C7995 Advanced Methods of Biomolecular Chemistry
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Task 1 – Preparation for Measurement

TASK 1A: Shimming

Chloroform in acetone (1%) was used as a sample with parameter set ***lineshape***. Using command ***lock*** **we took** choice acetone. Signal was tunned automatically with command ***atma*** as well as manually with command ***atmm manwbsw***. Automatic command ***topshim*** was used for shimming in z – direction. Manual shimming (in x, y, xy, xz, yz, …) was performed until lock signal was stabilized. Command ***loopadj*** was used for optimalization of lock phase and lock gain. Resulting peak was evaluated using command ***humpcal***. Results are shown in Table 1.

	Before shimming	**After shimming**
0.11 %	19.8 Hz	13.3 Hz
0.55 %	16.3 Hz	6.4 Hz
50 % half width	3.16 Hz	0.64 Hz

Tab. 2: *Results from manual shimming procedure.*

TASK 1B: Pulse Calibration

For this task, a dopped water was used as a sample and ***zg*** as a parameter set**.** Using command ***lock*** **we used choice** #### Spectrum was measured immediately after shimming and wobbling procedures. Using command ***paropt***, intensity of acquired peaks was modulated by sinus function. Phase 360° was observed in time 34,4 μs, length of 90° pulse is calculated as follow: 34,4 μs/4 = 8,6 μs. (Fig. 1.) Using command ***pulsecal***, which calculates the 90° pulse automatically, we obtained value 8,3 μs.

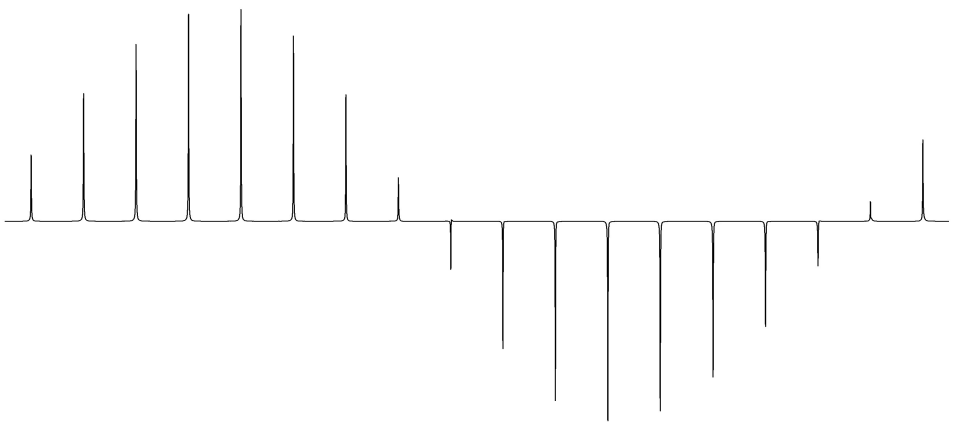

Fig. 1: *Set of peaks modulated by sinus function obtained from command* ***paropt****.*

TASK 1C: Temperature Calibration

For last task, a 4% methanol was used as a sample. ***zg*** was used as a parameter set**.** First, we lock the signal (form the lock table **we choose** solvent ###) and we also provided wobbling and shimming procedures using commands ***atmm manwbsw*** and ***topshim***, respectively.

Methanol gives 2 signals – one from methyl group with chemical shift δ_1 and one from hydroxyl group with chemical shift δ_2. Exact peak position is given by command ***pp***. The difference in position of peaks is a temperature function. This function allows temperature calibration.

First, we used NMR-TempCal.xls, an equation in Excel's table. With help of this spreadsheet, we were able to translate chemical shift difference into temperature. Another way is to use command ***calctemp***. Results are shown in Table 2. Tha aim of this task was to calibrate the temperature at 25 °C (298,15 K) and 10 °C (283,15 K). However, we couldn't set temperature to 10 °C due to the technical reasons. Therefore we used temperature 20 °C (293,15 K).

T [°C] / [K]	δ_1 [ppm]	δ_2 [ppm]	$T_{NMR\text{-}Temp.calc.xls}$ [K]	$T_{calctemo}$ [K]
20 / 293,15	3,3676	4,9217	293,39	294,87
25 / 298,15	3,3686	4,8729	299,11	301,24

Tab. 2: *Results from temperature calibration using command* ***calctemp*** *and spreadsheet NMR-TempCalc.xsl.*

SUMMARY:

Optimization and calibration methods were performed. We met several technical problems resulting in not perfect optimization of shimming and temperature calibration.

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TASK 2 – 1D spectroscopy in water

TASK 2A: Solvent suppression test

The very high concentration of water compared to the very low concentration of biomolecules necessitates the use of solvent suppression methods. Solvent suppression techniques are very efficient techniques used to suppress strong water signals from proton. 2 mM sucrose in water (90 % H_2O , 10 % D_2O) was used for measurement. Shimming was provided carefully using automatic command ***topshim 3D*** and manual approach. Water signal was suppressed using presaturation and WATERGATE with parameter set ***zgpr*** and ***p3919gp***, respectively (fig. 1 and 2).

The dublet of anomeric proton is used for shimming quality evaluation. The dublet is more separated for better shimming, the shimming quality is defined as ratio of the least intensity in dublet to maximal intensity in dublet. Using macro ***suppcal*** we obtained result with value 0.27 (27 %). Signal to noise ratio (SINO) was 293.0.

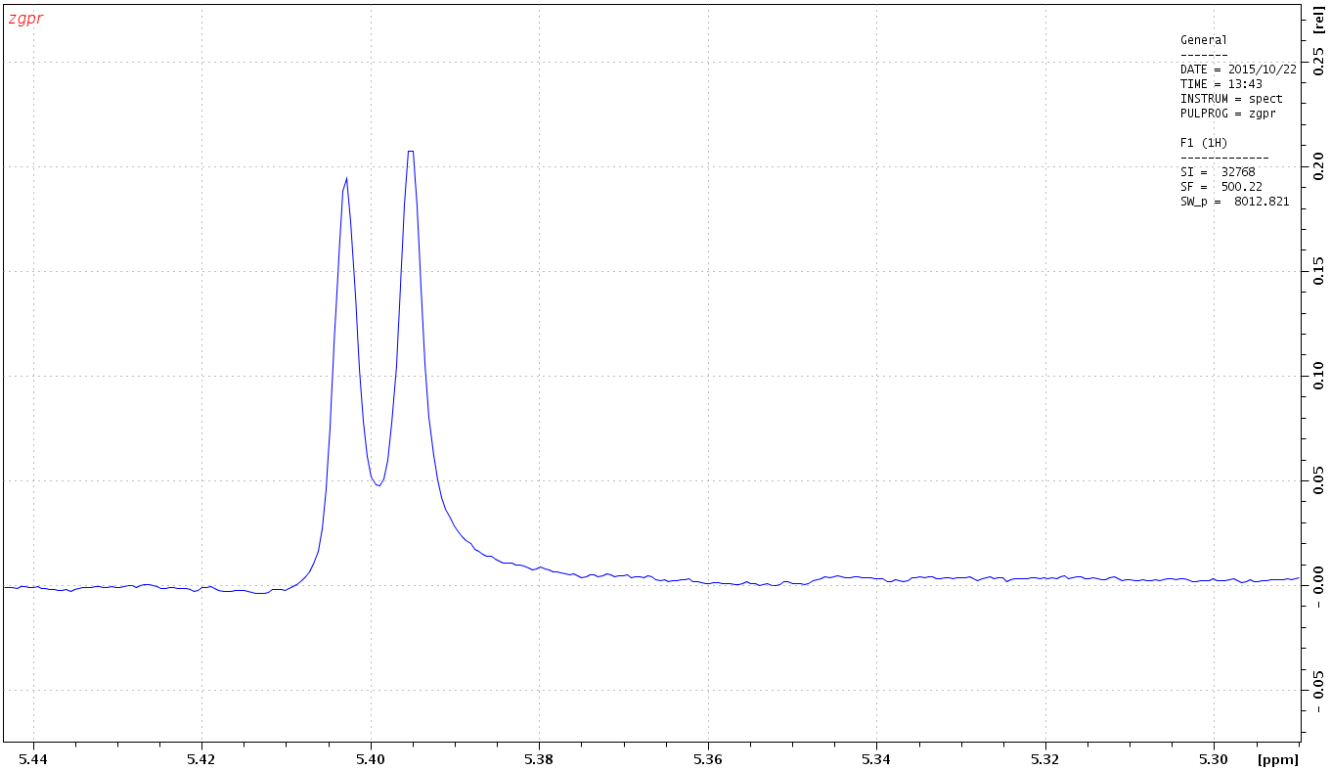

Figure 1: *Detail for anomeric proton from 1D spectrum of 2 mM sucrose using presaturation, parameter set: zgpr, 25 °C.*

