Determination of critical micellar concentration by NMR spectroscopy

Laboratory Practice,

700 MHz NMR spectrometer, -1.108 lab

Practical: Dr. Bodor Andrea

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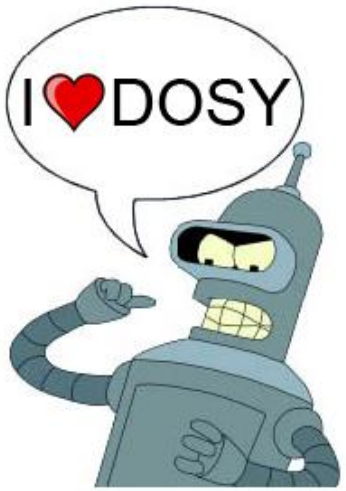
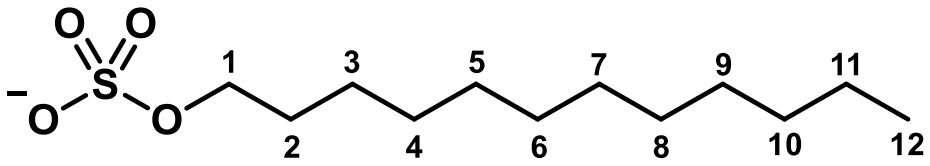
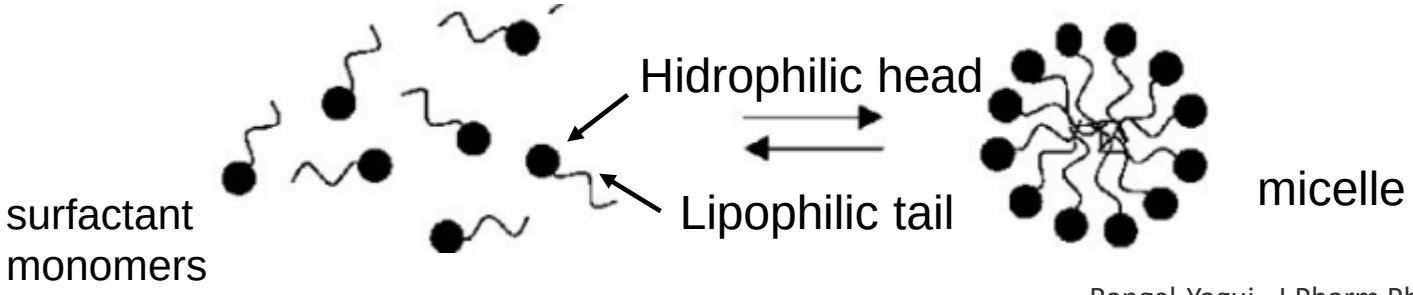


Goal

Critical micellar concentration of an anionic surfactant, sodium dodecyl sulphate (SDS) is going to be determined using diffusion NMR measurements.



Micelle formation

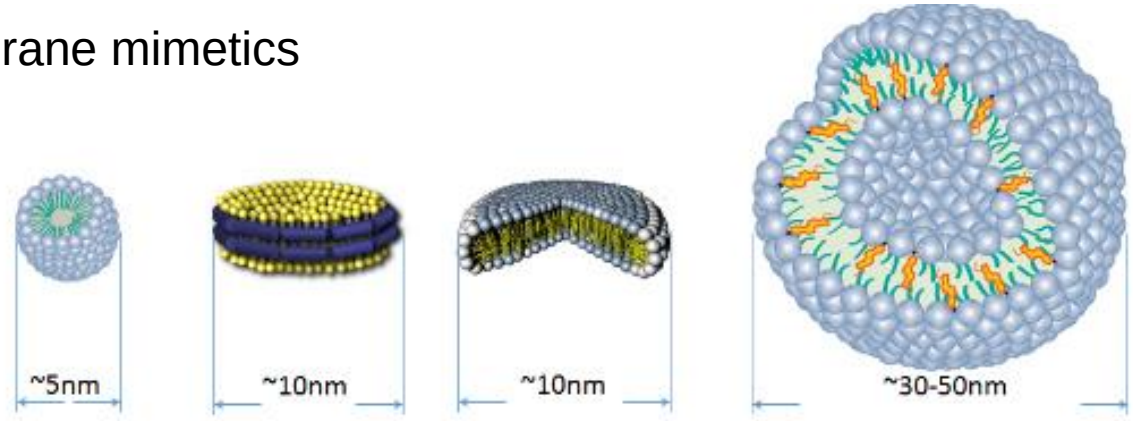


Rangel-Yagui, J Pharm Pharm Sci 2005

Critical micellar concentration (CMC)

The minimum required concentration of the surfactant to initiate micelle formation. Above this concentration the amphiphilic molecules form micelles.

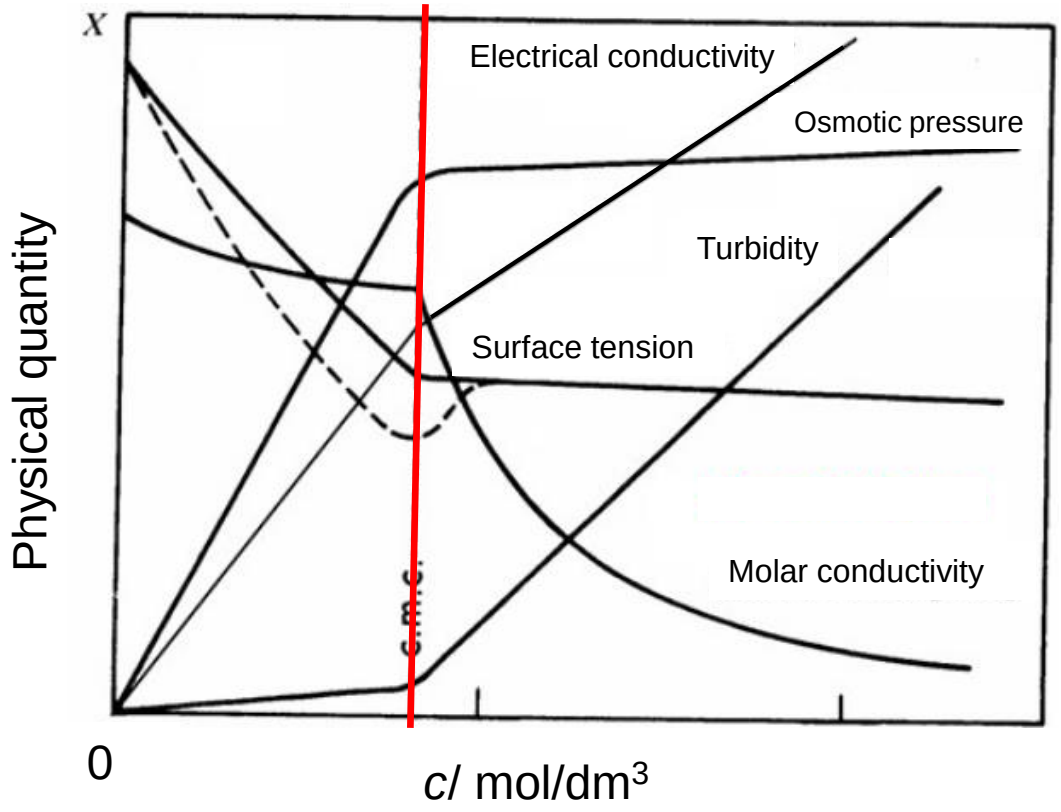
Membrane mimetics



micelle nanodisc bicelle liposome

Hagn, F.; Etzkorn, M.; Raschle, T.; Wagner, G. J Am Chem Soc 2013, 135, 1919–1925.

Determination of cmc



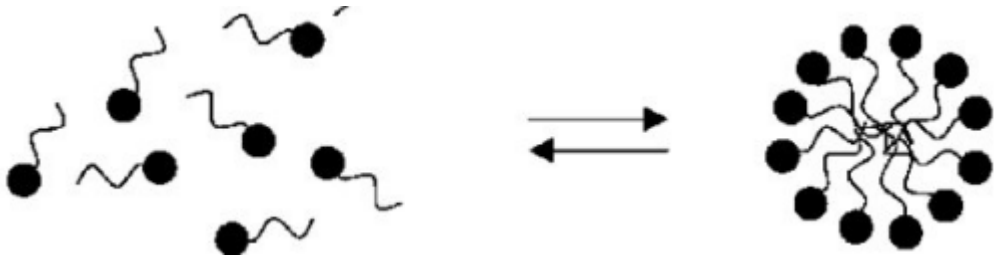
Today: cmc is going to be determined from diffusion coefficients.
Diffusion coefficient determination using NMR.

Stokes-Einstein equation

$$D = \frac{kT}{6\pi\eta r_{\mathrm{H}}}$$

D – diffusion coefficient
k – Boltzmann constant ($1.38 \cdot 10^{-23}$ J/K)
T – temperature
η – viscosity
r_{H} – hydrodynamic radius

The diffusion coefficient is inversly proportional to the size of the particle.



Free surfactant molecule
smaller size, larger D

Micelle
Larger size, smaller D

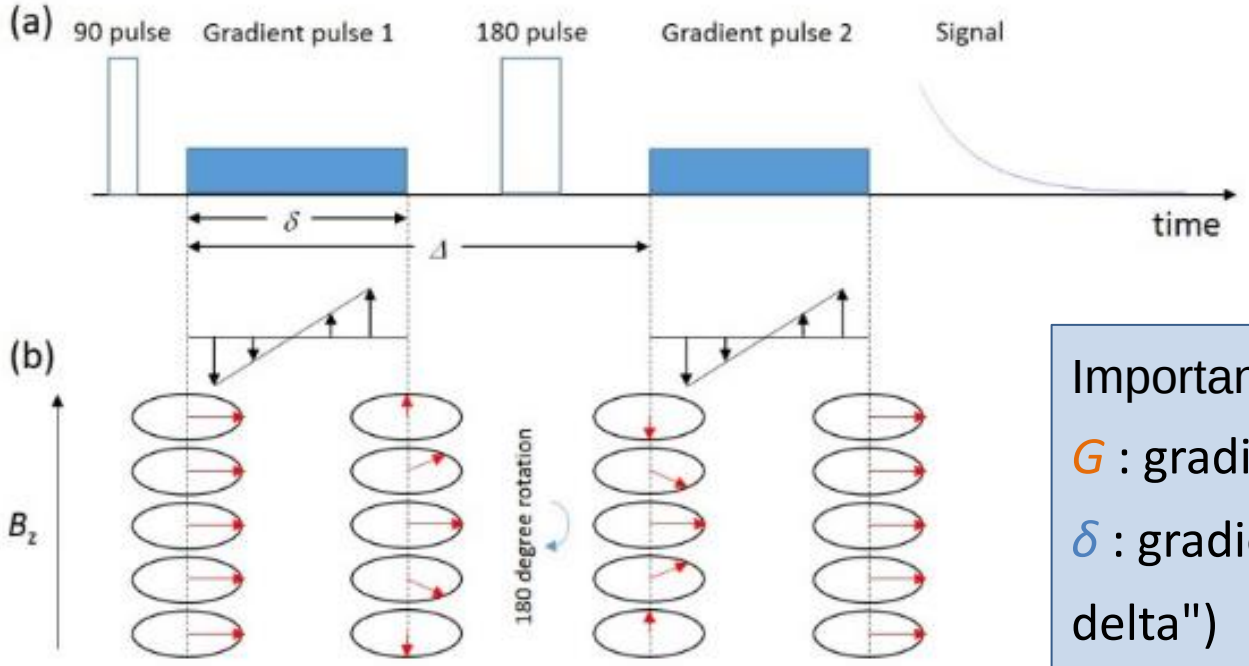
The measured diffusion coefficient is the mole fraction weighted average of free and in micelle surfactants.

$$D_{\mathrm{measured}} = x_{\mathrm{free}} D_{\mathrm{free}} + x_{\mathrm{mic}} D_{\mathrm{mic}}$$

How does the diffusion coefficient change, when the total surfactant concentration increases?

