Practical NMR Spectroscopy of Biomolecules

Protocols from the practical sessions have to be submitted at least 2 days before the examination!

Questions for Examination

A - Theory

1. Requirements for NMR samples – volume, concentration, purity, solvents, buffers, salt, ions, locking and reference substances; types of NMR sample tubes
2. Why is magnet homogeneity important in NMR spectroscopy and how is it achieved? Methods of magnet shimming.
3. What is the purpose of the field/frequency lock and how does it work? How do you set and optimize the lock parameters?
4. Pulse calibration in direct and indirect channels. Use of the prosol table. Recalculation of the power for different pulse lengths and shapes.
5. Sample temperature control and calibration. What are suitable calibration samples? Explain the calibration procedure.
6. Describe the set-up of proton 1D measurement. What parameters should be set? Explain the interdependence among spectral width, number of points and acquisition time (parameters SW, TD, AQ).
7. Describe the set-up of one-dimensional ^{13}C spectrum. What is the purpose of decoupling, how it works and what parameters have to be set?
8. Name the basic water suppression techniques and explain their principles.
9. Acquisition schemes in 2D spectroscopy – States, TPPI, Echo-Antiecho. Explain the purpose and differences.
10. Homonuclear through-bond 2D correlation experiments (COSY, TOCSY). Explain the principles and applications.
11. Homonuclear through-space 2D correlation experiments (NOESY, ROESY). Explain the principle and applicability.
12. Heteronuclear 2D correlation experiments (HMQC, HSQC). Explain the principles, differences and applications.
13. Heteronuclear 2D correlation experiments for isotopically labeled samples. Explain the purpose and principles of sensitivity enhancement by preservation of equivalent pathways and of constant time t1 evolution.
14. Protein NMR spectra. Identify the signal ranges in proton, ^{13}C and ^{15}N dimensions (amide, alpha, beta, sidechain, and carbonyl).
15. Proton NMR spectra of nucleic acids. Identify the regions of methyl, H1', other sugar protons, pyrimidine H5, other base protons, amino, and imino signals.

B – practical measurement

1. Measure proton 1D spectrum of an organic compound (0.1% Ethylbenzene in $CDCl_3$ standard sample). Concentrate on achieving high resolution and sensitivity.
2. Measure ^{13}C spectrum of an organic compound (20% Ethylbenzene in $CDCl_3$ standard sample) with proton decoupling.
3. Measure ^{31}P spectrum with proton decoupling of a DNA sample in phosphate buffer. Verify the ^{31}P 90^0pulse length on the buffer signal.
4. Measure proton 1D spectrum of the standard sucrose sample using presaturation. Evaluate the signal-to-noise ratio and resolution of the resulting spectrum on the signal of anomeric proton (5.4 ppm), use macro suppcal.
5. Measure proton 1D spectrum of a DNA sample in 90% H_2O/10% D_2O. Use a method suitable for detecting imino signals.
6. Set-up the 2D COSY experiment on a sample of DNA in D_2O. Verify the setup by measuring the first increment. Perform the processing, including the phase correction, on a supplied data set measured previously.
7. Set-up the 2D TOCSY experiment on a sample of DNA in D_2O. Verify the setup by measuring the first increment. Perform the processing, including the phase correction, on a supplied data set measured previously.
8. Set-up the 2D NOESY experiment on a sample of DNA in D_2O. Verify the setup by measuring the first increment. Perform the processing, including the phase correction, on a supplied data set measured previously.
9. Measure and process ^{1}H-^{15}N correlation spectrum of the amide region of ^{15}N and ^{13}C labeled Ubiquitin sample.
10. Measure and process ^{1}H-^{13}C correlation spectrum of the aliphatic region of ^{15}N and ^{13}C labeled Ubiquitin sample.