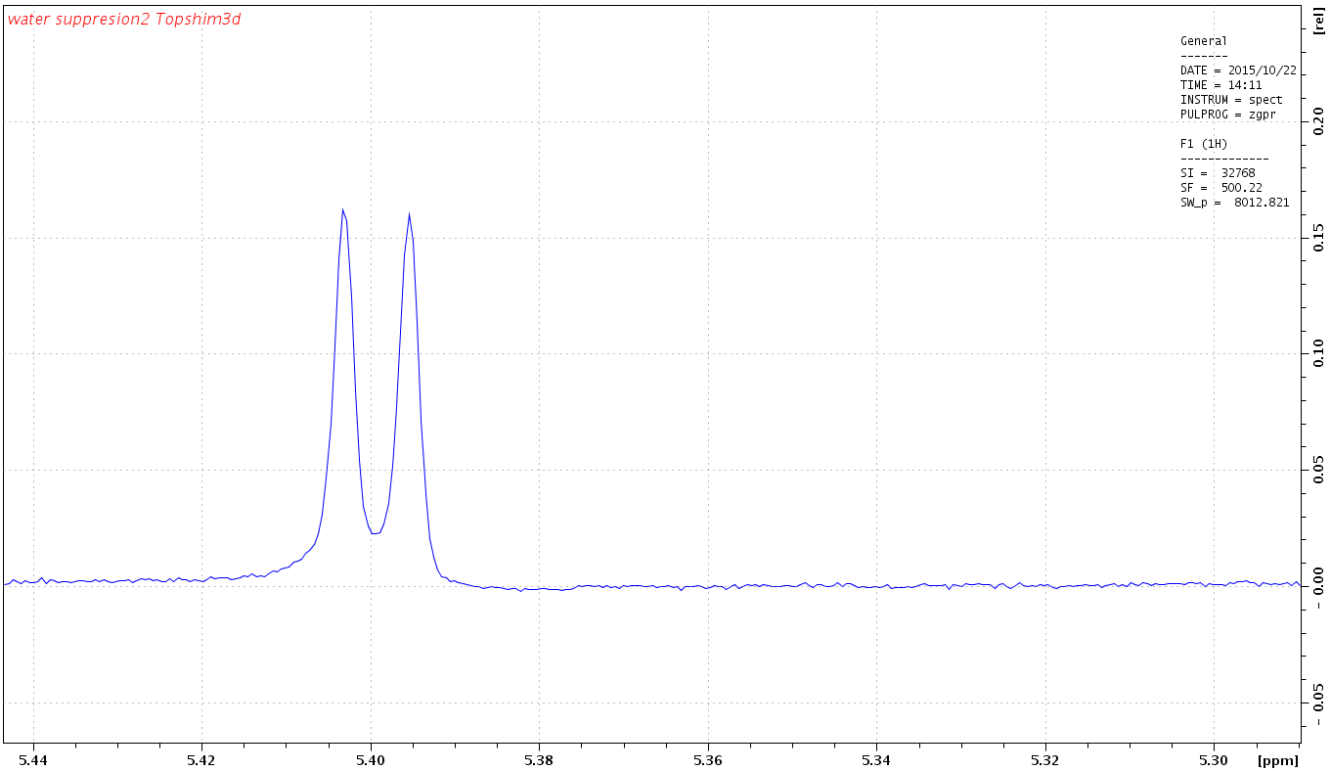

Figure 2: *Detail for anomeric proton from 1D spectrum of 2 mM sucrose using WATERGATE, parameter set: p3919gp, 25 °C.*

TASK 2B: Proton 1D spectra in water

Different approaches for water suppression were measured also with dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA). In case of presaturation (pulse sequence ***zgpr***), water frequence is irradiated by a long low power pulse. In addition to removing of water signal, exchangeable protons are also eliminated. WATERGATE is based on the gradient spin echo technique. We used two pulse sequences for WATERGATE ***zgpwg*** and ***p3913gp***.

Resulted spectra are shown in Fig. 3, detail for imino region is shown in Fig. 4. Both WATERGATEs, ***zgpwg*** and ***p3913gp***, achieve similar results and higher intensities in imino region compared to presaturation.

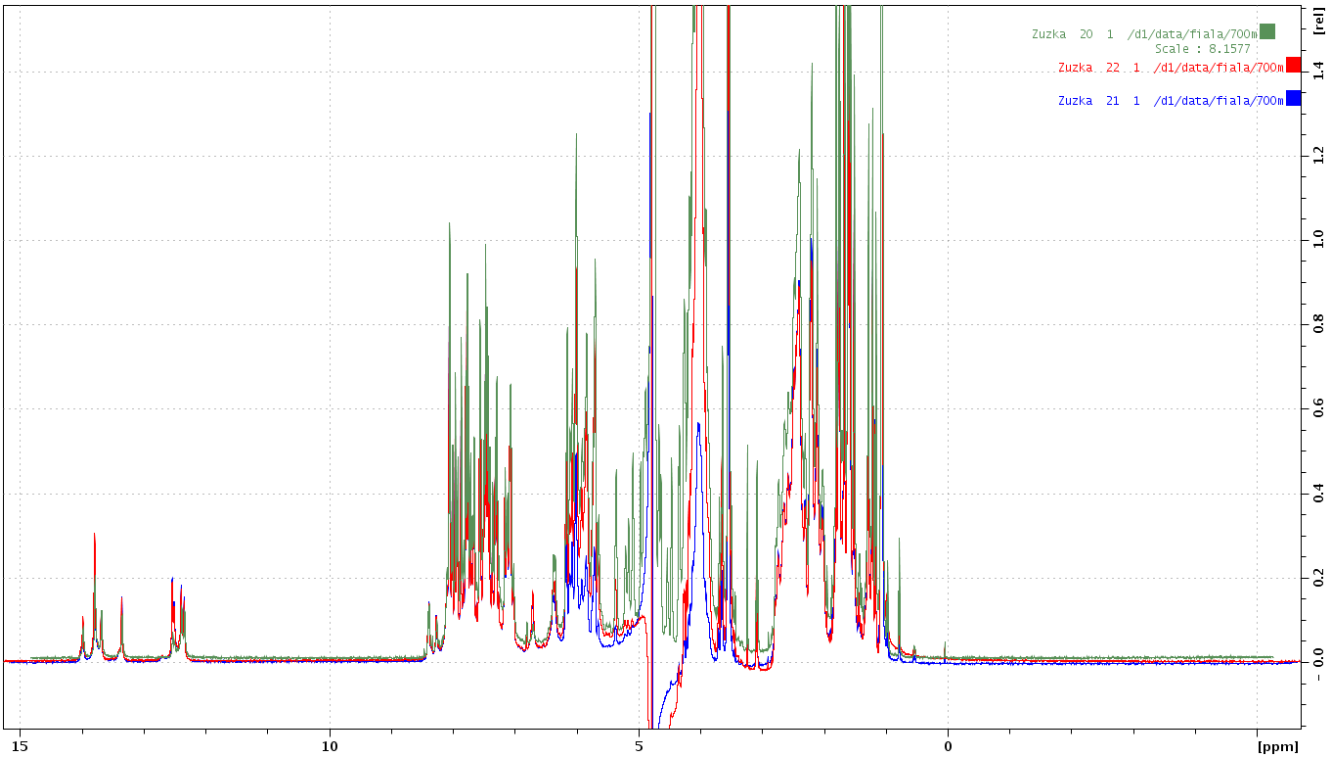

Figure 3: *Water supression using presaturation* ***zgpr*** *(green), WATERGATE* ***p3919gp*** *(blue) and flip - back* ***zggpwg*** *(red) . Sample: dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA), 10 °C.*