PGSE-Pulsed Gradient Spin Echo

Schematic representation of the pulse program



Important parameters:

G : gradient strength (Hz/G)

δ : gradient length („little delta")

Δ : diffusion time („big delta")

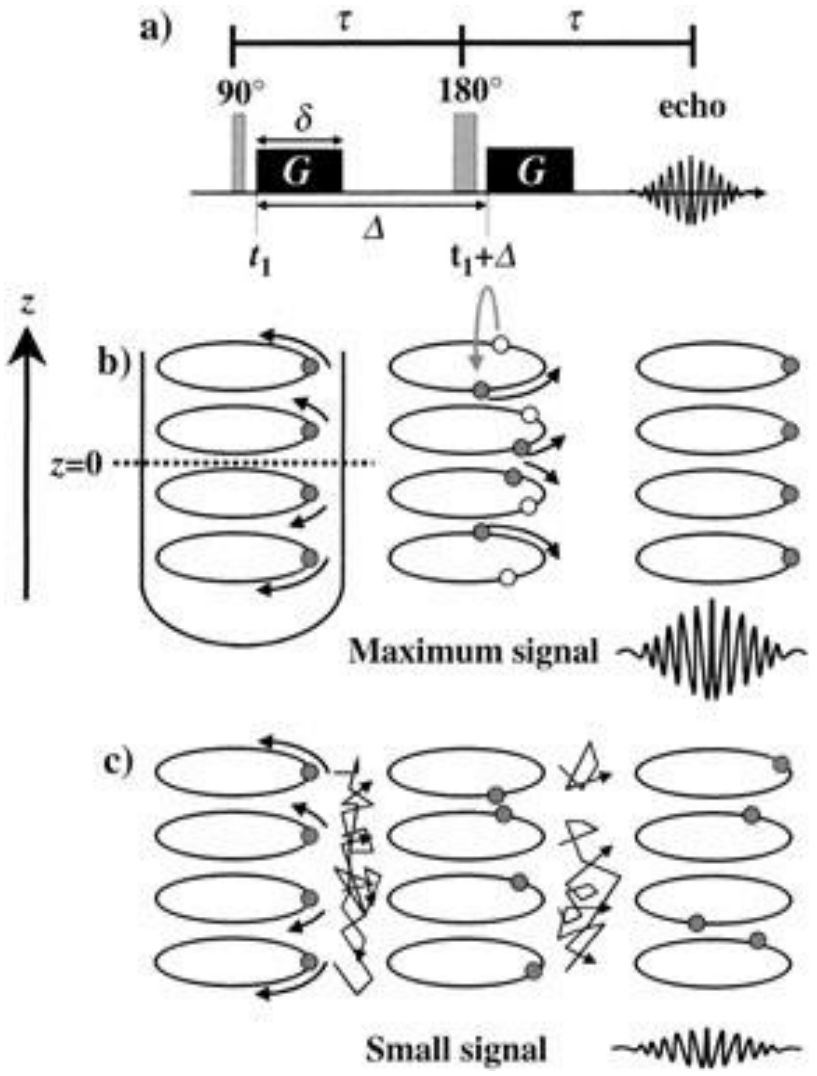
We used **stebpgp1s19** pulse program for the DOSY measurements

DOSY: **D**iffusion **O**rdered **S**pectroscop**Y**

DOSY measurements

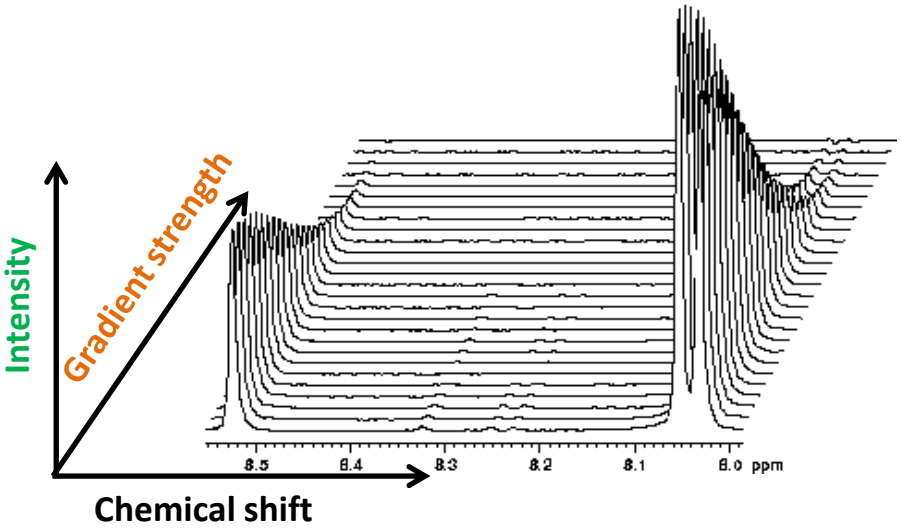
without diffusion

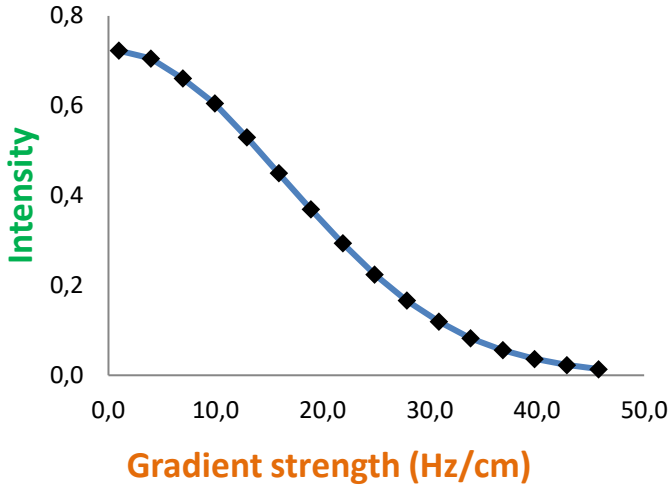
with diffusion



Determination of Diffusion coefficients: Stejskal-Tanner equation

$$I = I_0 \exp[-D\gamma^2\delta^2 G^2 \left(\Delta - \frac{\delta}{3}\right)]$$



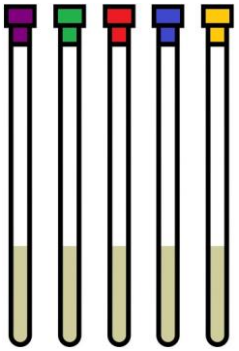


Parameters to set up: δ, Δ
In one experiment we change: Gradient strength
From a non-linear fitting we get: **Diffusion coefficient**.

The steps of our present practical

1. Sample preparation
2. NMR measurements
    1. Lock
    2. Atma proton
    3. Shim
    4. Acquisition of 1D ^{1}H spectrum
    5. DOSY measurement
3. Evaluation:
    1. Fitting the Stejskal-Tanner equation (1D evaluation)
    2. EDDOSY (2D evaluation)

Sample preparation



Reagents:

- 0.1 M SDS stock solution
- Distilled water
- D_2O

Prepare 5 diluted SDS solutions (Find the concentrations given in the table below)! Each sample has a total volume of 600 µl and each contains 10 % D_2O!

Calculate the amount of SDS, H_2O and D_2O in each sample!

c_{SDS} / mM	V_{SDS} / µl	V_{H2O} / µl	V_{D2O} / µl
3			
6			
9			
12			
15			

NMR measurements

Software used for data processing and evaluation:

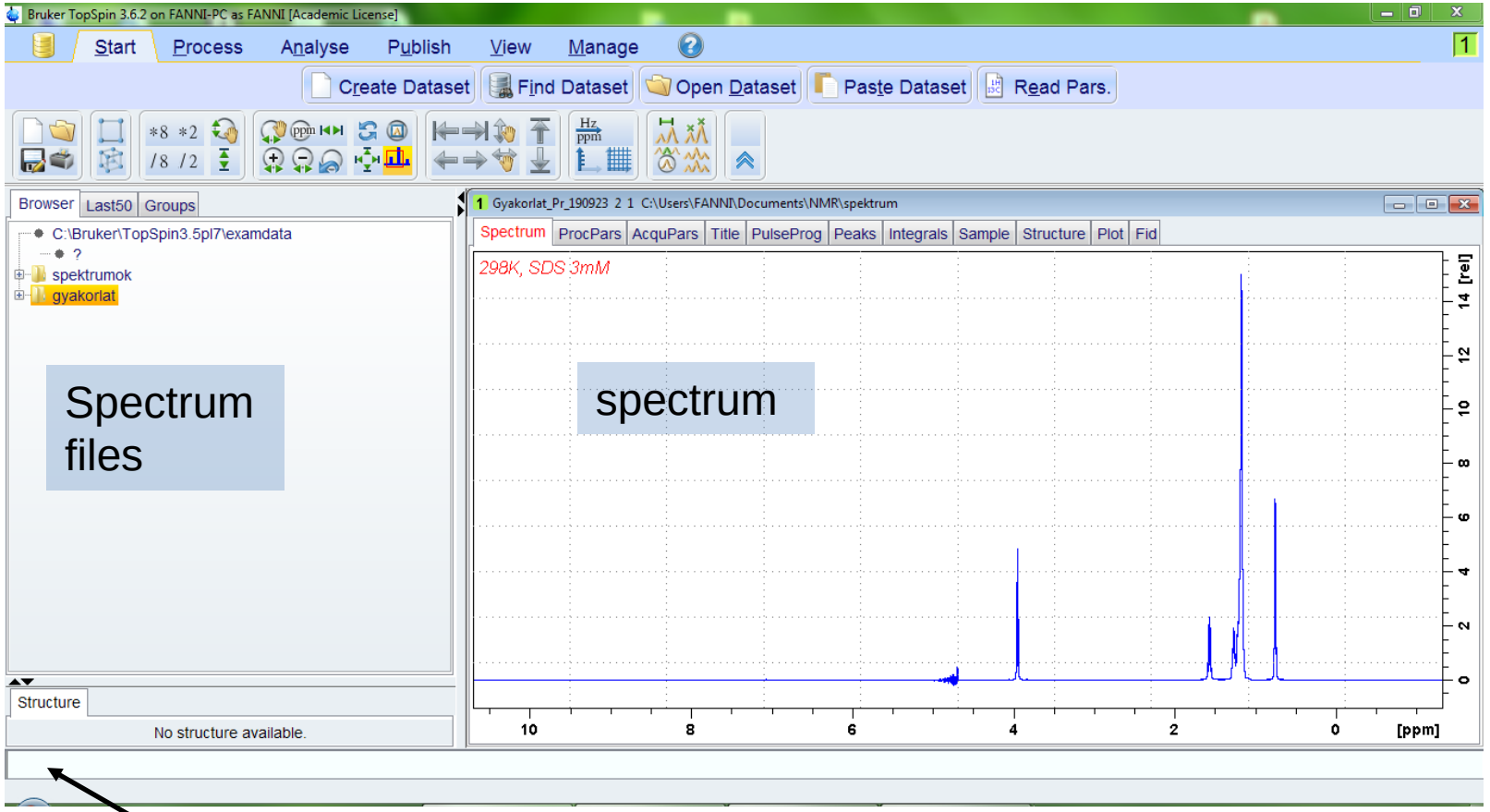
TopSpin 3.6.2

Download (free for students):
https://www.bruker.com/service/support-upgrades/software-downloads/nmr/free-topspin-processing/nmr-topspin-license-for-academia.html

Download **version 3.6** not version 4!!!
Choose "Data processing only" („Setup type")

TopSpin 3.6.2

A brief tutorial:
https://www.youtube.com/watch?v=FocoABJ2rvw



Write commands here

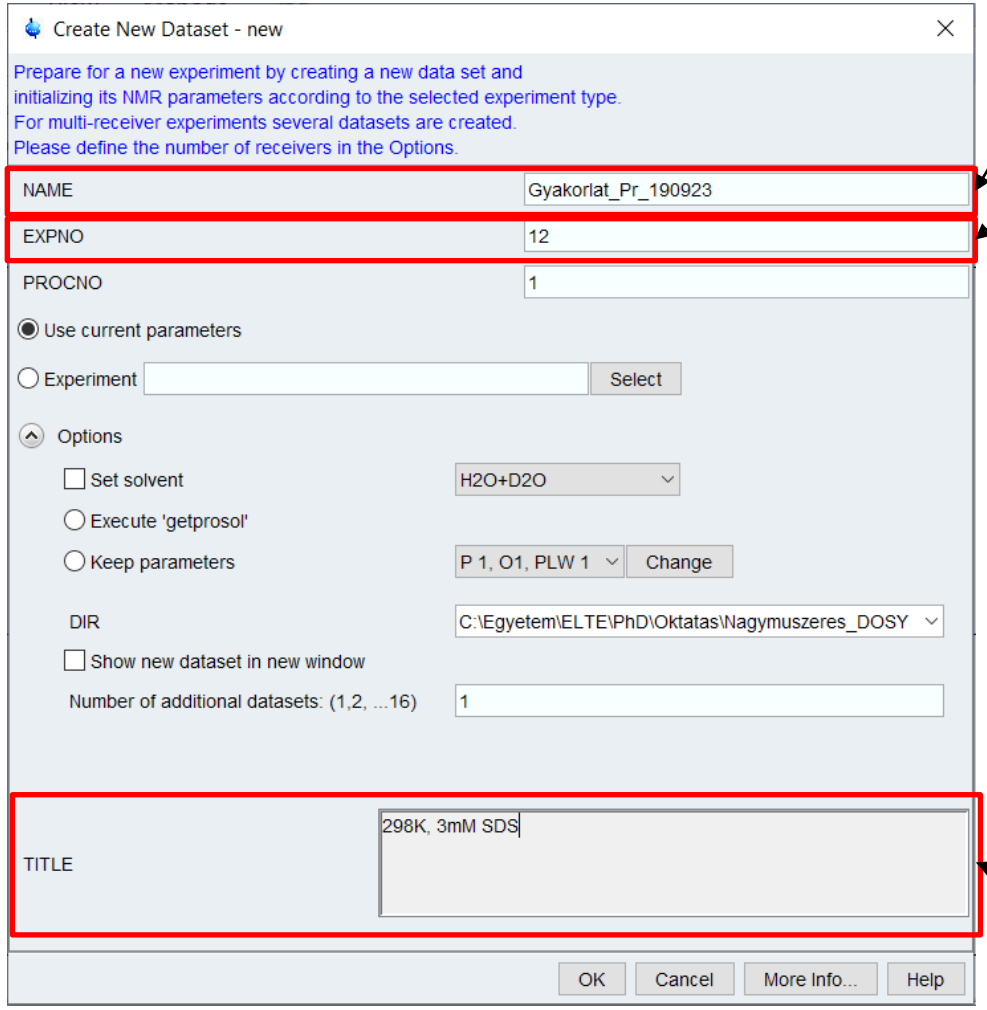
Adding a folder: Right click on Spectrum files → Add new data dir.
Load a Spectrum: drag-and-drop to the spectrum window

In the virtual laboratory practice you will be guided through the steps of recording ^{1}H 1D and diffusion NMR spectra (slides 14-25).

You will get a detailed hands-on on how to evaluate the spectra (slides 27 -40) and you have to perform this data analysis based on the spectra you receive in a separate file.

