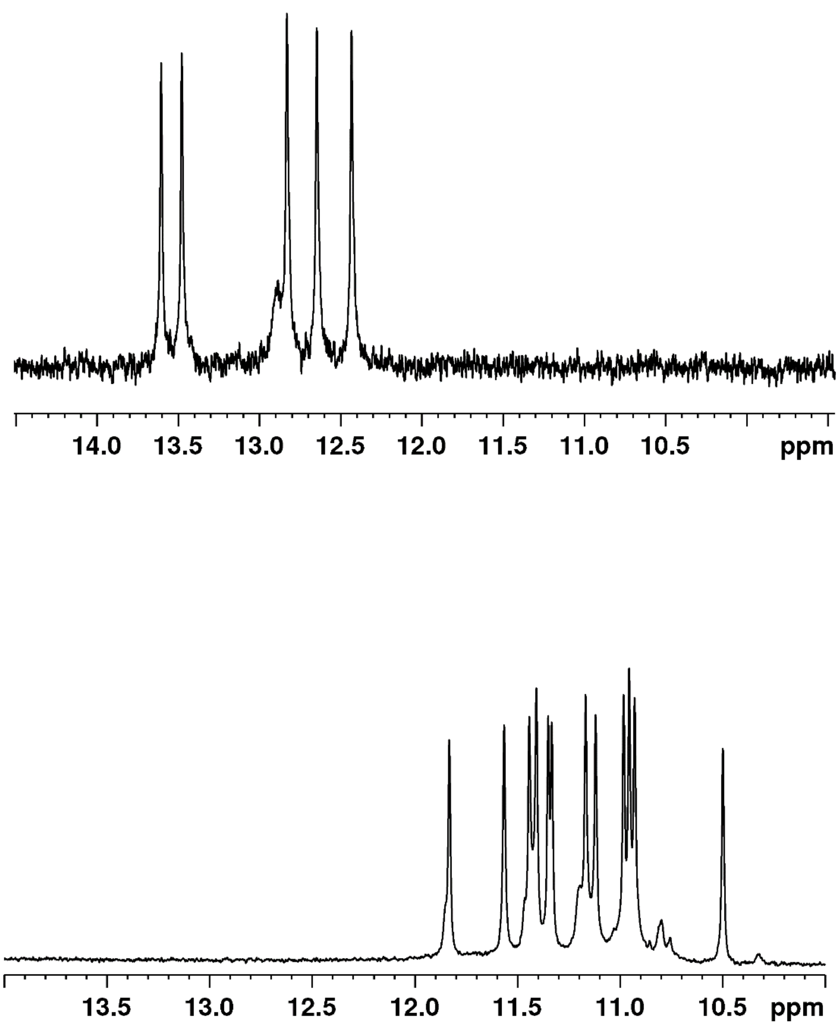
26. With the help of the table of typical carbon chemical shifts, assign the following chemical shifts (obtained from an HNCACB experiment) to alanine, isoleucine, histidine, glycine, and serine: (62.3 ppm and 38.4 ppm), (53.3 ppm and 28.4 ppm), (50.3 ppm and 19.4 ppm), (44.3 ppm), and (60.3 ppm and 57.4 ppm).

27. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.23/62.1, 2.01/31.9, 0.63/20.4, and 0.81/21.9.

28. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.93/59.1, 4.35/62.9, and 4.13/62.9.

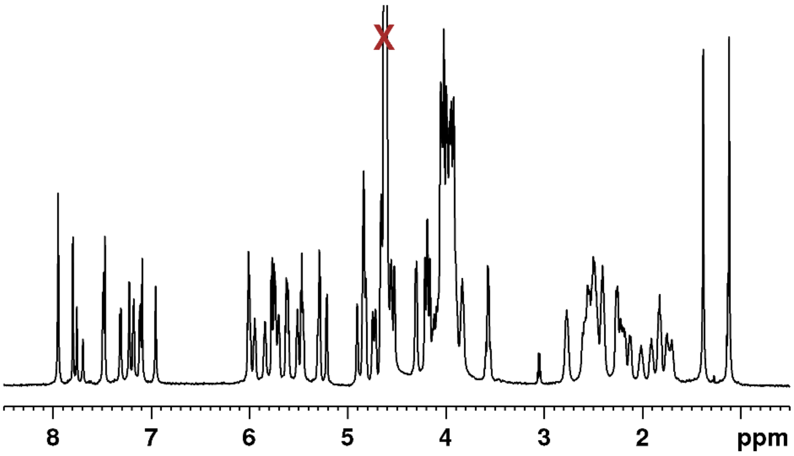
29. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.53/62.1, 5.35/69.9, and 1.23/22.9.

