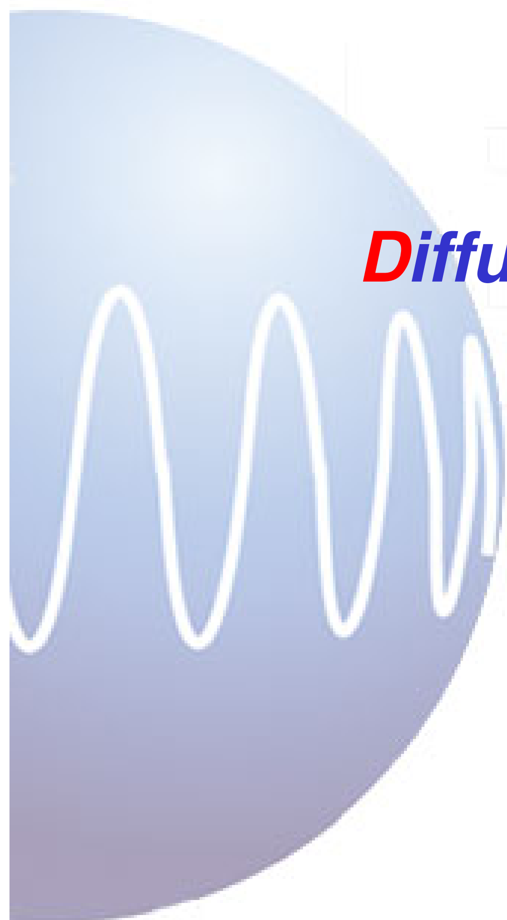


DOSY

***D**iffusion **O**rdered **S**pectroscop**Y** (**DOSY**)*

An Introduction

DOSY and Diffusion

DOSY:

- Separation of signals from different components
- Separation is based on differences in diffusion rates of the compounds
- A reasonable difference in the diffusion rates is required for successful separation
- Relative diffusion rates are determined
- Careful calibration of gradient strength of the spectrometer is NOT required

In contrast to the DOSY experiment:

Diffusion Experiments

- Absolute diffusion rates are determined
- Careful calibration of gradient strength of the spectrometer IS required