Measurement I – new dataset

edc: creating a new dataset with copying the parameters of a previous one



Name of folder:

Practice_Pr_[YYMMDD]

Number of experiment in folder

Following datasets should be created:

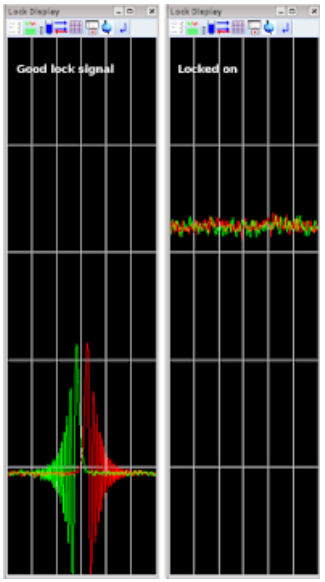
- **zgpr**: 1D proton with water suppression, only for setting O1 and p1 (only 1)
- **zgesgp**: 1D proton with better water suppression (1 for each sample)
- **stebpgp1s19:** DOSY experiment with water suppression (1 for each sample)

Title: add sample properties here

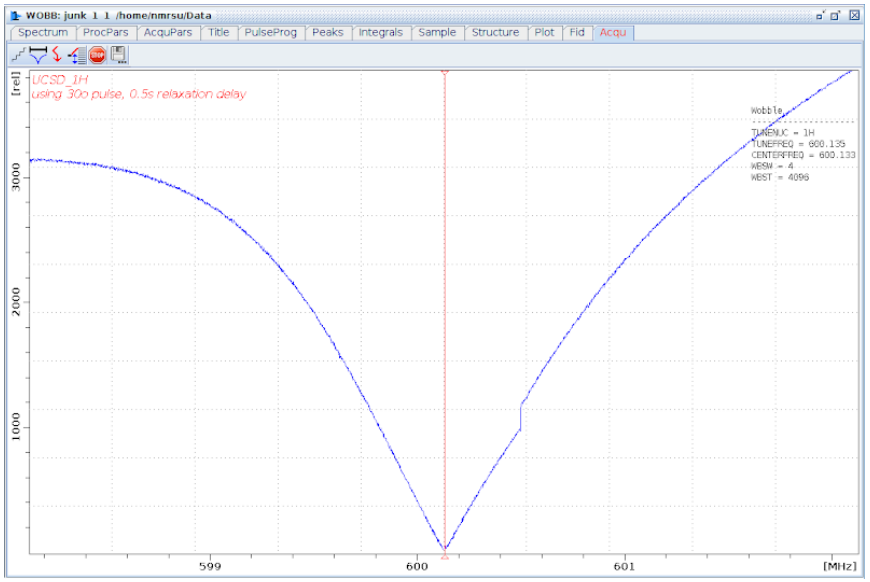
Measurement II – preparations

1. *lock*: monitoring the solvent's deuterium signal in order to compensate the temporal variations in the magnetic field → we have to set the solvent
2. *atma proton*: Tuning the RF coil to the ^{1}H resonance frequency
3. shim: ensures the spatial homogenity of the magnetic field → we compensate the inhomogenities of the 14.6 T magnetic field with shim-coils (procedure: Z, Z^2, Z, Z^2… then Z^3, X, Y)

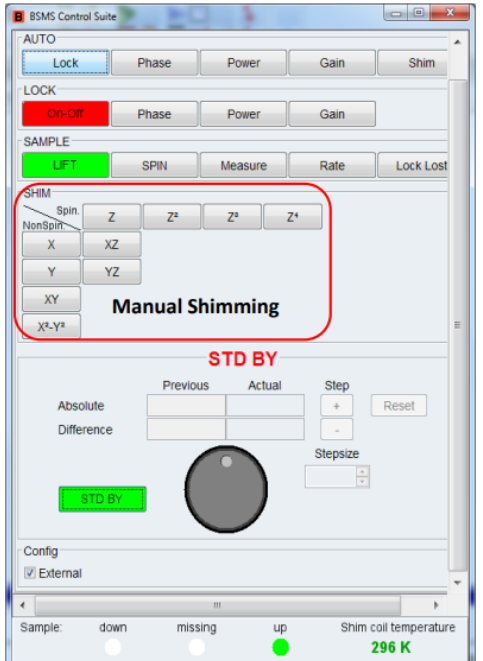
1. lock



2. Correct tuning

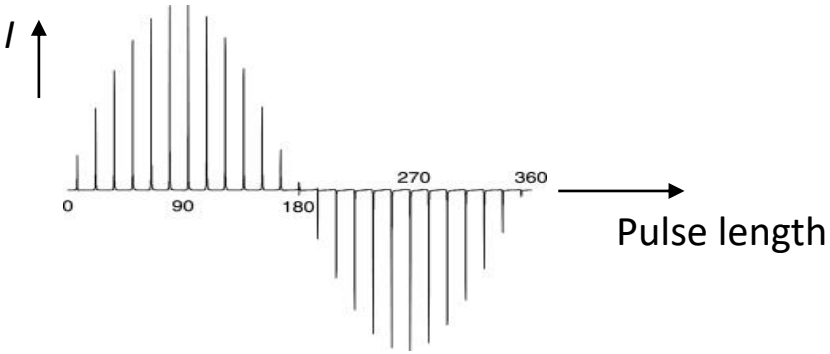


3. Shim

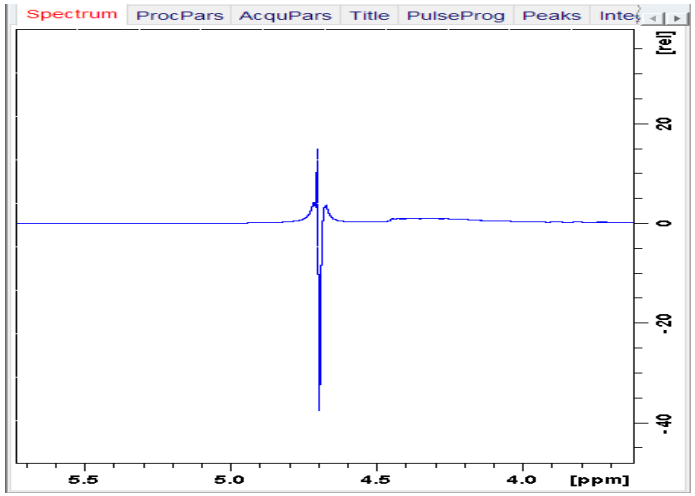


Measurement III – p1, O1, 1D ^{1}H spectrum

- *zg*: starting the measurement ← start the previously created **zgpr**
- *pulsecal*: calculates the length of the 90 ° hard pulse on the proton channel



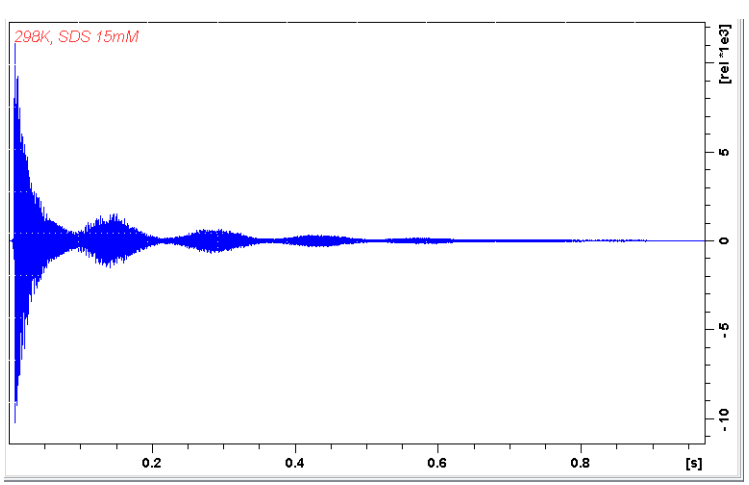
- *O1*: Transmitter frequency offset in Hz. We should set it to the maximum of the water signal to achieve maximum efficiency of water suppression.



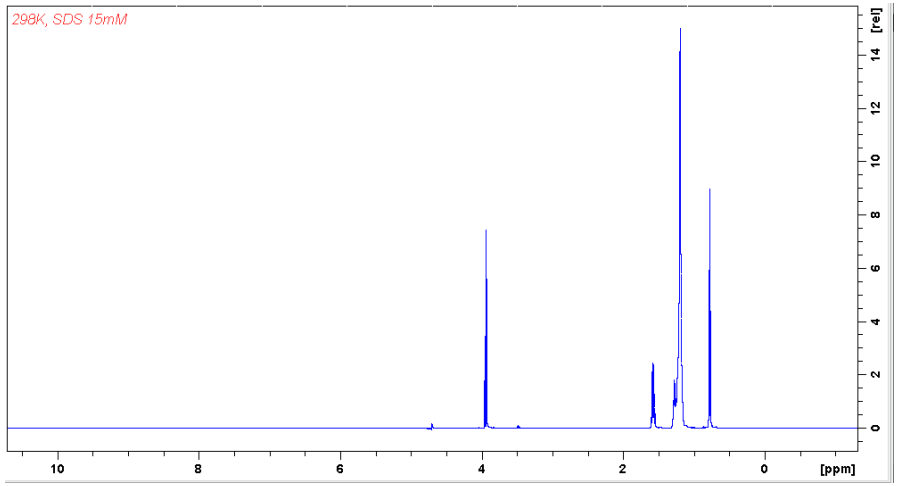
Good O1 - effective water suppression: residual water signal is small and distorted

Measurement IV – 1D proton

- Create a **zgesgp** dataset
- *getprosol 1H [p1] -12.55:*
  - Insert p1 value determined with *pulsecal* to *[p1]*
  - a command that sets *p1* and the power of shaped pulses
- *O1* - *S*et the O1 value determined from the **zgpr**
- *zg* – start experiment → what do we get?
- *efp* – Exponential multiplication + Fourier-transformation + phase correction → what do we get after this command?

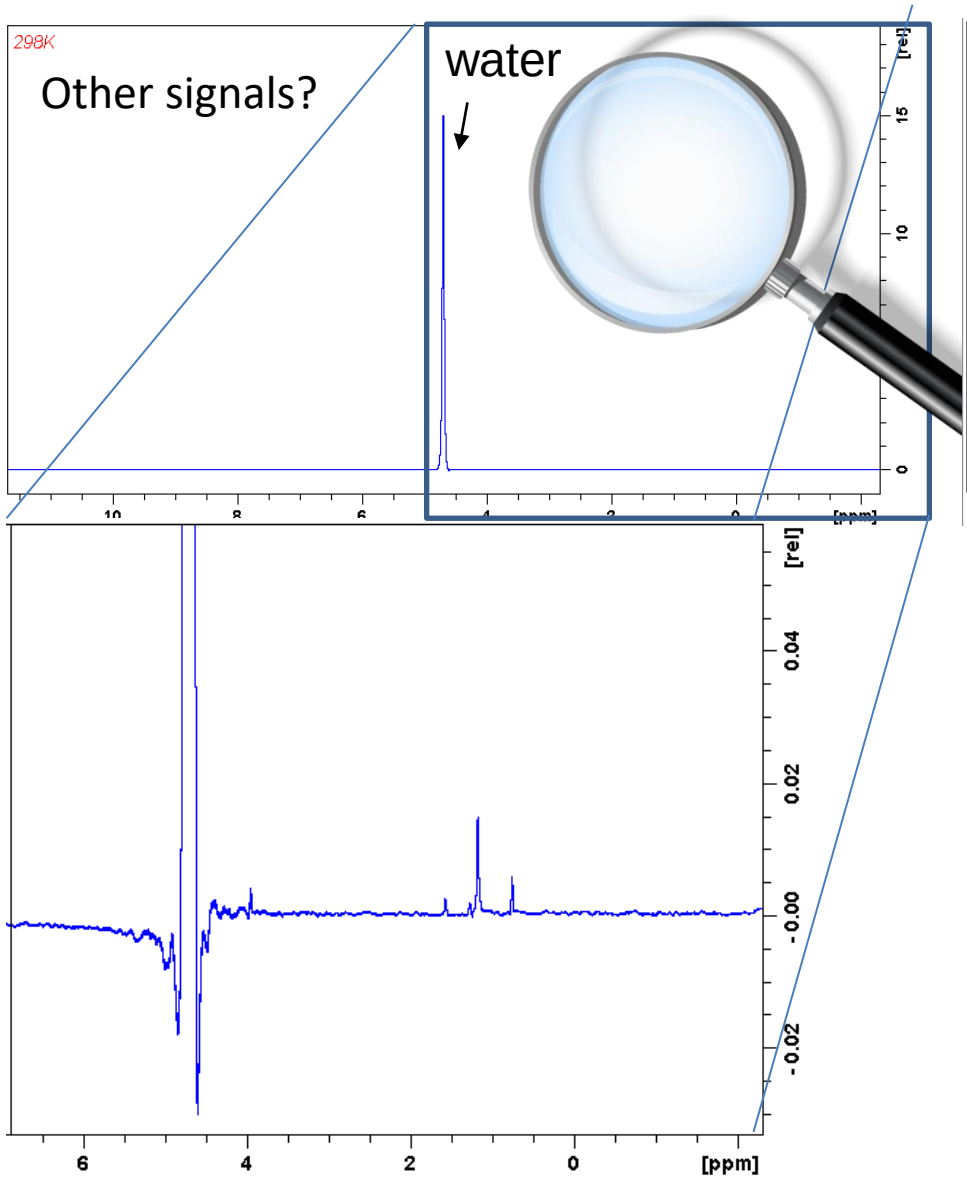


FT

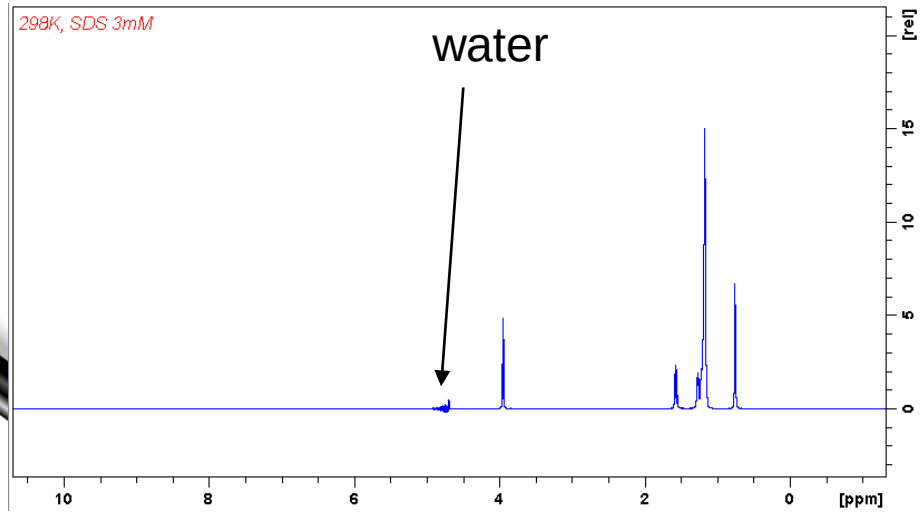


Importance of water suppression

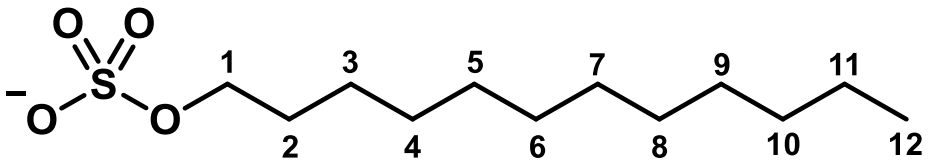
1D ^{1}H spectrum without water suppression

