C7995 Practical NMR Spectroscopy of Biomolecules

The following topics will be covered

1. NMR sample, preparation and handling
    a. Requirements for NMR samples – volume, concentration, purity, solvents, buffers, salt, ions, locking and reference substances
    b. NMR sample tubes – types, handling, cleaning
    c. Effects of temperature and pH

2. Spectrometer setup
    a. Probe tuning, automatic, manual
    b. Locking, optimizing lock parameters
    c. Magnet shimming – manual, auto (simplex, gradient)
    d. Temperature control, gas flow, temperature calibration

3. Spectrometer calibrations
    a. Proton pulse calibration using manual and automatic procedures
    b. Pulse calibration in indirect channels (^{13}C, ^{15}N, ^{31}P)
    c. Pulse power handling and calculation for rectangular and shaped pulses, using ShapeTool

4. 1D proton spectroscopy and water suppression techniques
    a. Proton spectroscopy
    b. Presaturation
    c. Selective excitation including WATERGATE
    d. Gradient water suppression

5. 1D NMR spectroscopy of nuclei other than H-1
    a. Properties of common nuclei (19F, 13C, 15N, 31P)
    b. Choice of probes
    c. Power settings and calibrations

6. Homonuclear 2D experiments
    a. Through-bond correlation experiments (COSY, TOCSY)
    b. Through space correlation (NOESY, ROESY)

7. Heteronuclear experiments for natural abundance and isotopically labeled samples
    a. Checking protein and nucleic acid samples using ^{1}H-^{15}N HSQC experiment; gradient sensitivity enhanced and TROSY versions of the experiment
    b. ^{1}H-^{13}C correlation experiment for ^{13}C-labeled compounds – constant time HSQC experiment.

8. Data processing, apodization functions, linear prediction, non-uniform sampling
    a. Apodization functions
    b. Baseline/ base plane correction and residual solvent signal subtraction
    c. Linear prediction

d. Special requirements for processing of non-uniformly sampled spectra

9. Analysis of protein NMR spectra
    a. Orientation in 1H-15N correlation spectra – identification of amide and sidechain signals
    b. Orientation in 1H-13C correlation spectra – identification of alpha, beta and sidechain signals. Chemical shifts of carbonyls.

10. Analysis of NMR spectra of nucleic acids
    a. Identification of sugar, base, amino and imino resonances in 1D spectra
    b. Identification of through-bond correlations in COSY and TOCSY spectra
    c. Identification of through-space correlations and exchange peaks in NOESY and ROESY spectra

Literature:

Roberts, G. C. K.: NMR of Macromolecules, A Practical Approach, IRL Press 1993.
Berger, S., Braun, S., Kalinowski H. O.: 200 and More NMR Experiments: A Practical Course, Wiley, 2004.
Teng, Q. and Lee, W. P.: Handbook of Structural Biology: Practical NMR Applications, Springer, 2005.

Additional Reading:

Cavanagh, J., Fairbrother, W. J., Palmer, A. G., Skelton, N. J.: Protein NMR Spectroscopy, Principles and Practice, Harcourt Brace & Company 1995.
Crosmut, W. R., Carlson, R. M. K.: Two-Dimensional NMR Spectroscopy, Applications for Chemists and Biochemists, Wiley 1987.
van de Ven, F. J. M.: Multidimensional NMR in Liquids: Basic Principles and Experimental Methods, Wiley 1995.