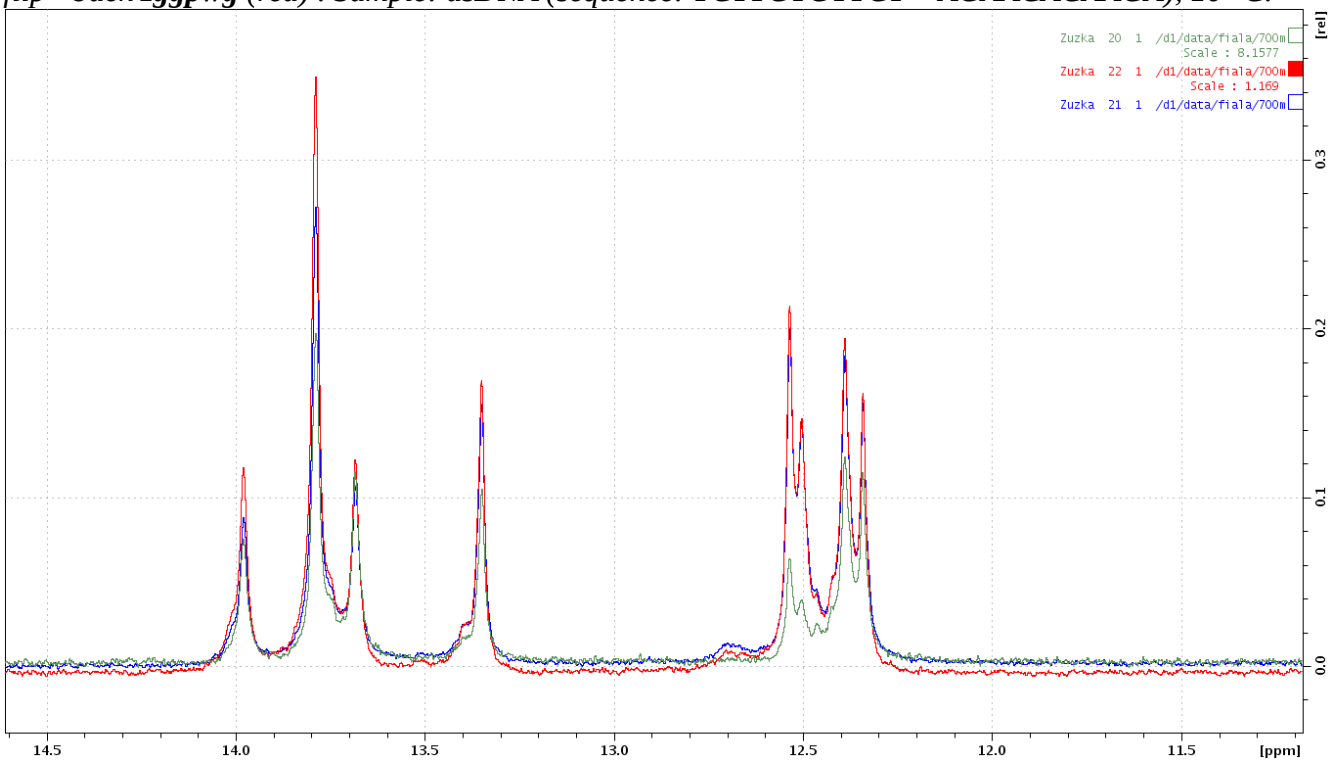

Figure 4: *Detail for imino region. Water supression using presaturation* ***zgpr*** *(green), WATERGATE* ***p3919gp*** *(blue) and standart W* ***zggpwg*** *(red). Sample: dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA), 10 °C.*

TASK 3 – 2D Homonuclear Spectroscopy

TASK 3A: Through – bond correlation experiments

For this experiment a dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA) in D_2O was used as a sample. Spactra were measured at 25 °C. For 1D spectrum was used ***zgpr*** pulse sequence with spectral width of 9.9925 ppm (*Fig. 1*).

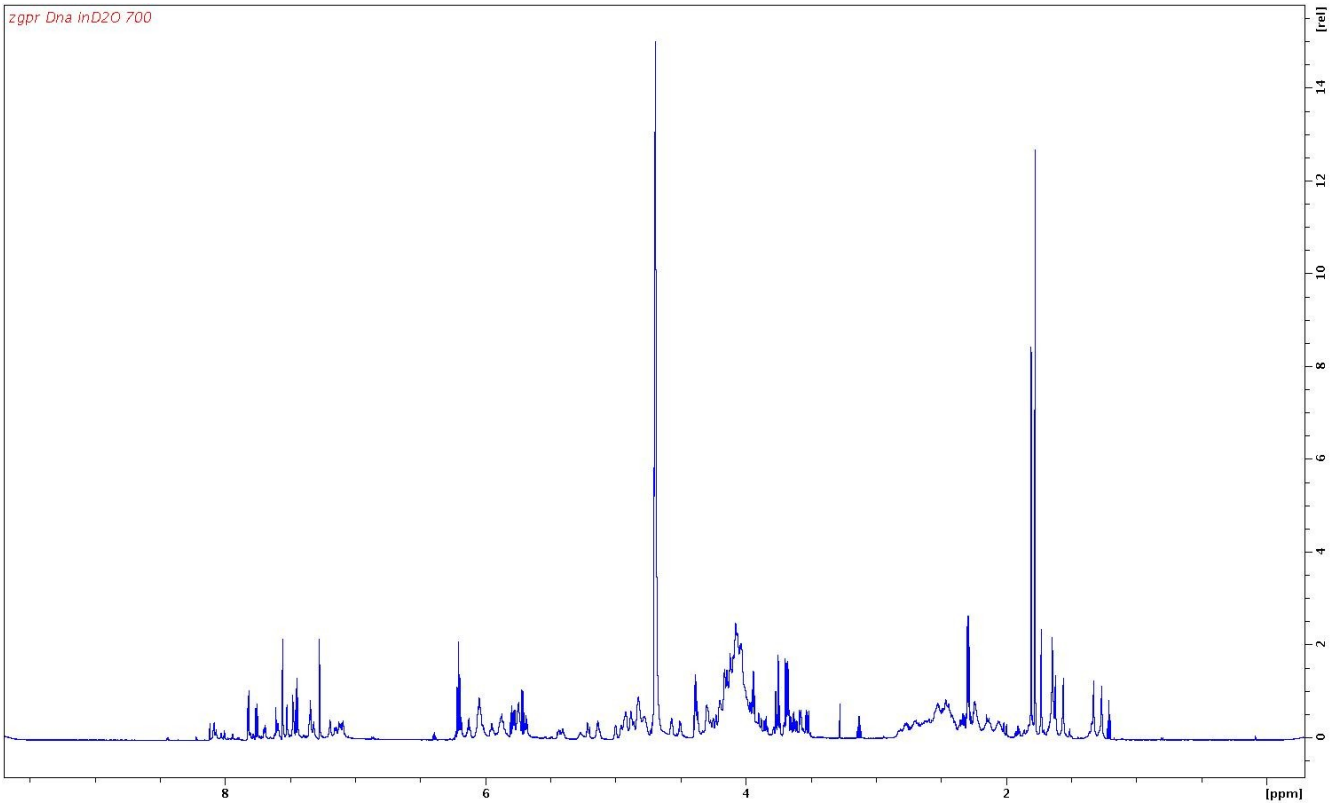

Figure 1: *1D spectrum for dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA). 25 °C.*

Pulse sequence ***cosyphr*** was used for COSY measurement. Acqu- and Proc-parameters are shown in *Tab. 1*. Obtained spectrum is shown in *Fig. 2*.

AcqusParameters		
Nucleus	1H	1H
Dimension	direct	indirect
Number of real points	2048	1600
Spectral width [ppm]	9.0084	9.0084
Observed frequency [MHz]	700.80329	700.80329
Carrier shift [ppm]	4.701	4.701
ProcParameters		
Nucleus	1H	H
Dimension	direct	indirect
Size of real spectrum	4096	4096
Spectrometer frequency [MHz]	700.8	700.8
Window function	SINE	SINE

Table 1: *Selected acquisition and processing parameters for COSY spectrum.*

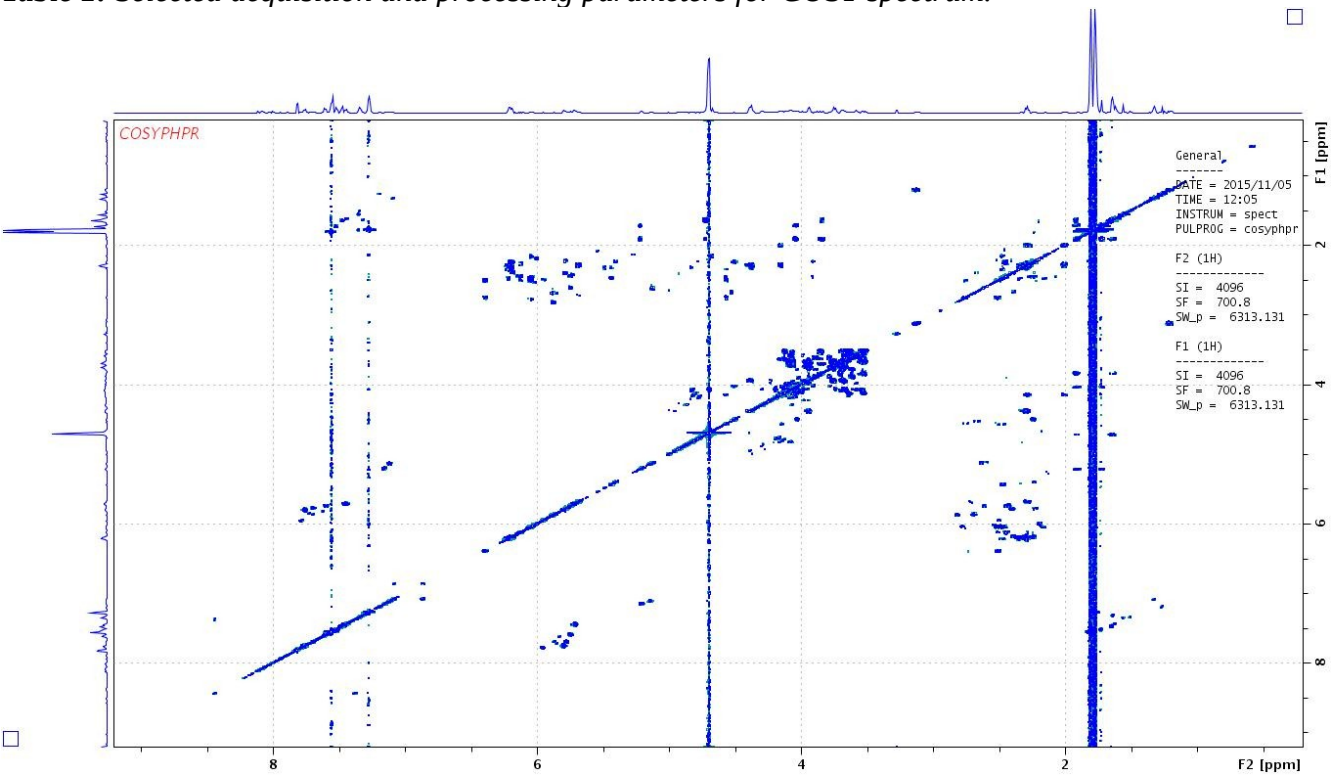

Figure 2: *COSY spectrum for dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA).*

TOCSY spectrum was measured using pulse sequence ***dipsi2phr***. Acquisition and processing parameters are shown in *Tab. 2*. Obtained spectrum is shown in *Fig. 3*.