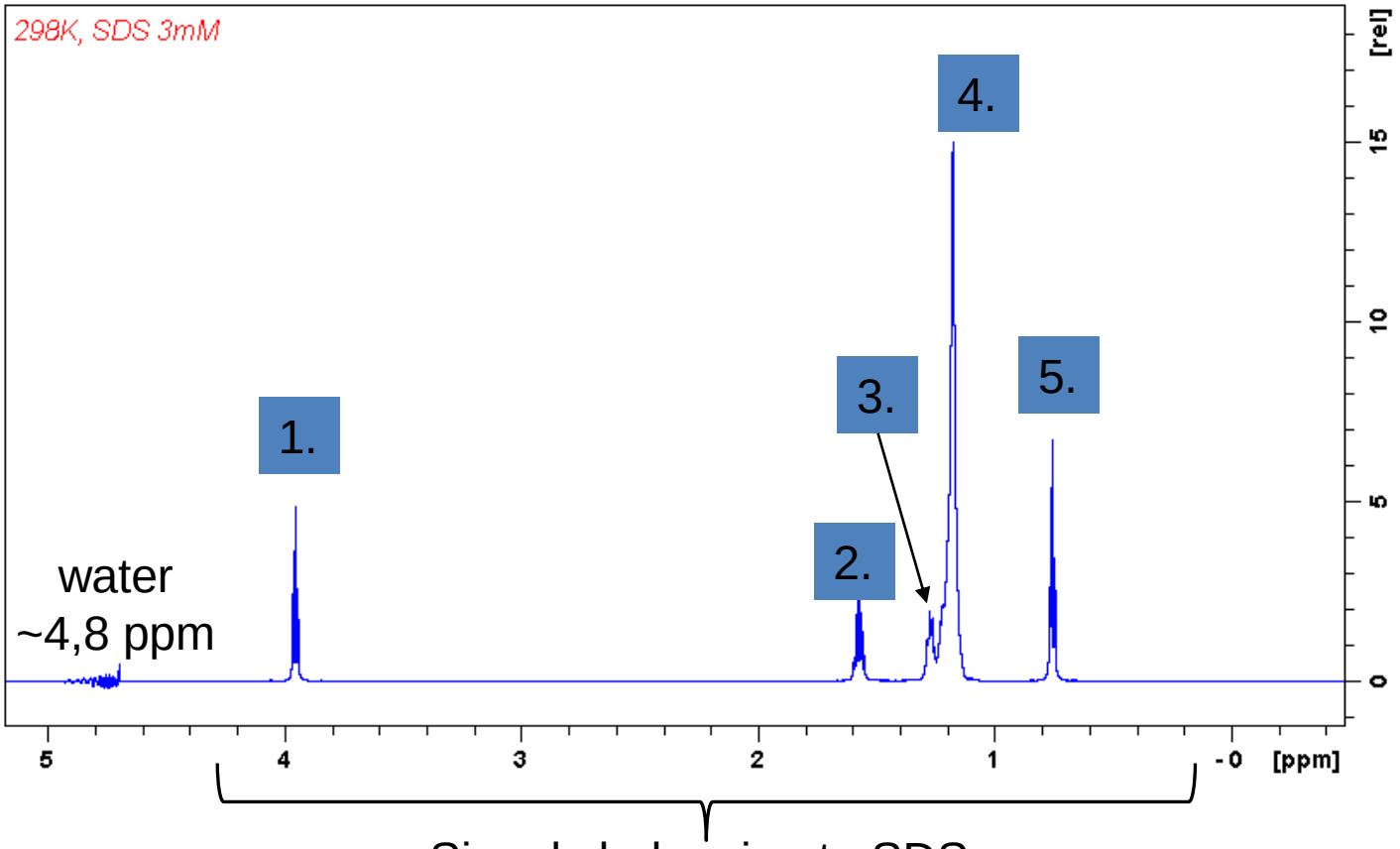


1D ^{1}H spectrum with water suppression



Assignment of 1D ^{1}H spectra





Signals belonging to SDS
We detect 5 signal groups

To which SDS protons do the signals belong?

Assignment should be included in the report

Number of signal (see previous slide)	Chemical shift (give accurate values in the report)	Assignment	Multiplicity	Relative integral
	~4,8 ppm	H_2O	-	-
1.	~3,9 ppm			
2.	~1,6 ppm			
3.	~1,3 ppm			
4.	~1,2 ppm			
5.	~0,8 ppm			

Diffusion measurements

Following parameters should be set:

General: p1, O1, SW etc.

DOSY parameters: δ, Δ, gradient strength *(G)* is changed according to what function and in how many steps?

Setting δ:

command: P30: we must give the half of δ in µs!

Setting Δ:

command: D20: we must give the Δ value in s!

Changing gradient strength:
See „Starting DOSY experiment” slide

Pay attention to the units!
When setting the parameters, *half of* δ is in µs, Δ is in s!
When evaluating DOSY data, δ and Δ values must be given in ms!

DOSY parameters

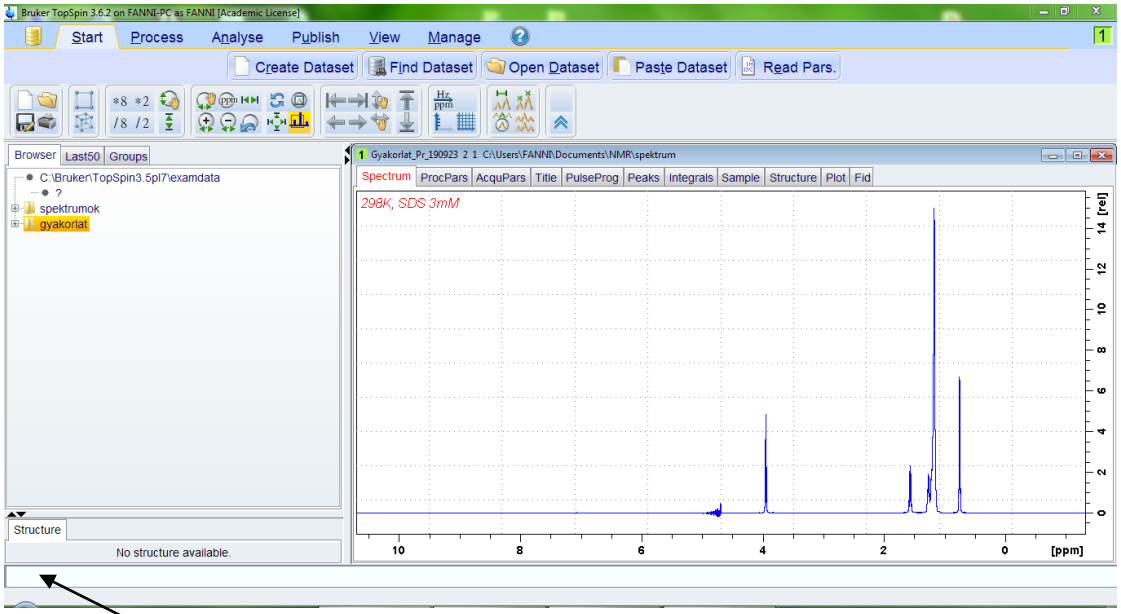
- Recommended values for δ and Δ:
  - Small molecules: δ = 2 ms, Δ = 75, 100 ms

    P30 = 1000, D20 = 0.75, 0.1
  - Larger molecules (10 kDa <): δ = 4 ms, Δ = 200 ms

    P30 = 2000, D20 = 0.2

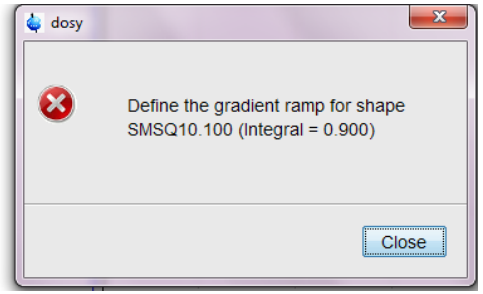
For SDS: δ = 2 ms, Δ = 150 ms

Starting DOSY experiment (not needed at home)



Type *dosy* command here

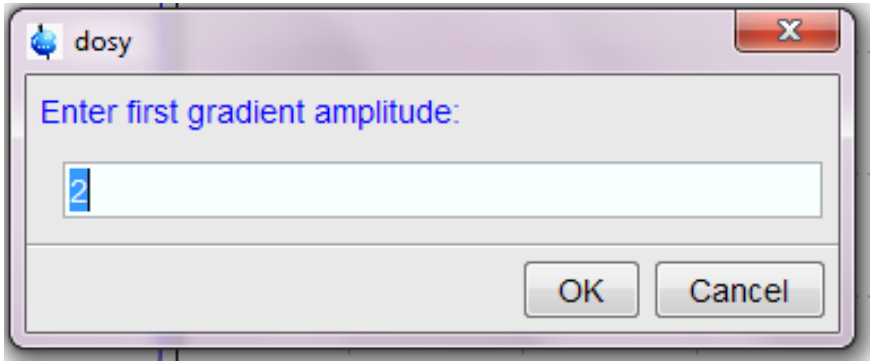
A new panel will appear, where we can set the parameters of how to change the gradient strength.



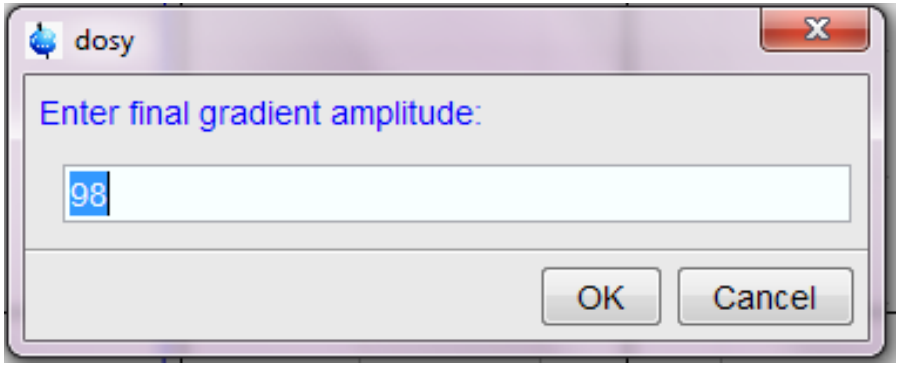
Press ENTER here.

