C – 7995 Practicals 1 – preparation for measurement

A) Shimming

Use sample: chloroform in acetone 1%

For NMR700m, you can use parameter set: exp. 101 in directory /d1/fiala/nmr/lineshape/

Shim the sample as best as you can by combination of methods; verify the result by measuring a spectrum and evaluating it by humpcal command.

Note: 5 mm probe specification is 10/20 Hz for non-rotating sample.

B) Pulse calibration

Use sample: doped water

Use parameter set: zg

Calibrate 90^{o} on ^{1}H manually (command paropt) and automatically (pulsecal)

Compare the values obtained at 180^{o}, 360^{o}, and by pulsecal

C) Temperature calibration

Use sample: methanol 4%

Use parameter set: zg

Calibrate the temperature at 25^{o}C and 10^{o}C, and calculate the correction parameters for the VT unit. Use calctemp command or NMR-TempCal.xls spreadsheet to translate the chemical shift difference into temperature.

Use the following procedure: set the temperature, wait till the temperature stabilizes, measure the spectrum and evaluate the temperature, wait for 5 min., measure and evaluate again, if the temperature difference is more than 0.1 K, wait another 5 min., repeat the measurement . . .

C7995 Practicals 2 – 1D NMR spectroscopy

1. NMR spectra in organic solvent

Measure proton spectrum of a simple compound in organic solvent

Use sample: 0.1% Ethylbenzene in $CDCl_3$ (Bruker standard test sample)

Pulse sequence: zg

Parameters: standard parameters – PROTON, change the pulse sequence to zg, adjust spectral width according to the needs, optimize 90^o pulse

^{13}C spectrum

Use sample: 20% Ethylbenzene in $CDCl_3$ (Bruker standard test sample for ^{13}C)

Pulse sequence: zgpg

Parameters: standard parameters – C13CPD, change the pulse program to zgpg, adjust spectral width according to the needs, use prosol pulse length for ^{13}C pulse and the proton pulse calibration from the previous step. Measure the spectrum with and without proton decoupling, compare the results. Note the splitting of the $CDCl_3$ signal (around 77 ppm) by deuterium.

2. NMR spectra in water solution

Measure proton spectrum of DNA dissolved in water using solvent suppression techniques

Use sample: Dickerson-Drew DNA dodecamer $d(CGCGAATTCGCG)_2$ in 90% H_2O/10% D_2O at 25^{o}C

Pulse sequences: zgpr, p3919gp and zggpwg

Parameters: standard parameters (ZGPR, P3919PG, ZGGPWG), adjust the spectral width (imino protons appear between 12 and 14 ppm, adjust sw and parameter d19 in p3919gp accordingly!), calibrate proton 90^o pulse using pulsecal.

Measure the spectrum using presaturation and WATERGATE techniques, compare the intensities of the imino signals.

On the same sample of DNA, measure ^{31}P spectrum

Pulse sequence: zgdc

Parameters: standard parameters – P31CPD, adjust spectral width (phosphate signals appear close to 0 ppm). Use ^{31}P pulse length from prosol and proton pulse length based on the calibration in the previous step. Verify the ^{31}P 90^{o}pulse length on the buffer signal.

C7995 Practicals 3 – 2D homonuclear spectroscopy

A) Through-bond correlation experiments

Measure COSY and TOCSY spectra of DNA in D_2O at 25^oC with presaturation of the residual water signal. Before the 2D, record also 1D spectrum.

Use pulse sequences: zgpr, cosyphpr, dipsi2phpr.

Based on the 1D spectrum, set a suitable spectral width (sw).

Measure the COSY spectrum with 16 scans and 1200 increments.

Measure the TOCSY spectrum with 50 ms mixing time, 8 scans and 600 increments.

Do not forget to scale down the presaturation power by 20 dB compared to water spectra.

B) 2D NOE spectra

Use sample: DNA in 90% H_2O/10% D_2O at 10^oC

Measure 2D NOESY spectrum of the sample above using WATERGATE solvent suppression (pulse sequences noesyfpgpphwg or noesyfpgpph19). To find a suitable spectral width check the spectrum measured in Practicals 2. Measure the spectrum with 200 ms mixing time, 16 scans and 1024 increments. When optimizing the water suppression measure the first increment with- and without the flip-back pulse and compare the effect on the imino signals.

Practical 4 – 2D heteronuclear spectroscopy of DNA and isotopically labeled protein sample

Samples:

Ubiquitin, ^{15}N and ^{13}C labeled, in 90%H_2O/10% D_2O

Dickerson-Drew DNA dodecamer $d(CGCGAATTCGCG)_2$ at natural isotopic abundance in D_2O

A) ^{1}H-^{15}N correlation

Measure **^{1}H-^{15}N HSQC spectra** using HSQC (pulse sequence sfhsqcf3gpph) and gradient sensitivity enhanced HSQC (hsqcetf3gpsi2) on the Ubiquitin sample. In the first case, use the spectral width sufficient to include signals from Arginine sidechains. In the second spectrum, set the spectral width for the amide region only. In the resulting spectrum, indicate the folded peaks from the Arginine sidechains. In both cases, use a refocusing carbon pulse to eliminate the effect of carbon coupling in the indirect dimension (flag LABEL_CN).

For the protein chemical shifts, refer to the attached table or go to BMRB database

http://www.bmrb.wisc.edu/ref_info/

B) ^{1}H-^{13}C correlation

On the Ubiquitin sample, measure **^{1}H-^{13}C HSQC spectra** of the aliphatic region (5 ppm to 75 ppm in the carbon dimension) using standard (e.g. hsqcetgp, hsqcetgpsi) and constant time pulse sequences optimized for proteins that allow selective refocusing of the carbonyl (e.g. hsqcctetgpsp, hsqcctetgpsisp). Run enough increments to see the carbon-carbon splitting in the standard experiment. In both cases, use a refocusing nitrogen pulse to eliminate the effect of nitrogen coupling in the indirect dimension (flag LABEL_CN).

C) ^{1}H-^{31}P correlation in DNA

Measure **^{1}H-^{31}P correlation spectrum** of 3', 4', and 5',5'' protons with the phosphate backbone in the DNA sample. Use the pulse sequence hp_rf. Refer to the proton and phosphorus 1D spectra measured previously for the spectral widths and pulse lengths. As there is no signal in the first increment in this type of experiment, check with d0 set to 50 ms before starting the measurement (pulse sequence hp_rf_tst).