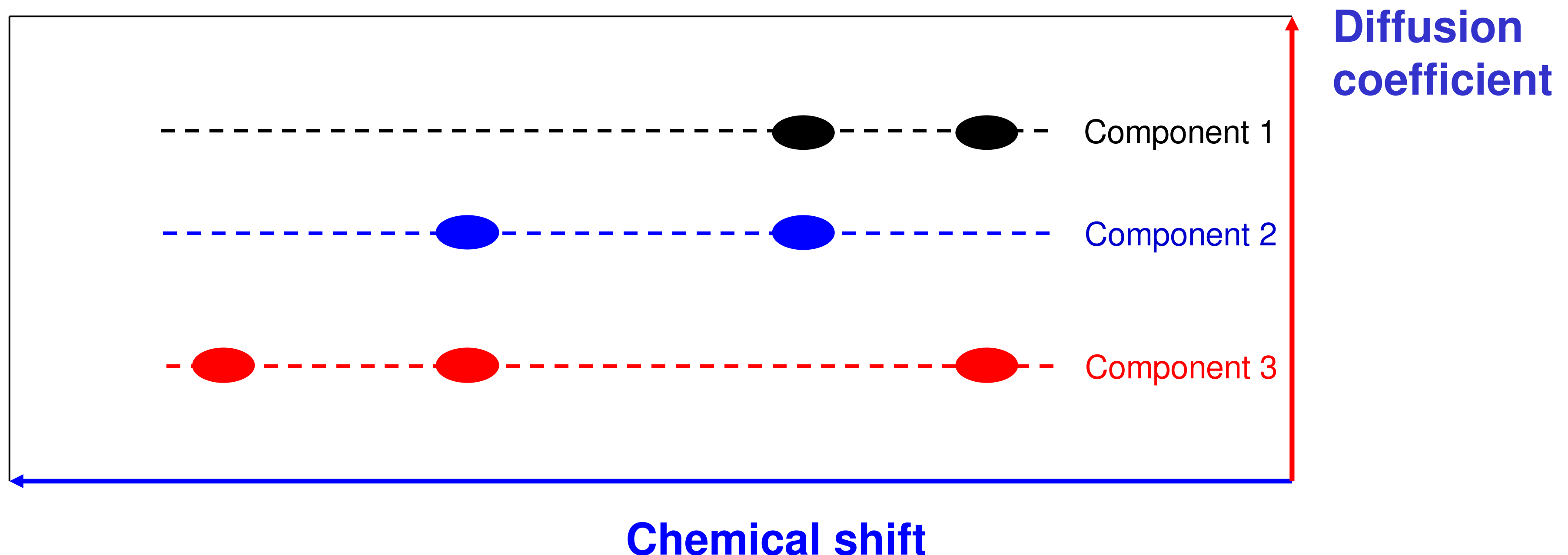
DOSY and Diffusion

DOSY and diffusion experiments have in common:

- identical experiments
- same pulse programs can be used

DOSY and diffusion experiments differ in:

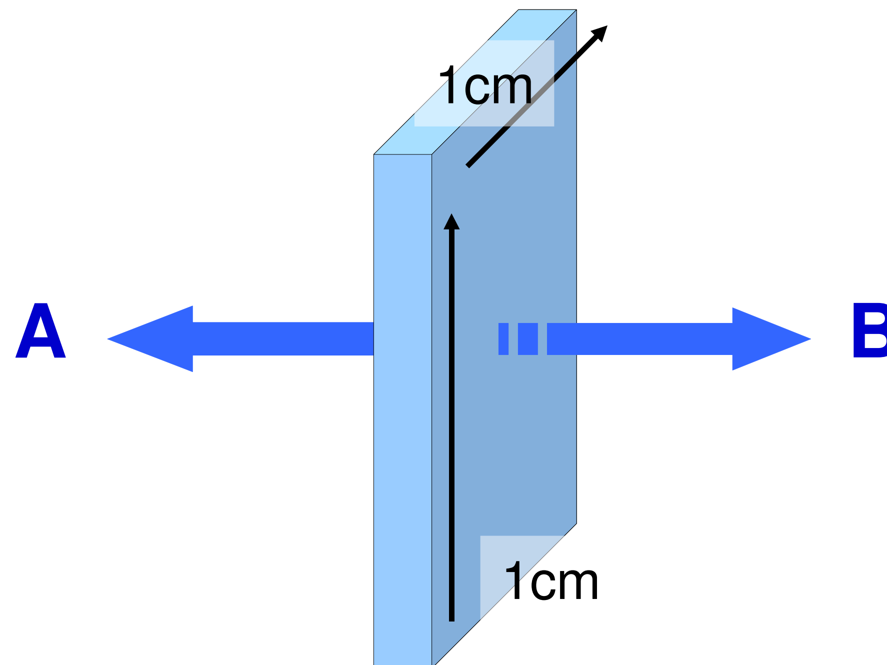
- processing
- diffusion experiments require careful calibration of the gradient constant



Translational Diffusion

$J = dn/dt/A$

J	=	*flux*
dn/dt	=	*number molecules transported/sec*
A	=	*sampling area of the reference plane*



Translational Diffusion

$$I(q) = I_o \, e^{-Dq^2 \Delta'}$$

D : diffusion coefficient

Δ' : $\Delta - \delta/3$

Δ : diffusion time and δ is a correction factor for finite gradients

q : $\gamma \, g \, \delta$

γ : gyromagnetic ratio

g : is the amplitude of the applied gradient

δ : duration of the applied gradient

I(q) depends on:

size, shape, aggregation etc. of molecule

temperature

viscosity

Translational Diffusion

π

rf

G_z

A

B

z

Effective Field

Sample

B

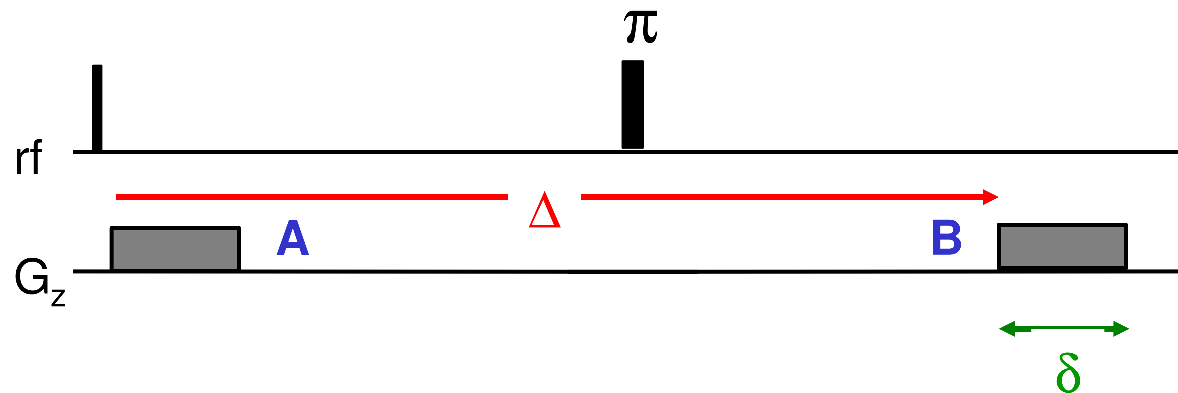
A

B

B_0

$B(z)$

Simple Pulse Sequence and Parameters



Acquisition parameters:

G_z: gradient strength

Δ: ‚big delta‘

diffusion takes place during this delay

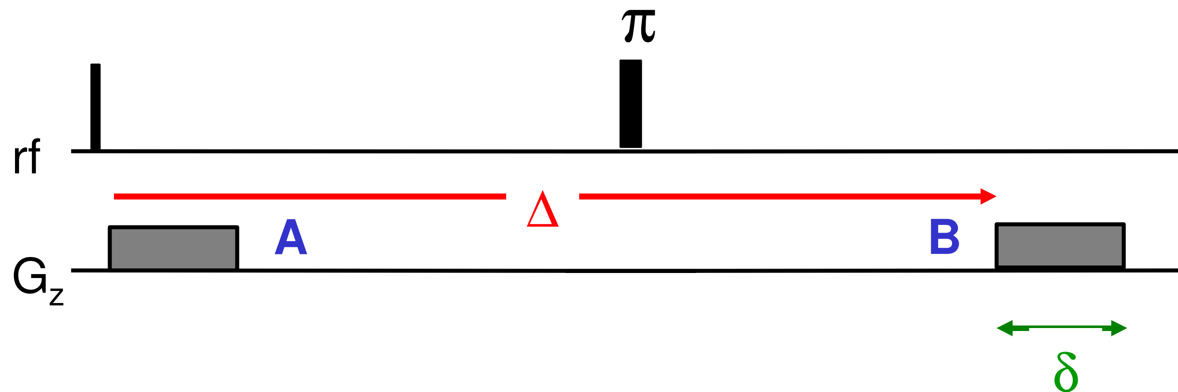
δ: ‚little delta‘

duration of the gradient pulse

diffusion also takes place during gradient pulses

As the name says, the duration for ‚big delta‘ is longer than ‚little delta‘

Simple Pulse Sequence and Parameters

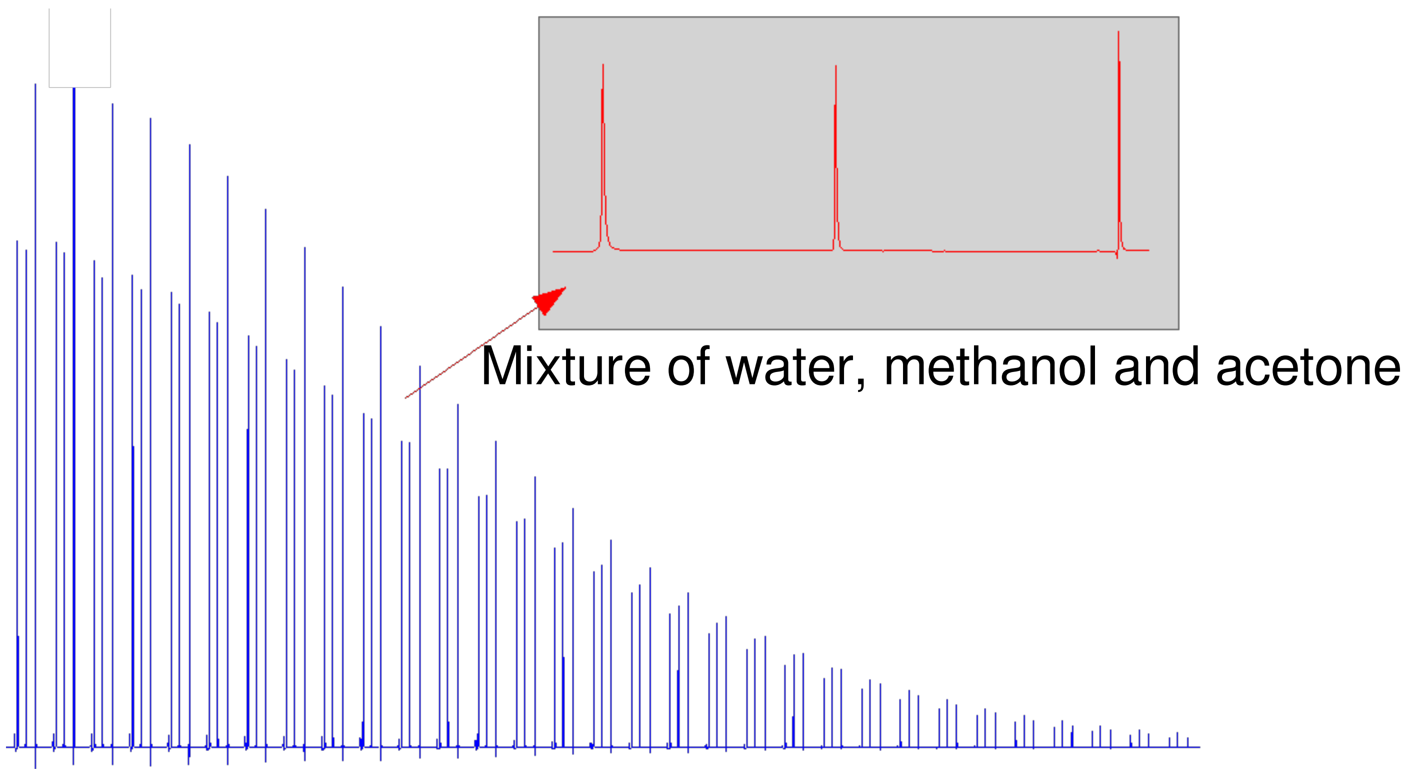


The DOSY / diffusion experiment:

- In principle, the parameters G_z, Δ or δ can be varied for a gradient experiment
- Varying Δ or δ will vary the length of the pulse sequence and T_2 relaxation effects will add to diffusion effects
- To avoid relaxation effects the gradient strength G_z is varied

Translational Diffusion

Decay of magnetization as function of gradient intensity



Setup DOSY / Diffusion Experiment

1. Start from a ^{1}H spectrum:

- Switch OFF sample rotation
- Create new data set, change to 2D-mode
- TD{F1}: 24 – 32 (gradient ramp with 24 – 32 different gradient values)
- PULPROG: stebpgp1s or ledbpgp2s
- TD: 16k - 32k
- D20: 100ms (‚big DELTA‘: needs to be adjusted according to diffusion rates of compounds)
- D21: 5ms (eddy current delay)
- GPNAM*: SINE.100 (square gradient can also be used, e.g. SMSQ10.100)
- P19: 600us (spoil gradient)
- P30: 2 – 5ms (‚little DELTA‘: needs to be adjusted according to diffusion rates of compounds)