We set the following parameters:

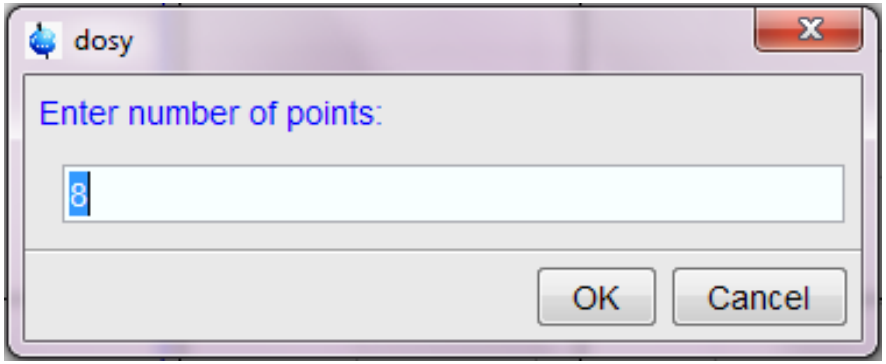
1. First gradient amplitude, percentage of the maximum gradient strength



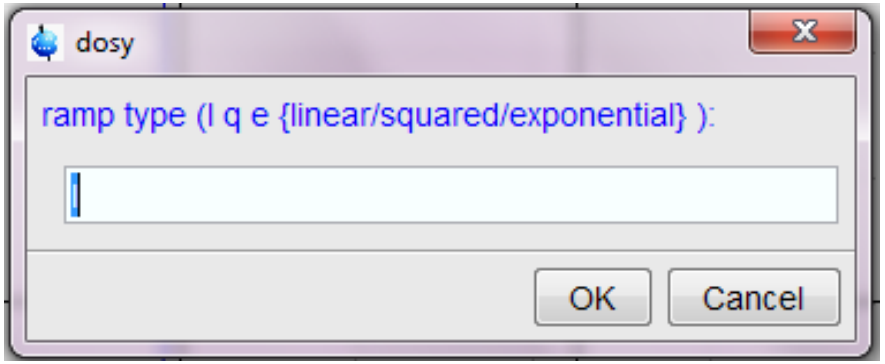
2. Final gradient amplitude



3. In how many points do we change gradient strength

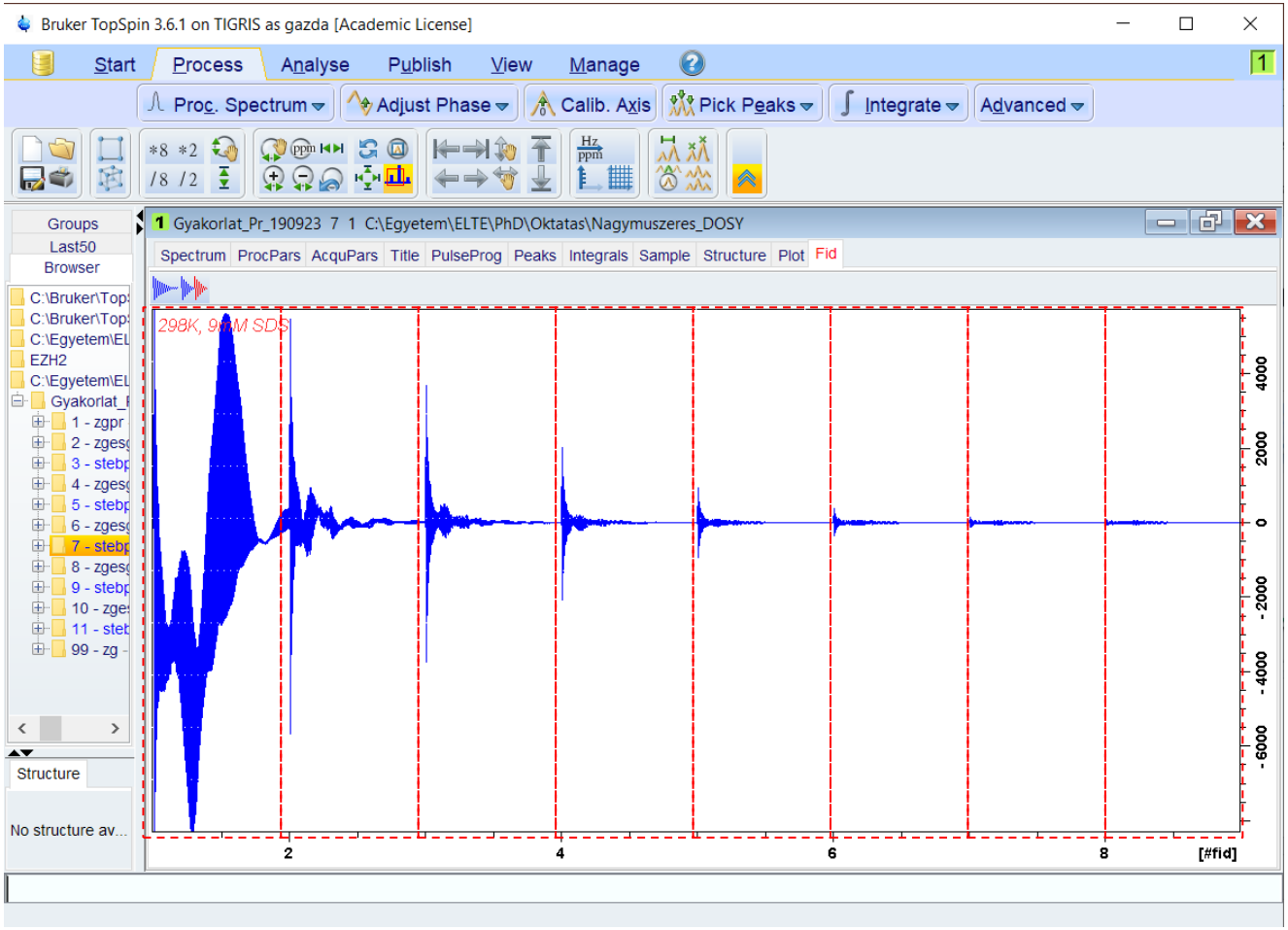


4. According to what function do we modify gradient strength



During practice we modify gradient strength linearly in 8 steps, between 5 and 95%.

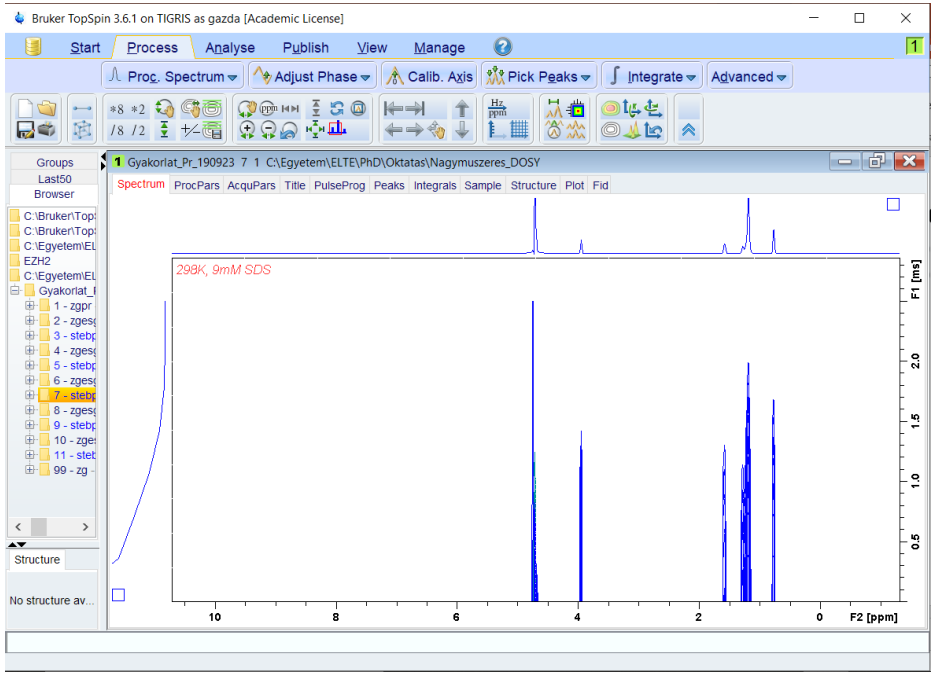
A FID of a DOSY experiment



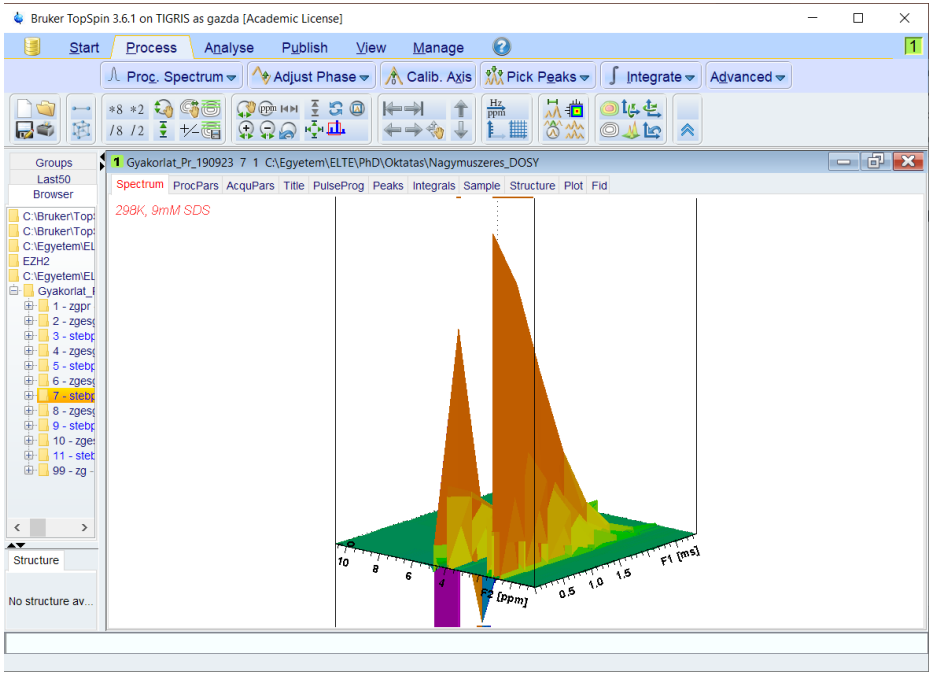
8 points, 8 FIDs are seen with decreasing intensities

Evaluation of the diffusion measurements

Pseudo-2D dataset: Fourier-transform with the *xf2* command

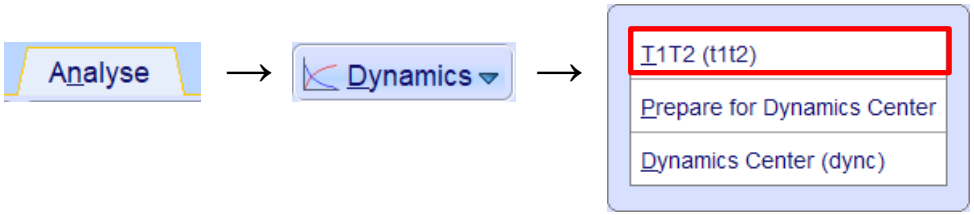


2D view

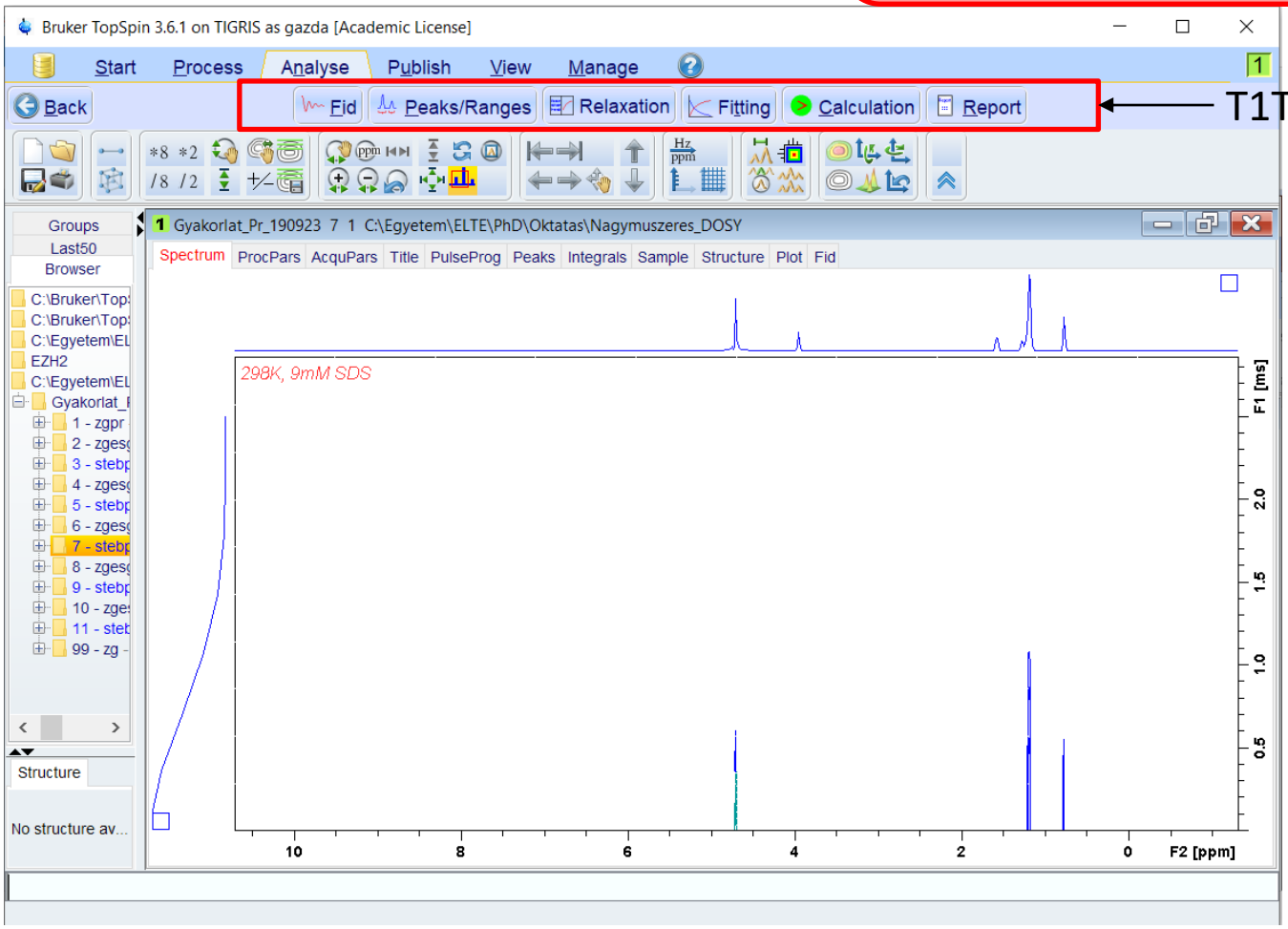


3D view

Evaluation based on fitting the Tanner equation

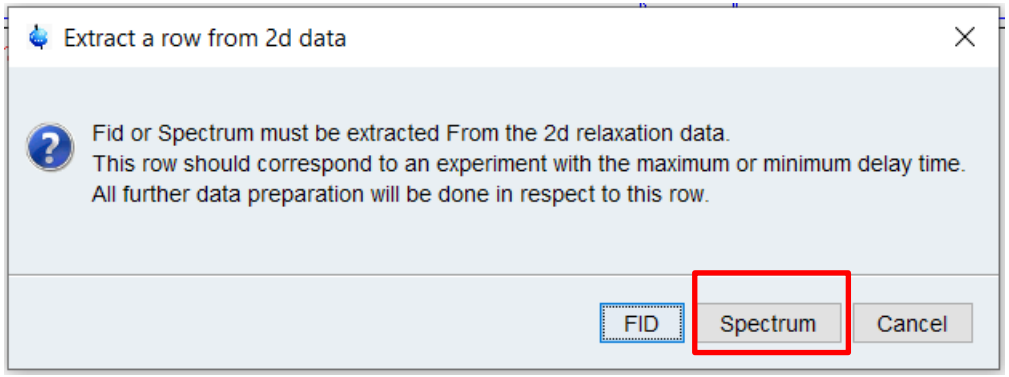


Click on the Dynamics button and not the DOSY button!!!!!

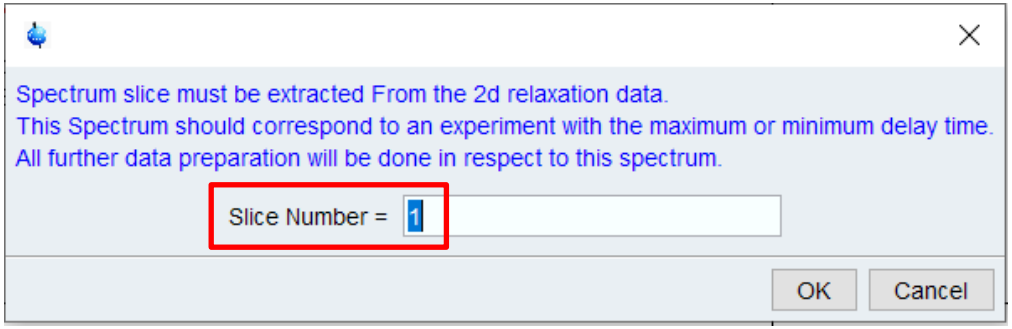


T1/T2 row : click on 

icon: what appears

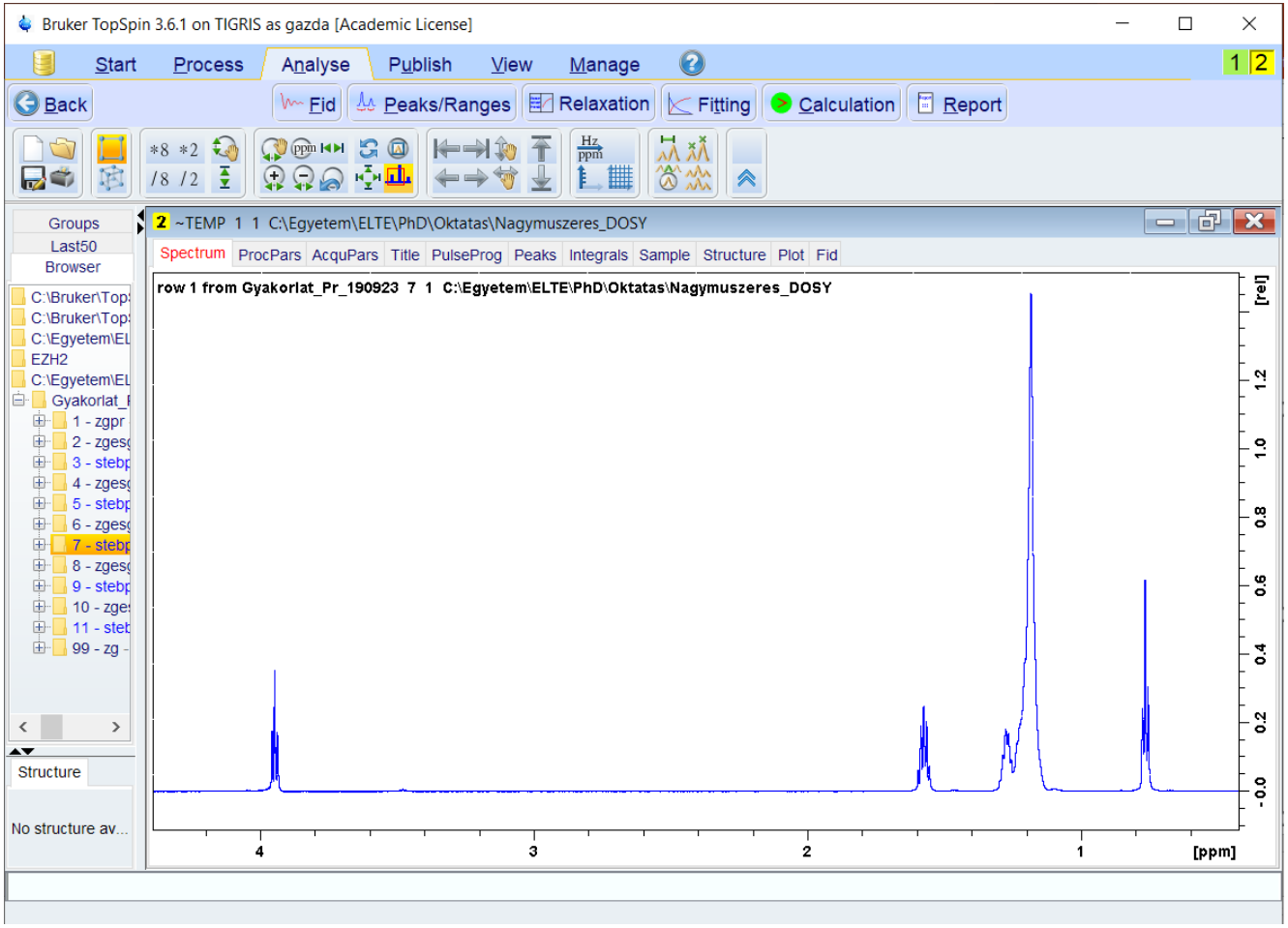


Choose „Spectrum”: this means you will pick one 1D spectrum from the pseudo 2D data



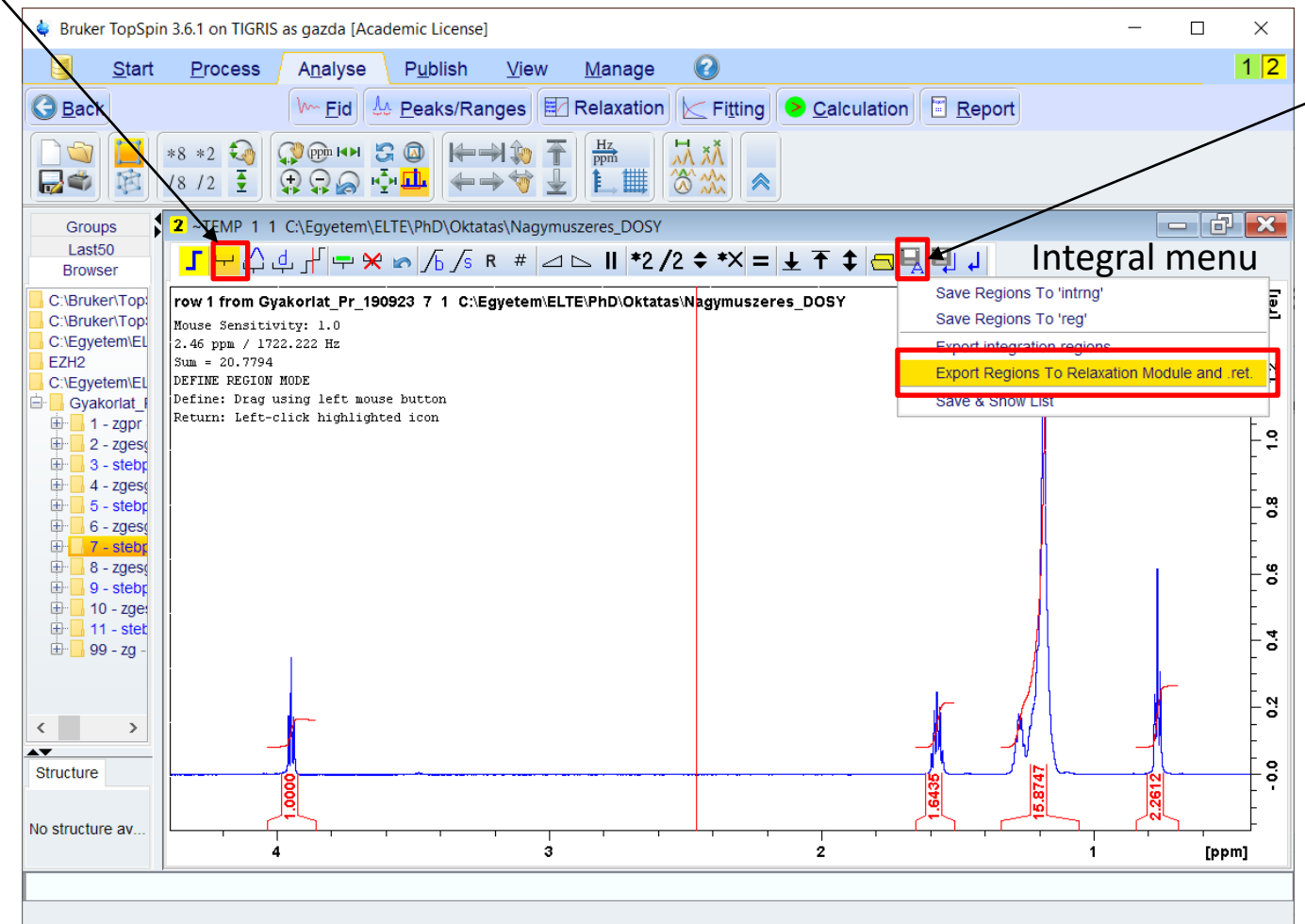
Choose the first spectrum (recorded with the lowest gradient intensity): 1
Then press OK.

The corresponding 1D ^{1}H spectrum appears.
Zoom on the aliphatic region of the SDS peaks.



Click now on the Peaks/Ranges menu, and from the appearing window choose the „Manual integration” (next button will be [„Prepare relaxation data”] OK).

choose the integration (second icon from left), if this is clicked, then you can integrate the selected regions (click and drag on the spectrum) and in red the integral values will appear



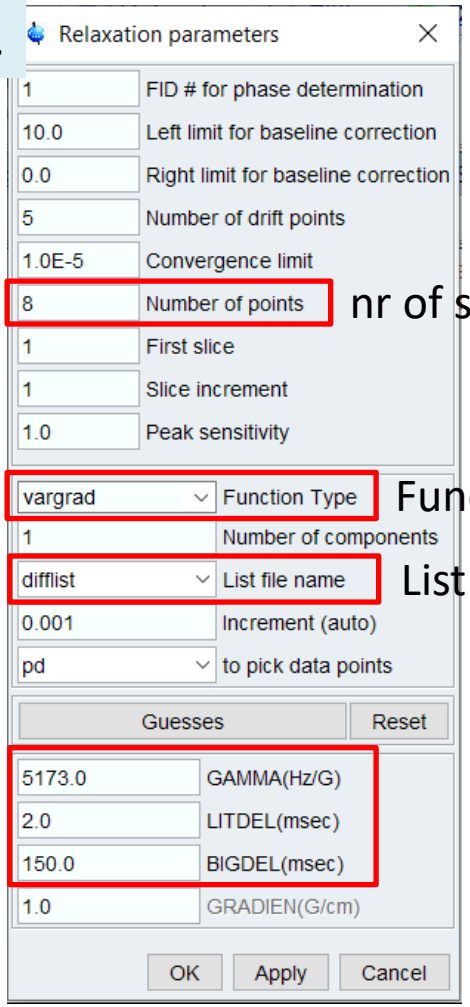
you have to save in a manner that these integral regions will be applied for all spectra from the pseudo2D set

Click on the Relaxation icon. Don't worry an error-message looking-like text (complaining about a vd file) will pop-up, just click on it, and ignore. And now you will see the following table.

The first 9 entries correspond to the already acquired spectra, and deal with how to integrate them. We want to integrate all 8 of them, starting from the first slice and moving in one increments.

The following 5 entries will help the program define what type of equation to fit on the integrated intensities. As we varied the gradient strength based on the predefined difflist these should be set (see red boxes). Now we chose the Stejksal Tanner equation, and we have to tell which were the experimentally used diffusion parameters in the coming 4 entries (out of which only 3 are active – shown also by red boxes): these are the GAMMA, Little delta and Big delta.

1.

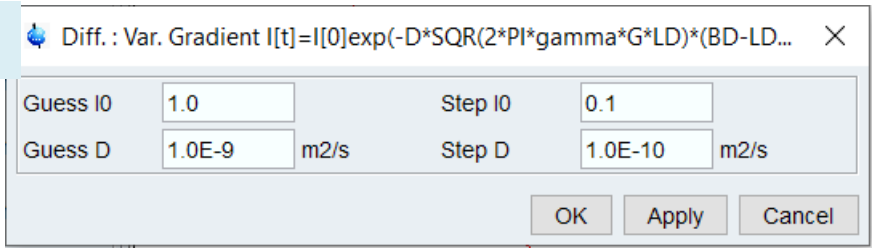


nr of spectra: 8

Function type: vargrad

List file: difflist

2.



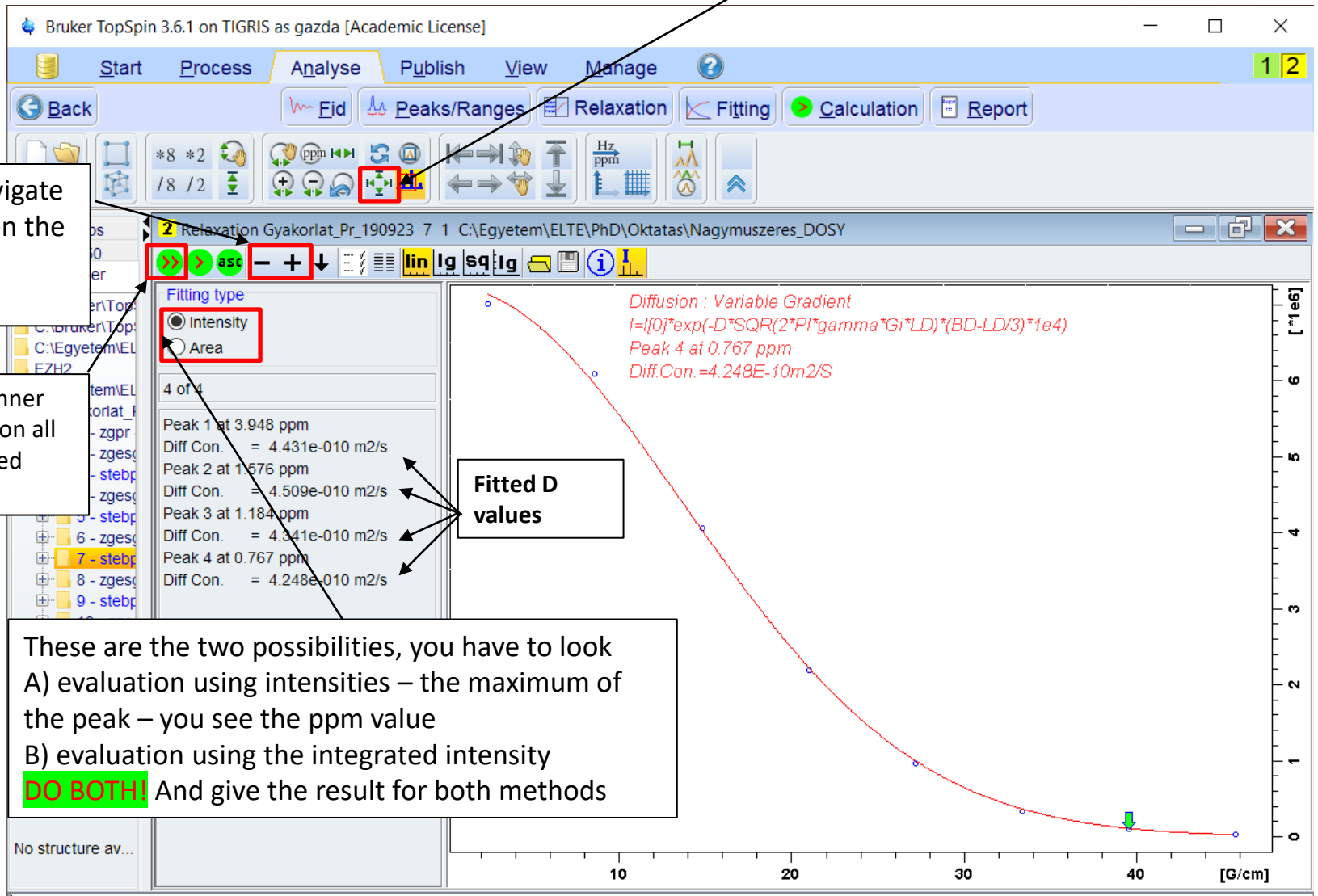
After the OK this window will pop-up, needs some initial parameters, click OK

Stejskal-Tanner equation fit need them:

- GAMMA (γ): 5173 (contains already corrections)
- LITDEL: little delta, δ ms
- BIGDEL: big delta, Δ ms

If all is set : OK

If you don't see the whole curve (8 dots first) then zoom with this button



You navigate between the chosen regions

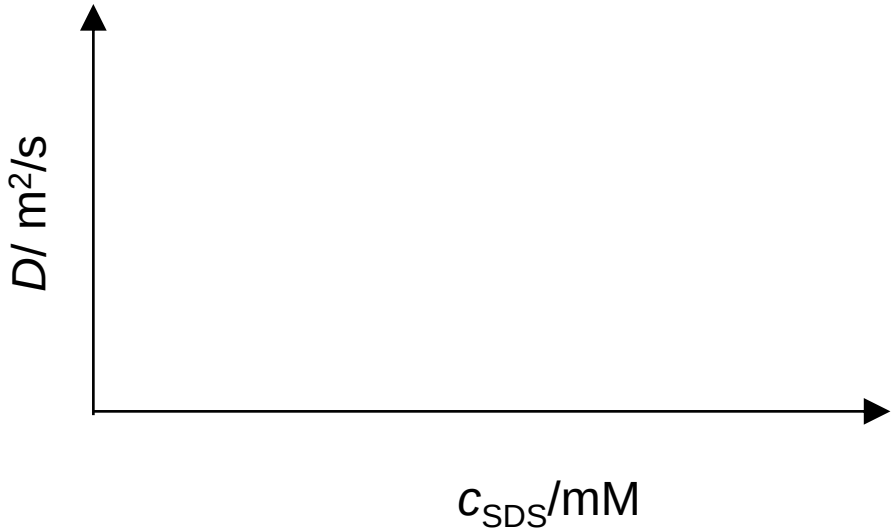
Fit the Tanner equation on all determined regions

Fitted D values

These are the two possibilities, you have to look
A) evaluation using intensities – the maximum of the peak – you see the ppm value
B) evaluation using the integrated intensity
DO BOTH! And give the result for both methods

Evaluation 1 of the diffusion parameters

Data analysis presented on slides 27-33 has to be performed for each solution, pick the corresponding measurement (in TopSpin 1D spectrum measurement files are shown in black the ones referring to diffusion data are pseudo 2D and are shown in blue).
In the end you will have a *D* value for each SDS concentration, and this is the graph you have to make. The intersection of the two fitted linear equations will give you the cmc.

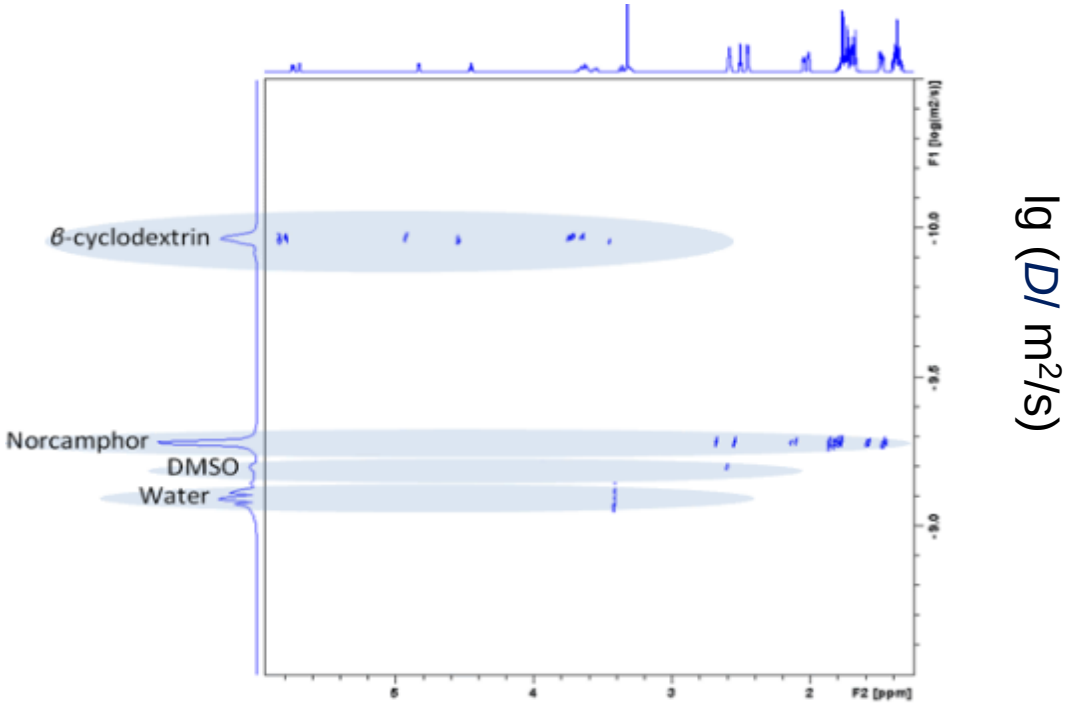


Note how given peaks vary in the 1D ^{1}H spectra! (chemical shift variation).
Which environments are mostly affected? Can you give an explanation?
(You can overlay the 1D proton spectra with the icon)

Evaluation 2 of the diffusion parameters

eddosy command

In the end you will get an NMR "cromatogram" looking like 2D table, where the vertical axis is the log of the diffusion coefficient (log*D*) and the horizontal axis is the ^{1}H 1D spectrum. In case of multicomponent system each molecule will show up as a horizontal line – its ^{1}H chemical shifts - with the corresponding log*D*. We show an example how can you determine the different components from a mixture:

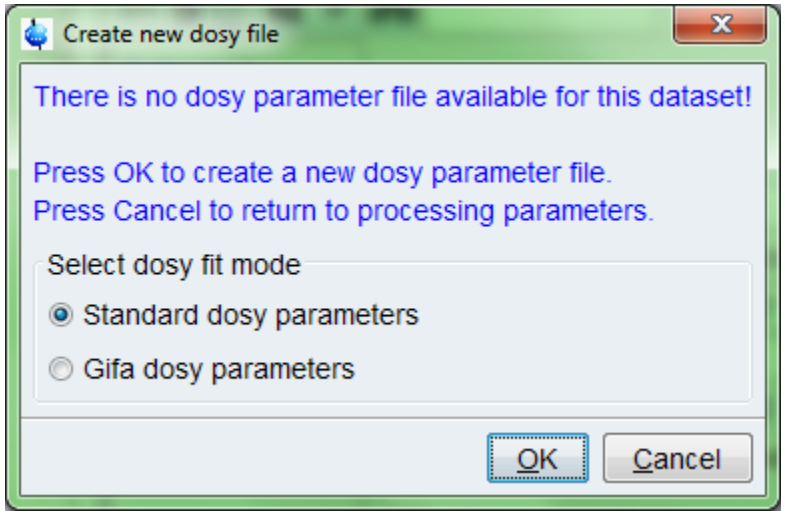


http://chem.ch.huji.ac.il/nmr/techniques/other/diff/diff.html

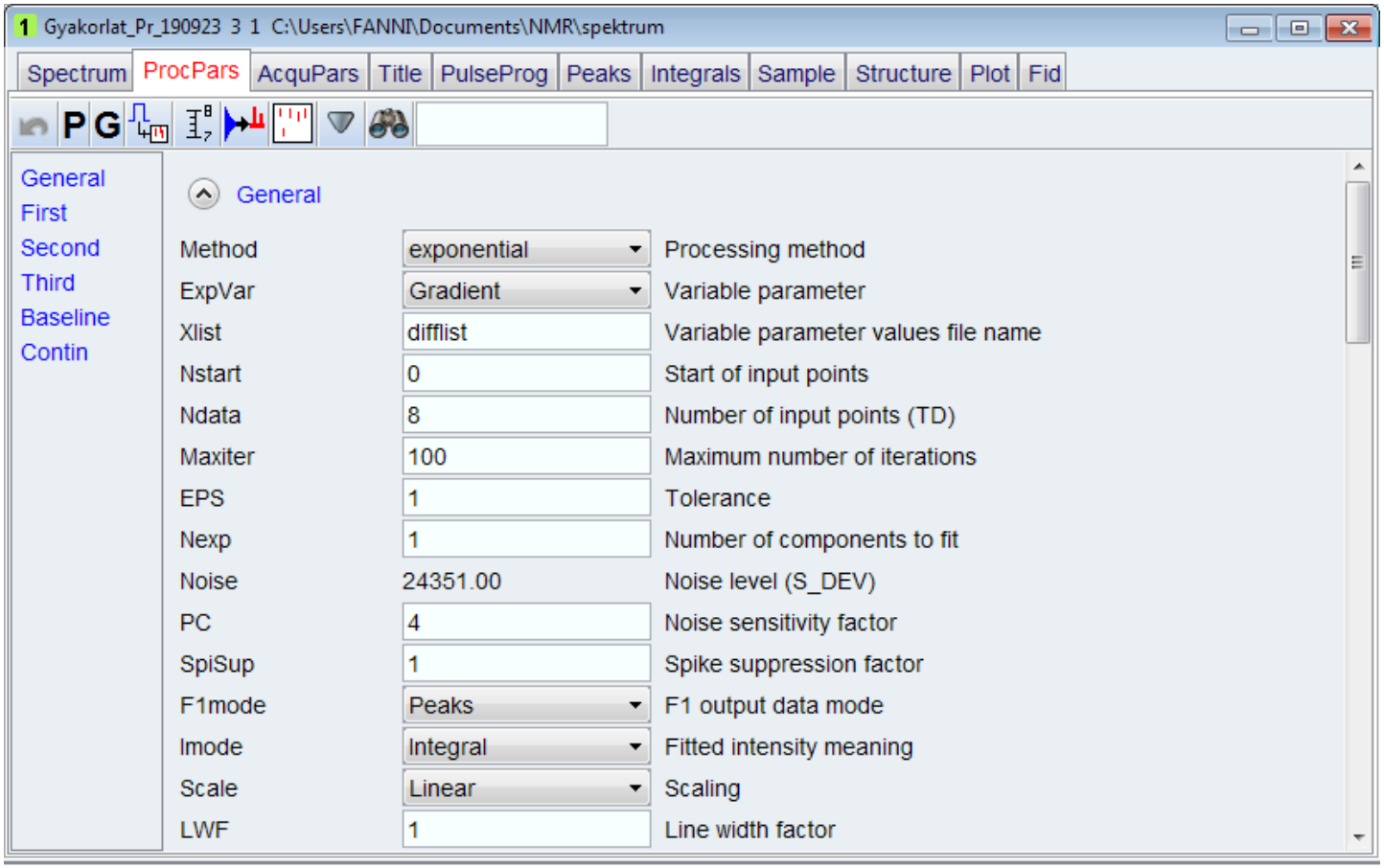
eddosy evaluation

1. Recall the original diffusion measurement (close all other windows opened in TopSpin)
2. *xf2* command (this is the processing, performs Fourier transformation for each spectra)
3. *eddosy* command