30. Identify amino acid which showed the following ^{1}H/^{13}C chemical shifts (in ppm) in the HCCH-TOCSY spectrum: 4.23/60.1, 3.35/41.9, 3.25/41.9, 2.00/30.7, 1.85/30.7, 1.92/28.4 and 1.63/28.4.



14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 ppm
13.5 13.0 12.5 12.0 11.5 11.0 10.5 ppm

Figure 1:



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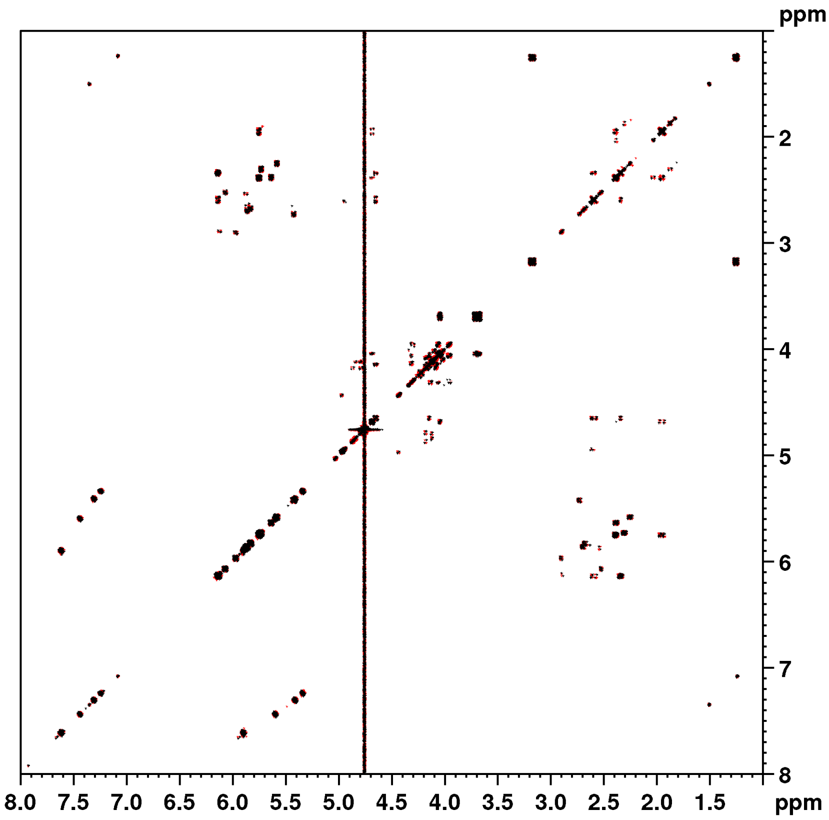

Figure 3:

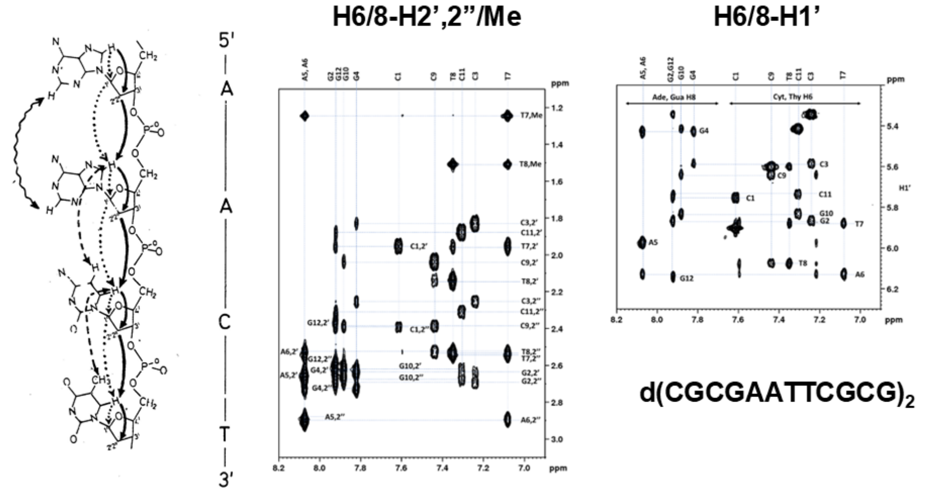

Figure 4: