GLYCOTwinning
Building Networks to
Excel in Glycoscience

Summer School
25th—28th September '23
Scientific Hands-on course
in protein/glycan interactions:
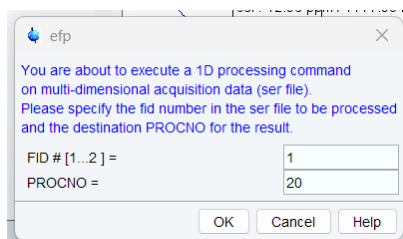a structural perspective.

STD-NMR PROCESSING

1. Open the acquired STD experiment (pseudo-2D)
2. Type **> efp**<ENTER> to process the first FID
   - TopSpin will open a dialog box asking which FID to process (according to the frequency list) and what procno to place the data. A processed spectrum will be saved inside the expno of the STD.


> **Note:** the number of FIDs that you need to process corresponds to the number of frequencies you defined on the FQLIST2.

3. Adjust the phase of the spectrum and <u>save for nD spectrum</u> (so the phase is saved and applied to the next spectra processed)

4. Type **> lb** <ENTER> to change the line broadening value to 1-2 Hz
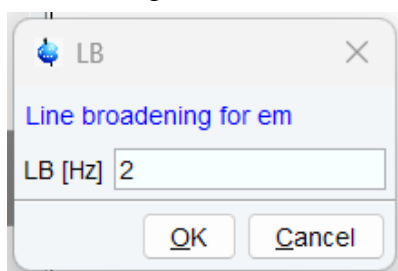
5. Type **> efp** <ENTER> to apply that lb value to that spectrum (TopSpin will ask the FID number)

6. Open the pseudo-2D spectrum again and repeat steps 2, 4 and 5 for the other FIDs.

Note: All FIDs should be processed exactly with the same phase, and Lb.

7. Calibrate each spectrum with the same chemical shift reference

8. In the Multiple Display Mode (**> .md** <ENTER>), open the off-resonance spectrum first and then the on-resonance spectrum. Click on the difference button (**Δ**) to subtract the on-resonance spectrum from the off-resonance. Save the Difference spectrum (**STD = OFF – ON**) to another procno.

STD = Off resonance − On resonance

$$I_{STD} = I_0 - I_{sat}$$
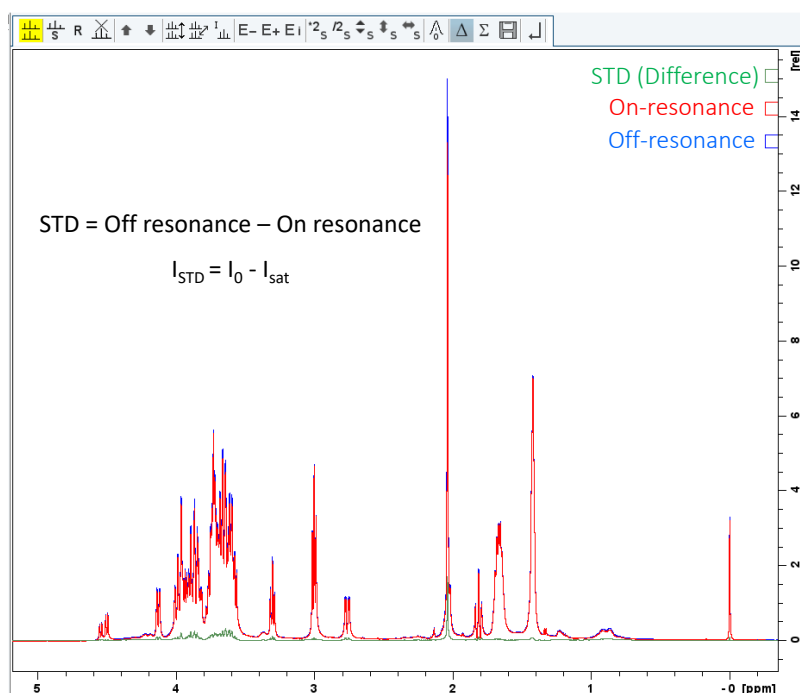
STD-NMR Analysis for Epitope Mapping Determination

The STD response of each proton is calculated by comparing STD spectrum with the off-resonance spectrum and estimated the fraction I_{STD}/I_0. The STD response of each proton is required to further build the STD-derived epitope map of the ligand.

Herein, we described two methods to estimate the STD response.

  a) Integrals method: measure the integrals of the signals in the off-resonance and in the STD spectra and make the ratio.
  b) Manually scaling intensity method: manually scale the off-resonance spectrum intensity to match the shape and intensity of each signal of the STD spectrum.

The first method is ideal when the ligand's signals are well resolved and do not have overlapping signals, which allows the use of the same integration regions for both spectra. However, when working with more complex ligands, such as oligosaccharides, the integral method could be much more difficult to apply and thus the manual scaling intensity method is preferred.

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a) Integrals method

1. Open the off-resonance spectrum and in the **Analyse** tab select the **Integrate** function
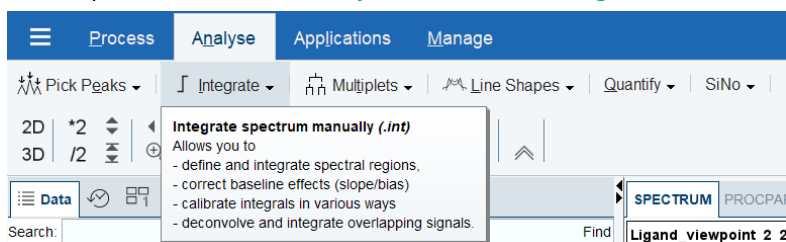
2. Click on the button *Define new region* and click and drag with the cursor to define an integration region for each signal. When every integration region is defined, click on *Save/Export integration regions,* and select *Export integration regions*. A window will pop up; you should click on *Write new* and give a name to the file and press *OK*. Then *return and save regions*. On the *INTEGRALS* tab of the spectrum, a table with the defined integrals should appear.

3. Open the STD spectrum and select the *Integrate* function. Click on *Read/Import integration regions*, select *Import integration regions* and select the file created in step 2. Then click on *return and save regions.* The regions defined for the off-resonance spectrum will be applied to the STD spectrum and the table with the values is found in the *INTEGRALS* tab of the spectrum.
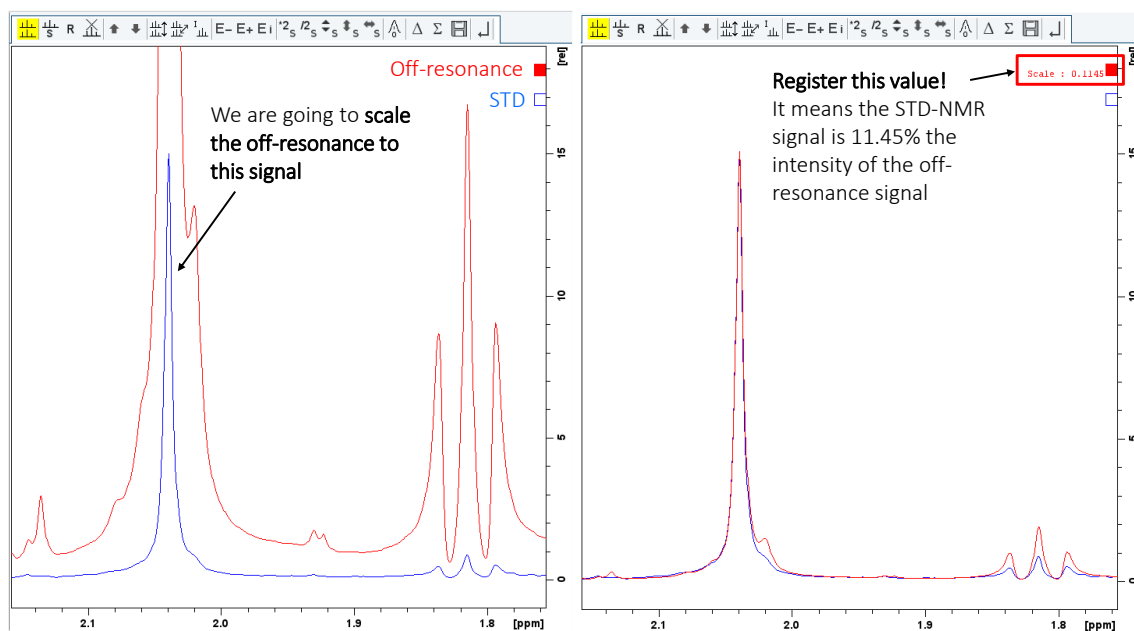
4. Copy the integral values of both tables to an Excel sheet and calculate I_{STD}/I_0, where I_{STD} is the integral of the signal in the STD spectrum and I_0 is the integral of the signal in the off-resonance spectrum.

5. To calculate the STD derived epitope map normalize the I_{STD}/I_0 values for each proton by setting the highest one to 100% and calculating all others accordingly.

b) Manual scaling intensity method

1. Open the STD spectrum and select multiple display to then open the off-resonance spectrum in the superimposed mode.

2. Select the off-resonance spectrum and scale the spectrum to match the shape and intensity of a signal of the STD spectrum. Register the scale value, which corresponds to the I_{STD}/I_0 ratio.

Off-resonance

STD

We are going to **scale the off-resonance to this signal**

Register this value!
It means the STD-NMR signal is 11.45% the intensity of the off-resonance signal

Scale : 0.1145

3. Repeat the scaling step for each signal of the spectra and register the values.

4. To calculate the STD derived epitope map normalize the I_{STD}/I_0 values for each proton by setting the highest one to 100% and calculating the others accordingly.