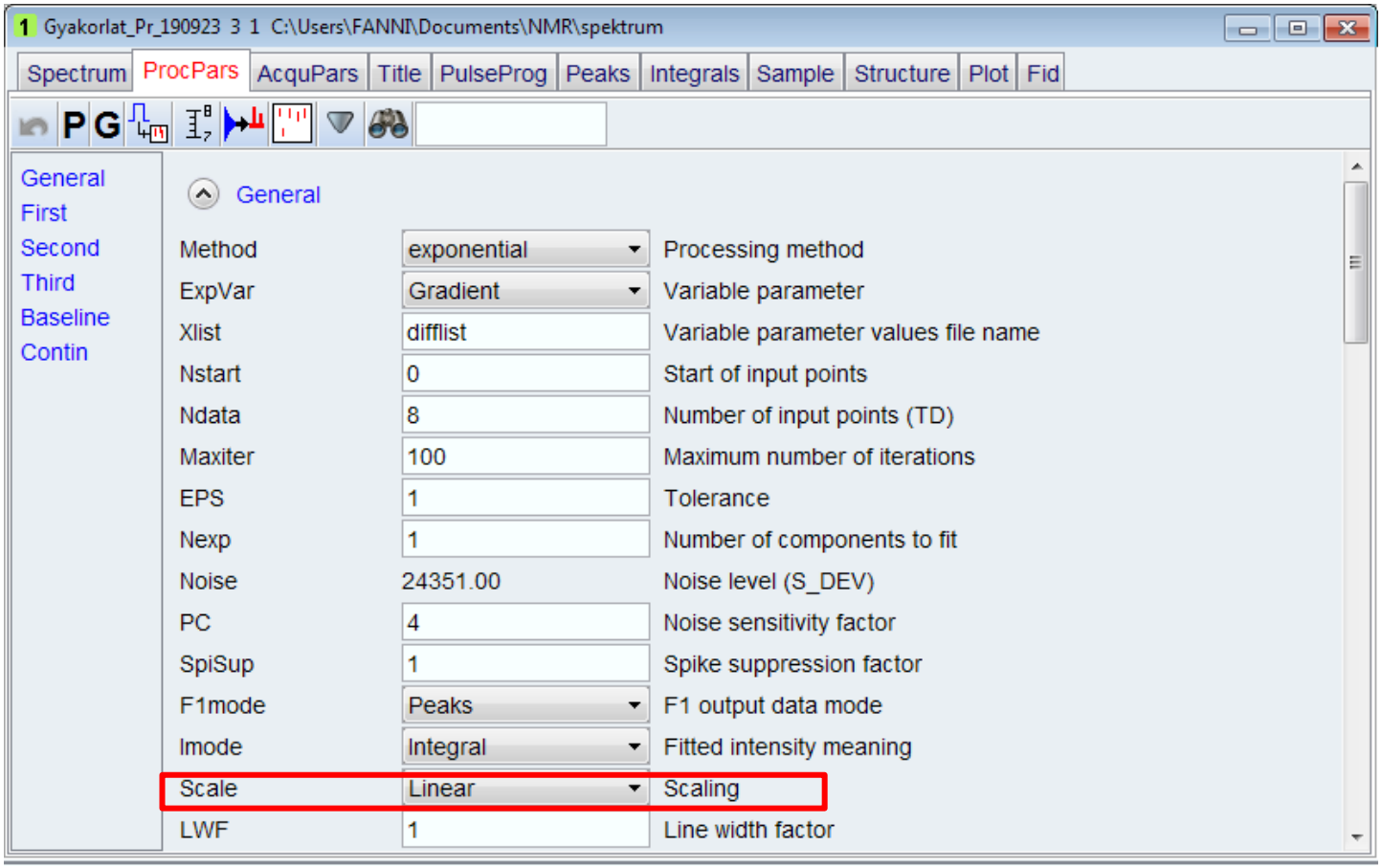
The following window appears, click on OK button



The appearing window:

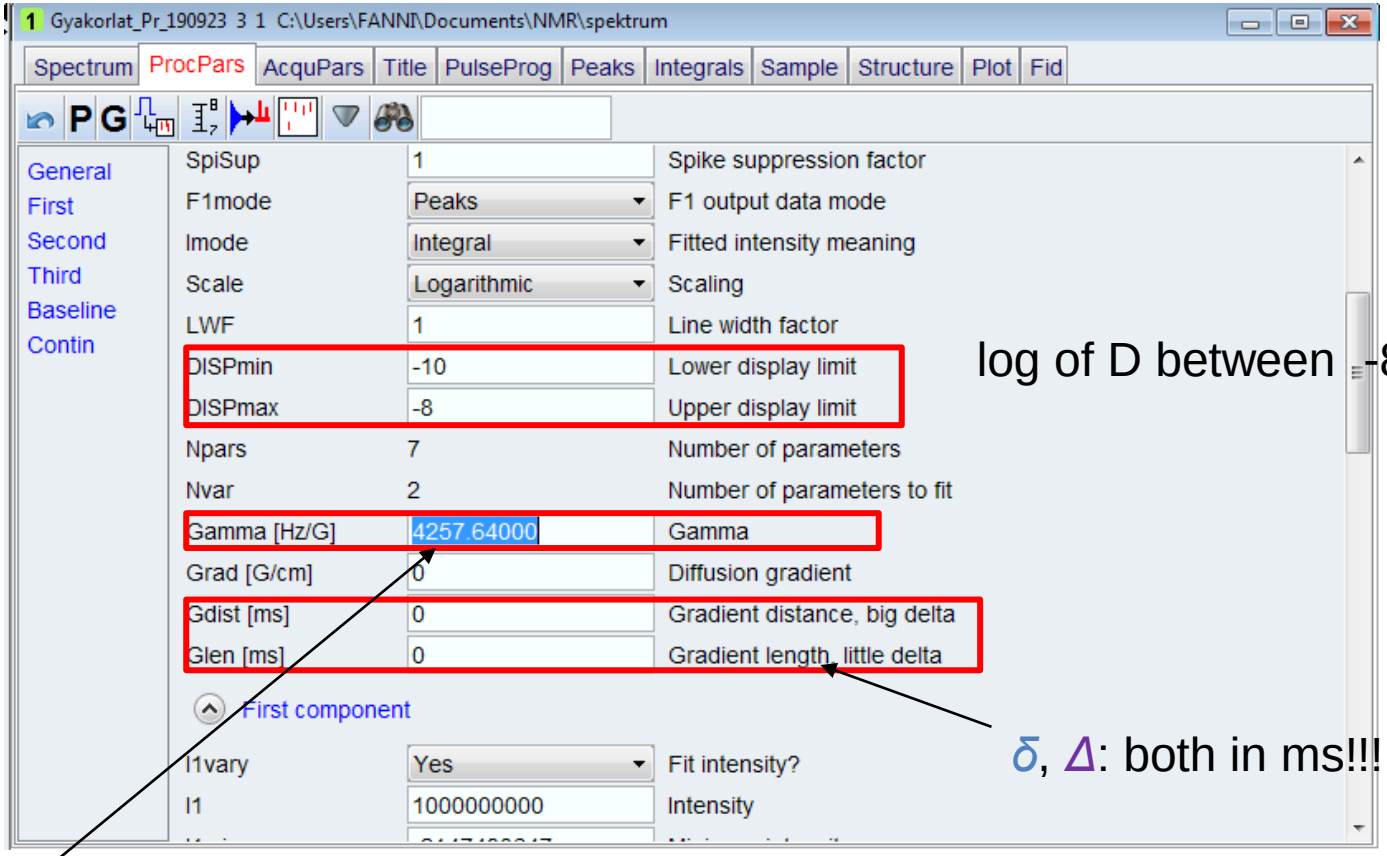


Follow the indications shown in the red boxes!



Change scale instead of Linear to Logarithmic!

The following entries should be updated

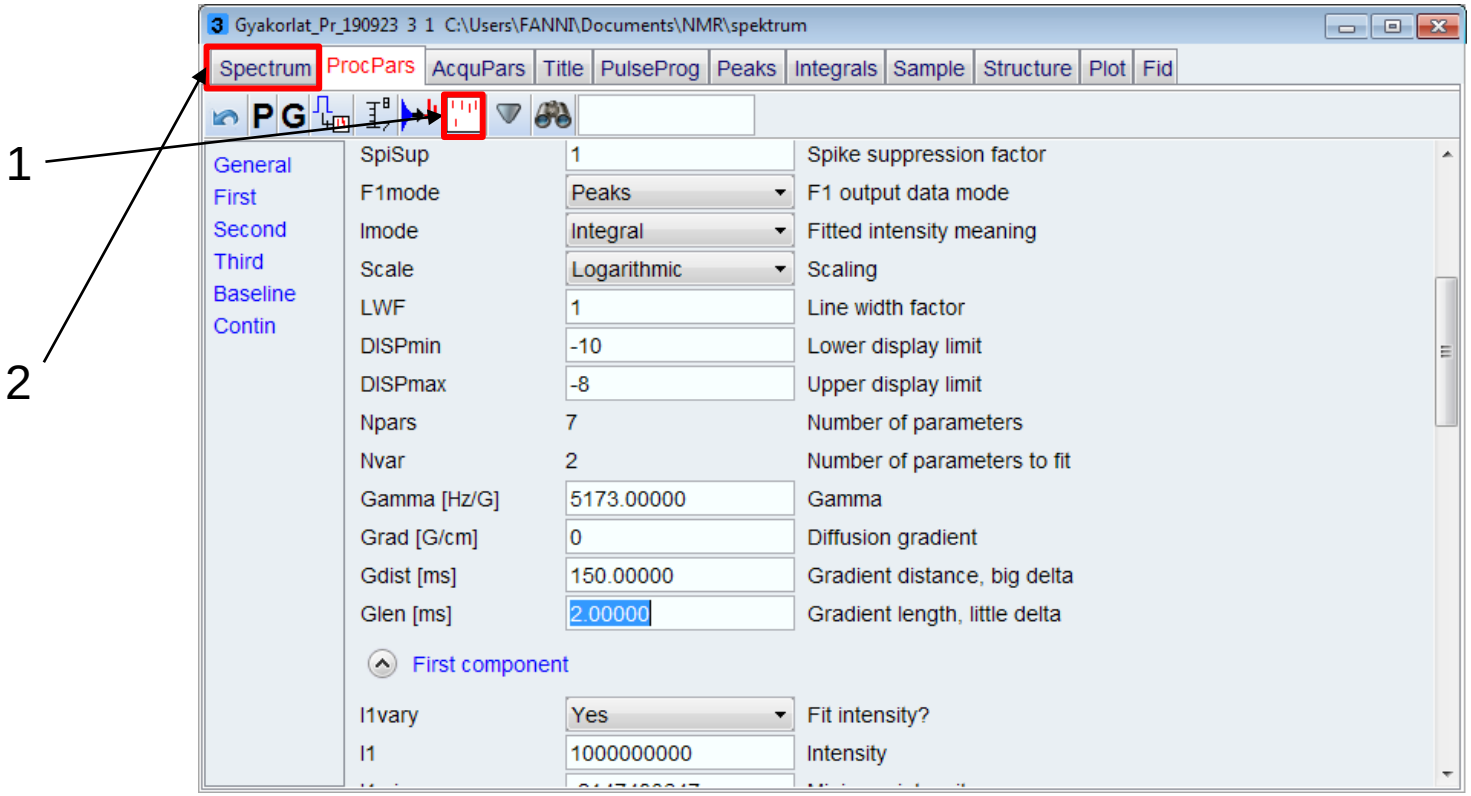


log of D between -8 and -10

δ, Δ: both in ms!!!

GAMMA: 5173 Hz/G

If all is set, then first click on button 1 – performs the calculation, then go to Spectrum (2) and look at the result
Zoom on the SDS signals and read the log*D* value with positioning the cursor in the middle of the signals. On the upper left corner you can read the precise value of the y axis position („Row”)



An example can be found on slide 35.

The report should contain:

- Your names and the date
- Title, and goal of the practical
- Short theoretical description (SDS, micelles, cmc and ways of determination, DOSY, Stejskal-Tanner-equation)
- Steps of the measurement
- Stock solutions, sample preparations
- diffusion parameters (δ,Δ,γ, p1, o1)
- eddosy 2D lg *D*
- 1D spectra for each solution, discussion
- Show a Stejskal-Tanner fitting from your data
- Results in table (*c*, chemical shift (ppm), *D*, average, standard deviation), 1D (perform it for both area and intensity), results from 2D evaluation
- Concentration-*D* curves, calculated cmc
- Check the literature values, comment the discrepancies
- Comments

Send the report in 1 week,
email: abodor@caesar.elte.hu

Recommended literature

https://www.cis.rit.edu/htbooks/nmr/inside.htm

The Basics of NMR

Joseph P. Hornak, Ph.D.