AcqusParameters		
Nucleus	1H	1H
Dimension	direct	indirect
Number of real points	2048	800
Spectral width [ppm]	9.0084	9.0084
Observed frequency [MHz]	700.80329	700.80329
Carrier shift [ppm]	4.701	4.701
ProcParameters		
Nucleus	1H	H
Dimension	direct	indirect
Size of real spectrum	1024	1024
Spectrometer frequency [MHz]	700.8	700.8
Window function	QSINE	QSINE

Table 2: *Selected acquisition and processing parameters for COSY spectrum.*

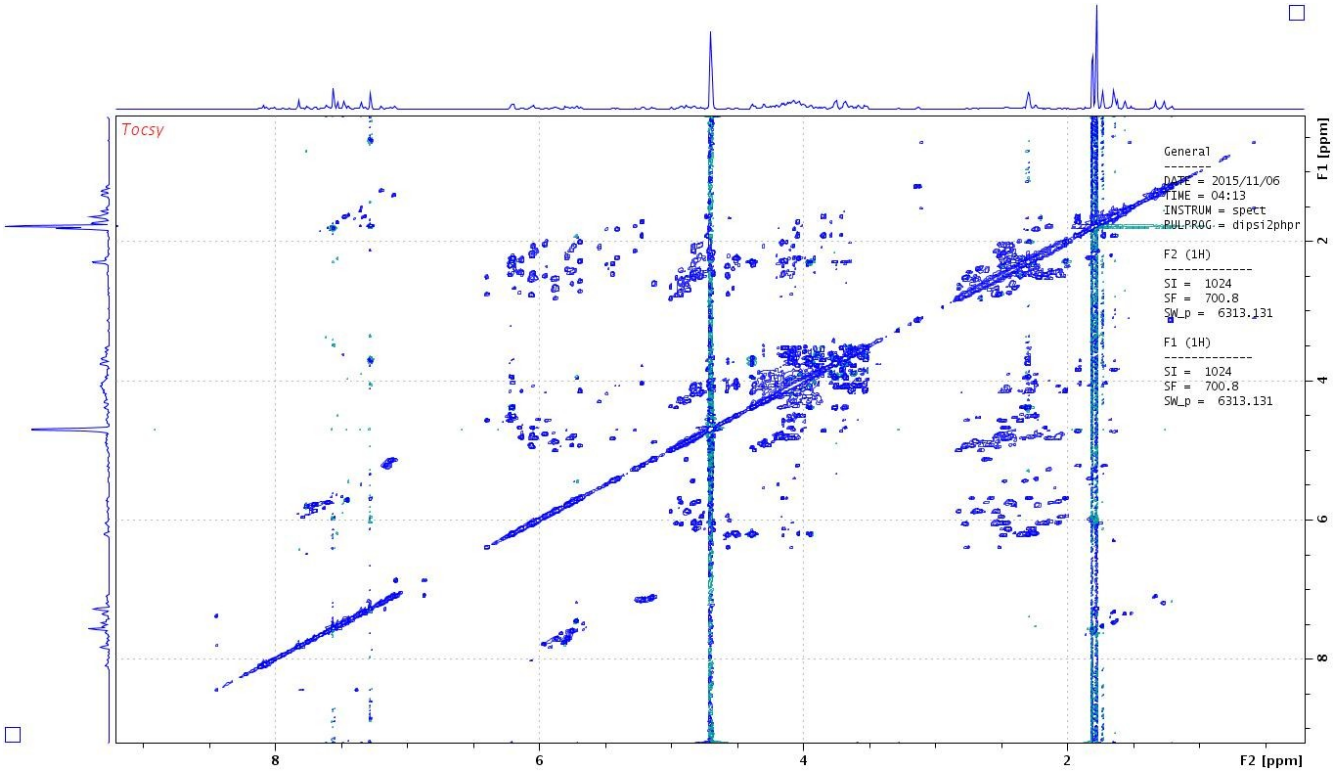

Figure 3: *TOCSY spectrum for dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA). 25 °C.*

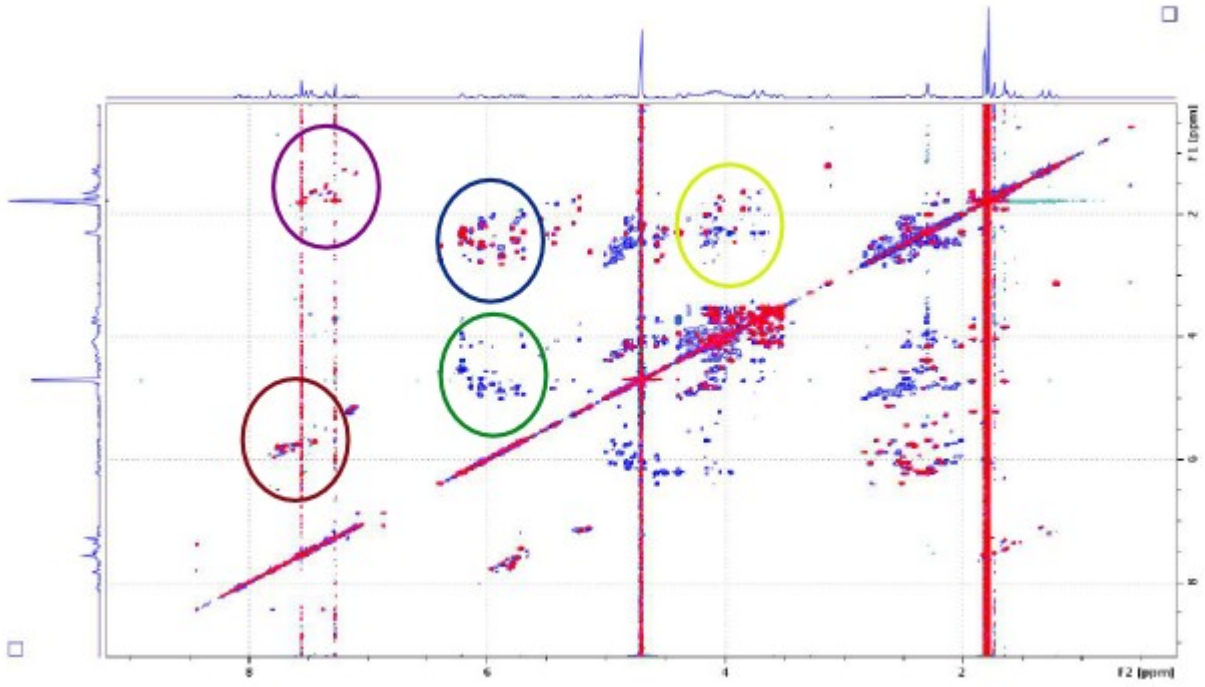

Figure 4: *Comparison of COSY (shown in red) and TOCSY (shown in blue) spectra for dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA). Highlighted regions: A) base-to-base - CH_3-H6 from Thymine (violet), H5-H6 from Cytosine (red), B) sugar-to-sugar H1'-H2',H2'' and H2',H2''-H3' from sugar (blue), H3'-H4' from sugar (yellow). 25 °C.*

TASK 3B: 2D NOE Spectra

Pulse sequence ***noesyhsqcetgpsi3d*** *was used for measurement of through-space correlation.*

AcqusParameters			
Nucleus	1H	13C	1H
Dimension	direct	indirect	indirect
Number of real points	2048	64	1
Spectral width [ppm]	13.9994	75	13.9994
Observed frequency [MHz]	500.22235	125.785324	500.22235
Carrier shift [ppm]	4.706	39	4.706

ProcParameters		
Nucleus	1H	13C
Dimension	direct	indirect
Size of real spectrum	2048	128
Spectrometer frequency [MHz]	500.22	125.780419
Window function	QSINE	QSINE

Table 3: *Selected acquisition and processing parameters for NOESY spectrum.*

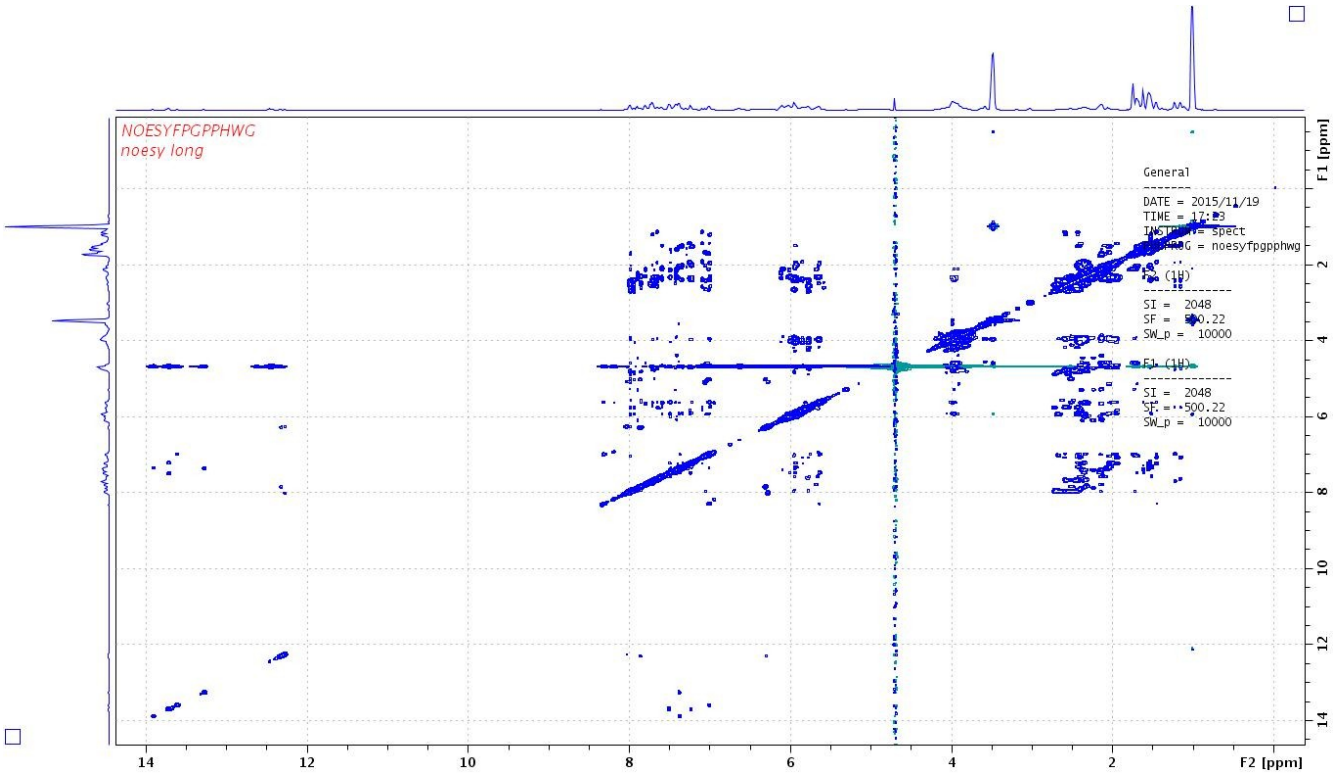

Figure 5: *NOESY spectrum of dsDNA (sequence: TCTTGTGTTCT * AGAACACAAGA). 10 °C.*

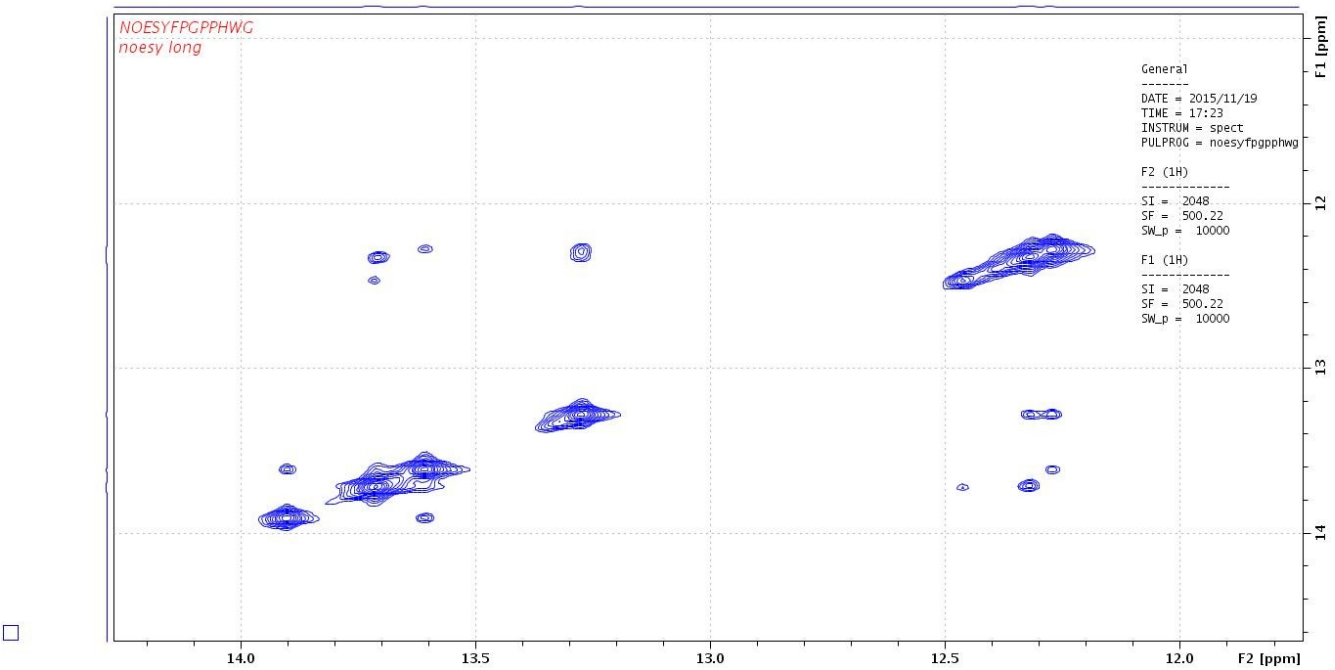

Figure 6: *Detail for imino - imino region in NOESY spectrum of measured dsDNA. 10 °C.*

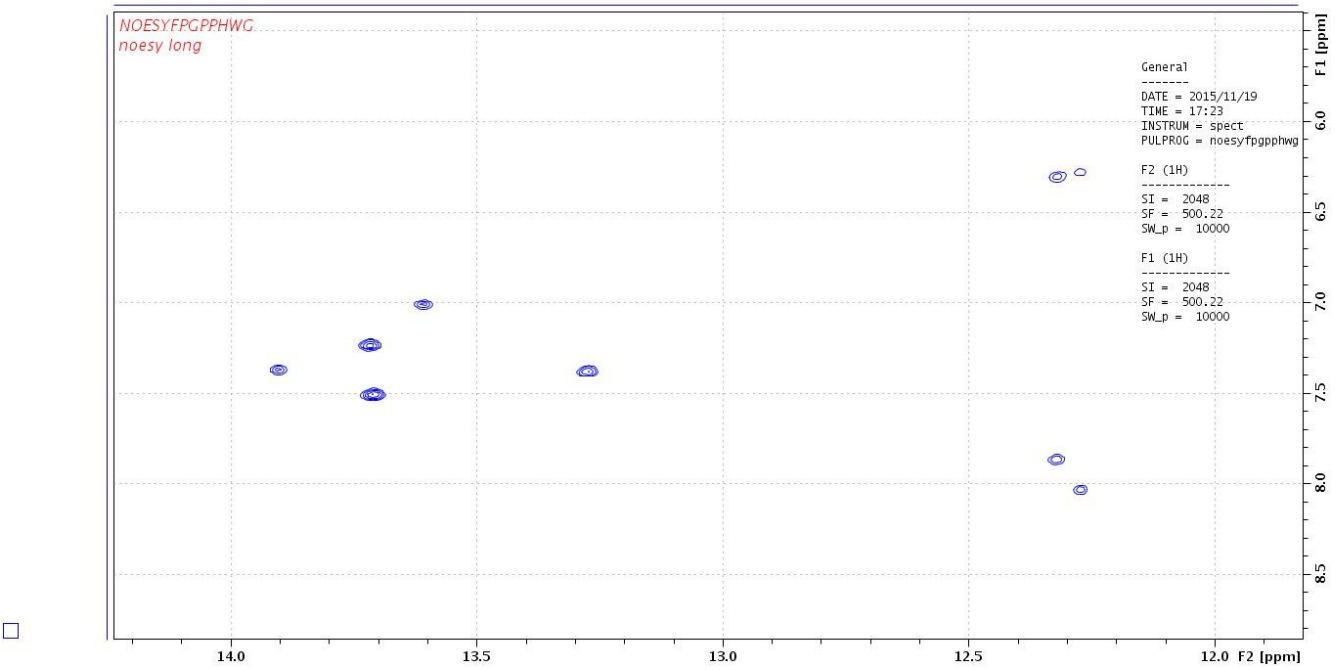

Figure 7: *Detail for imino - amino region in NOESY spectrum of measured dsDNA. 10 °C.*

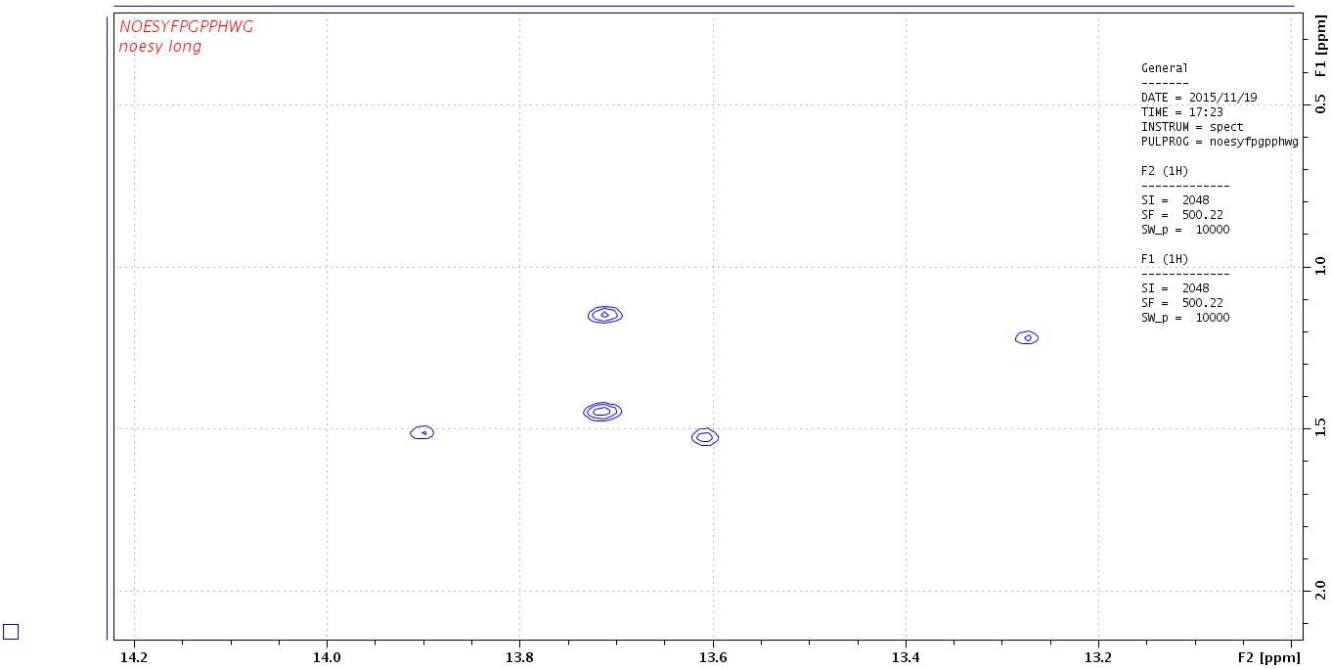

Figure 8: *Detail for imino - methyl group region in NOESY spectrum of measured dsDNA. 10 °C.*

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TASK 4 – 2D heteronuclear spectroscopy of isotopically labeled protein sample

TASK 4A: ^{1}H - ^{15}N correlation

Heterouclear Single Quantum Coherence, where ^{1}H and ^{15}N atoms are correlated, was measuerd using pulsesequence ***hsqctfpf3gp***. Spectrum was measured two times: 1) wider spectral witdh, which includes also signal from Arginines (spectral width in 15N dimension: 31.999 ppm). However, arginines are not visible in obtained spectrum (Fig. 1) and 2) spectral width, which is sufficient for amide signals only (spectral width in 15N dimension: 70.0009 ppm, Fig. 2). Selected acquision and processing parameters are listed in Tab. 1.

AcqusParameters		
Nucleus	1H	15N
Dimension	direct	indirect
Number of real points	2048	256
Spectral width [ppm]	16,0185	31,9999
Observed frequency [MHz]	500,222351	50,692833
Carrier shift [ppm]	4,7	118
ProcParameters		
Nucleus	1H	15N
Dimension	direct	indirect
Size of real spectrum	2048	2048
Spectrometer frequency [MHz]	500,22	50,6868524
Window function	QSINE	QSINE

Table 1: *Selected acquisition and processing parameters for* ***hsqctfpf3gp*** *experiment.*

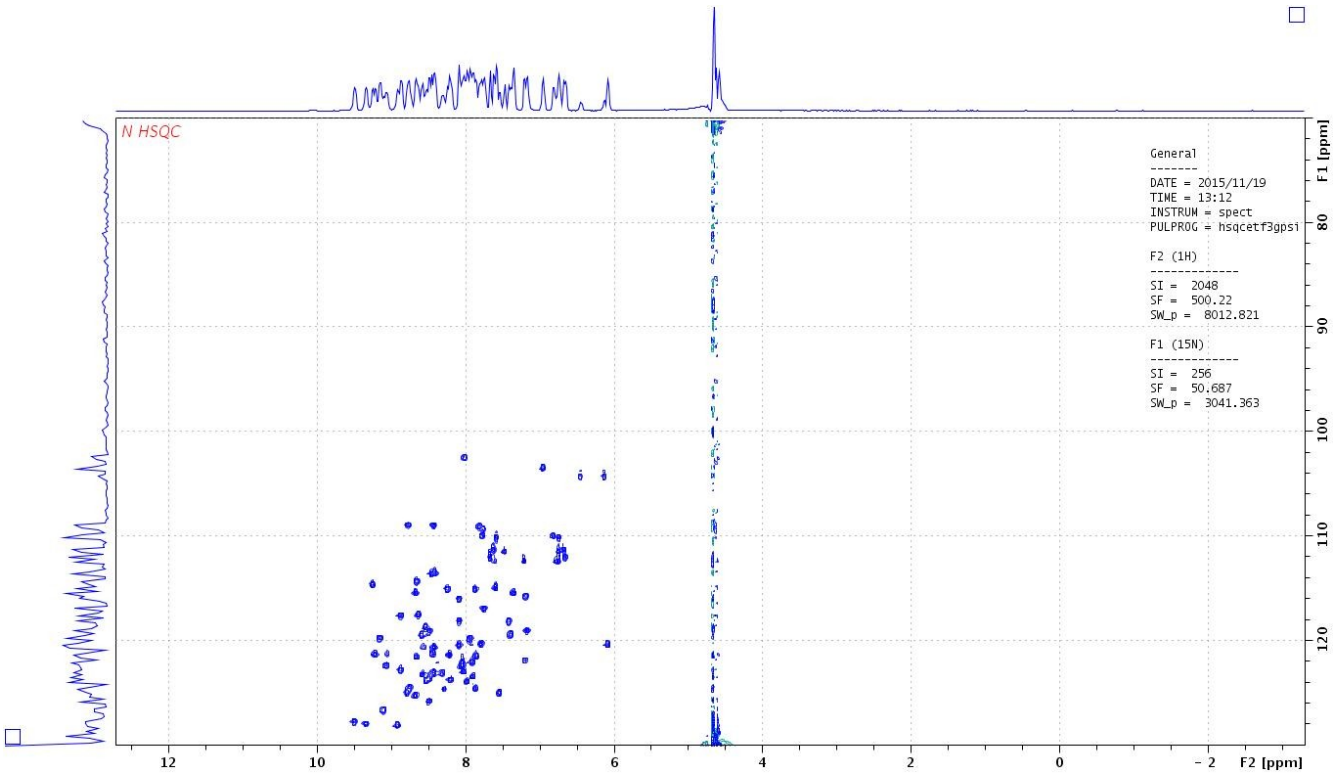

Figure 1: *^{1}H-^{15}N HSQC for ^{15}N^{13}C labeled ubiquitin with wider spectral width. Signal for arginine's sidechains are not visible. 25 °C.*

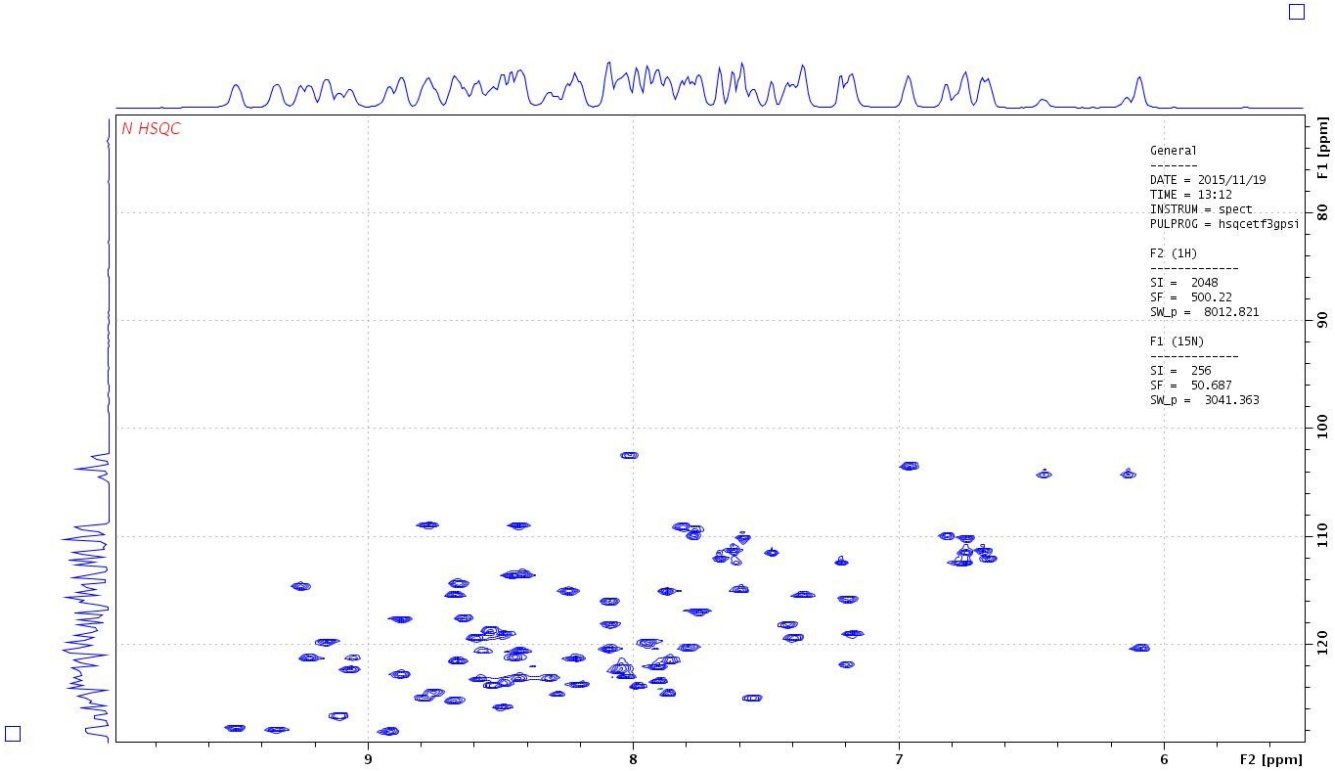

Figure 2: *^{1}H-^{15}N HSQC of ^{15}N^{13}C labeled ubiquitin for amide region. 25 °C.*

TASK 4B: ^{1}H - ^{13}C correlation

hsqceptg was used as a pulse sequence for Heterouclear Single Quantum Coherence with ^{1}H - ^{13}C correlation. Selected acquision and processing perameters are listed in Tab. 2, obtained spectrum is shown in Fig. 2.

AcqusParameters		
Nucleus	1H	13C
Dimension	direct	indirect
Number of real points	1024	256
Spectral width [ppm]	13,015	79,9995
Observed frequency [MHz]	500,222351	125,7854502
Carrier shift [ppm]	4,7	40
ProcParameters		
Nucleus	1H	13C
Dimension	direct	indirect
Size of real spectrum	1024	1024
Spectrometer frequency [MHz]	500,22	125,780419
Window function	QSINE	QSINE

Table 2: *Selected acquisition and processing parameters for* ^{1}H - ^{13}C *HSQC spectrum.*

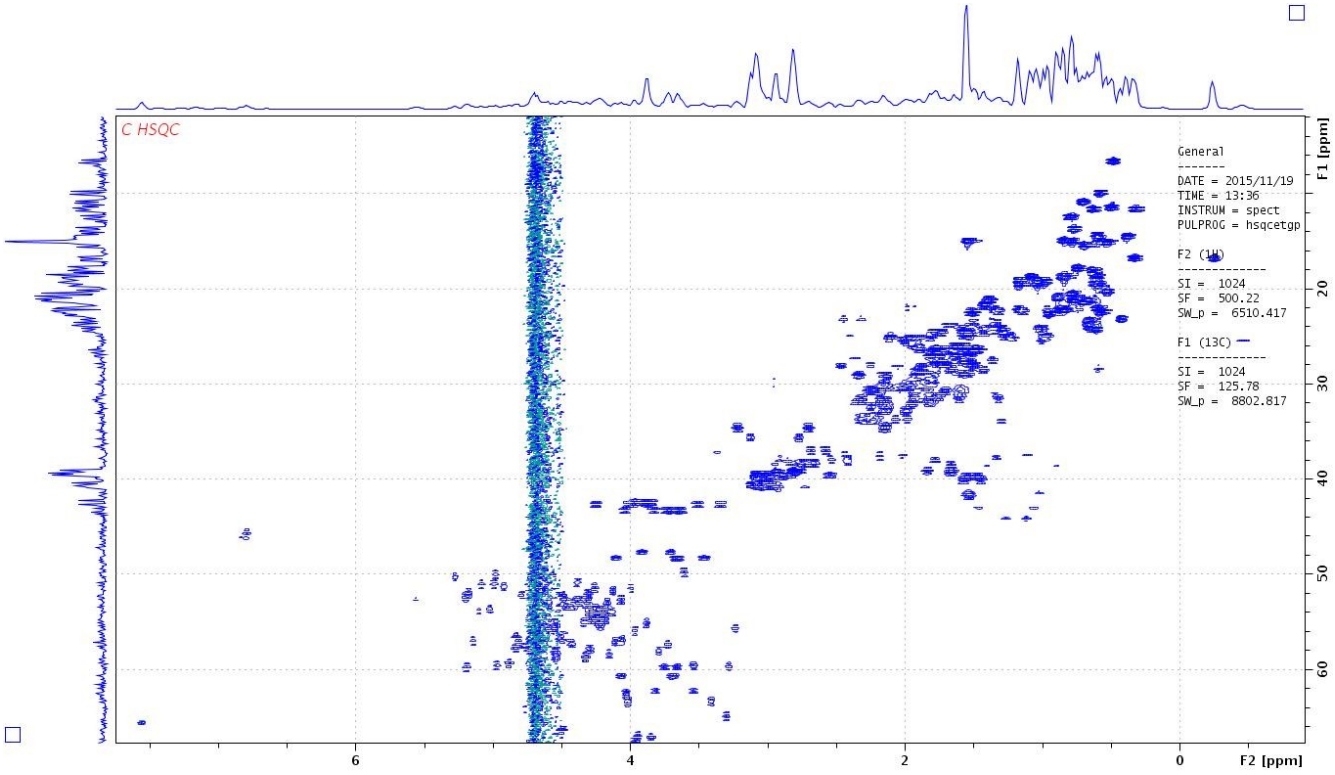

Figure 3: ^{1}H - ^{13}C *HSQC spectrum for double labeled ubiquitin. 25 °C*

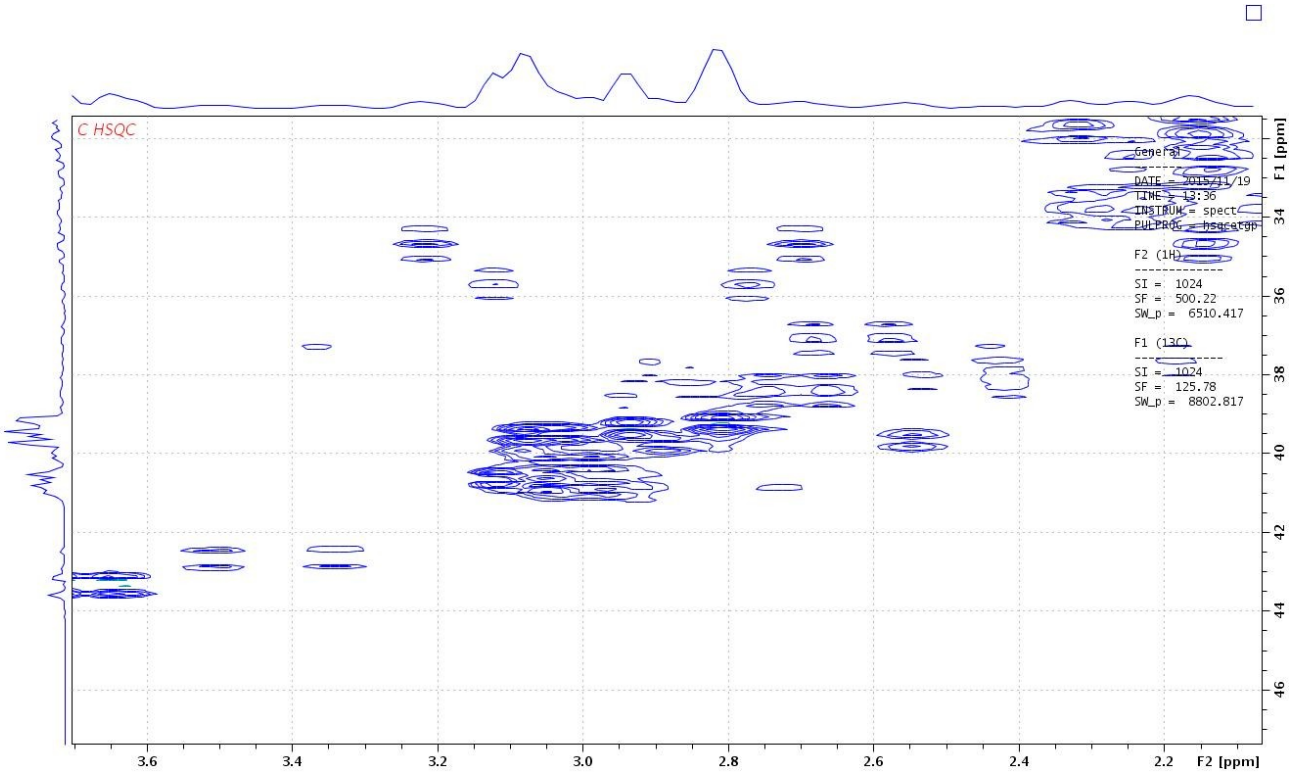

Figure 4: *Detail for slitting of signal in* ^{1}H - ^{13}C *HSQC spectrum for double labeled ubiquitin. 25 °C*

We can observe splitting of signals because of strong scalar coupling interaction (J interaction, Fig. 3 and 4). To remove splitting of signals we measured the 2D Constant-Time HSQC (CT-HSQC). CT-HSQC experiment is a version of the conventional 2D HSQC experiment in which the typical variable 13C evolution period is replaced by a constant-time evolution period in which homonuclear 13C-13C coupling constants are refocused. Evolution period was held constant at value 28 ms. Negative peaks correspond to carbons with none or 2 bounded Hydrogens (Fig. 4). Aromatic carbons are shown in Figure 5.

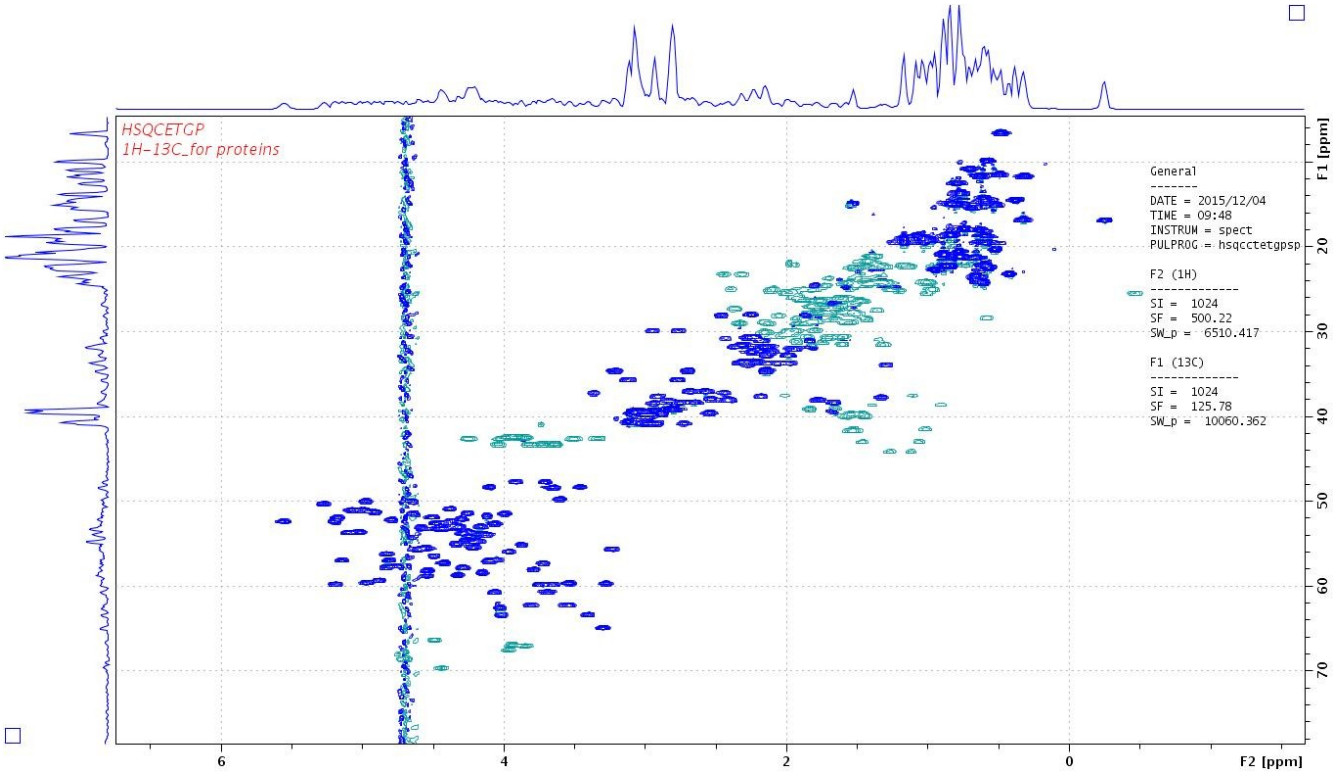

Figure 4: ^{1}H - ^{13}C *CT - HSQC spectrum for double labeled ubiquitin. 25 °C*

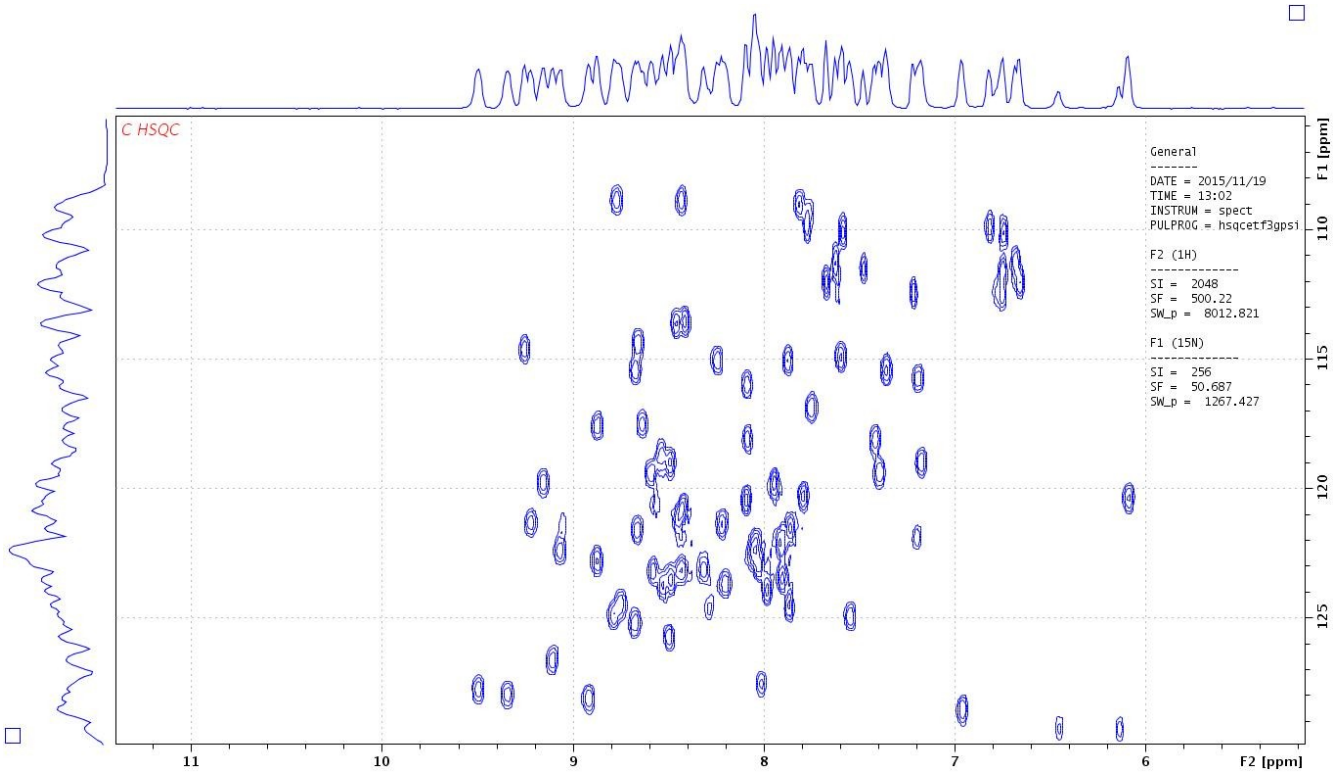

Figure 5: ^{1}H - ^{13}C *CT - HSQC spectrum for aromatic carbons in double labeled ubiquitin. 25 °C*