Note: for CryoProbes, max p30=4ms, GPNAM* = SINE.SHAPE

Setup DOSY / Diffusion Experiment

2. Calculate gradient ramp and start experiment:

- Use AU program *„dosy"* to calculate the gradient ramp:

  xau dosy 2 95 32 l n

  (first gradient: 2%, last gradient 95%, 32 gradient values using a linear ramp, do not start the acquisition)

- Adjust receiver gain: *"rga"*
- Start experiment: *"zg"*

3. Optimize values for little / big delta:

- Process data with *„xf2"* and phase correction
- Check signal decay:

  Signals should decay to 5-10% of the original intensity

  Decay incomplete: increase D20 and/or P30

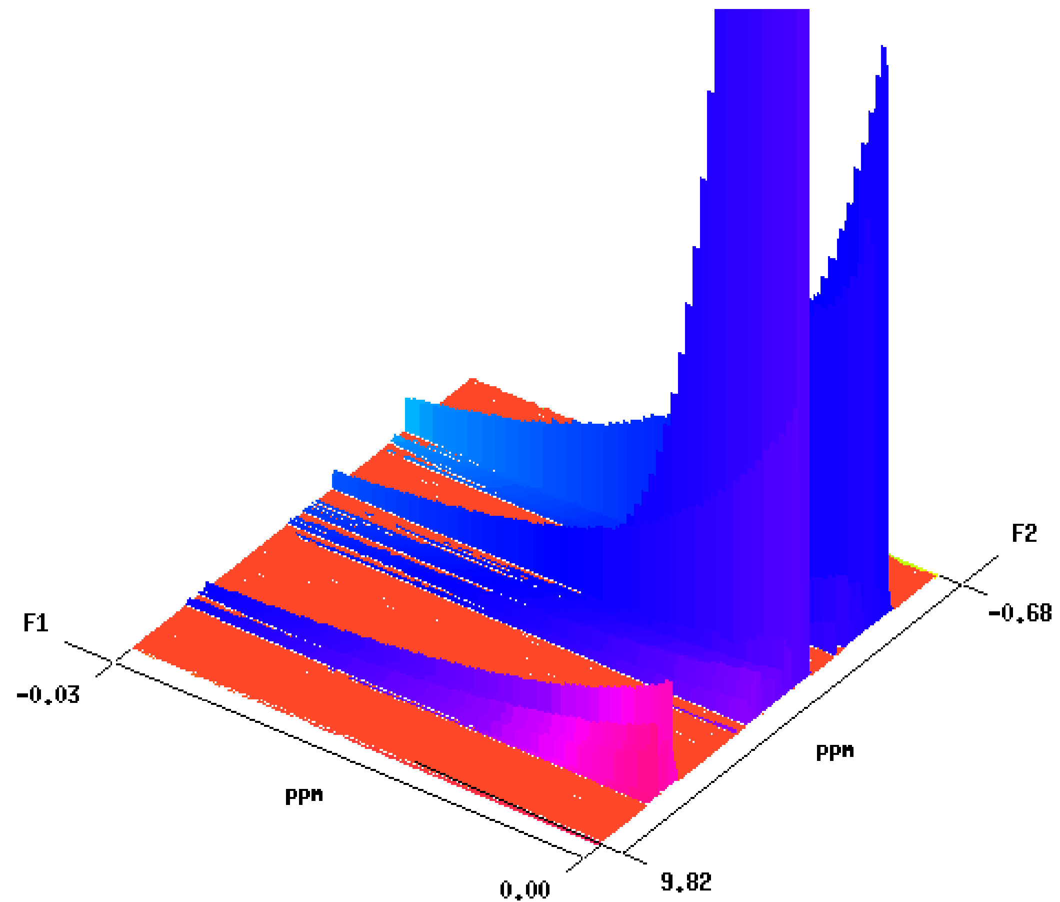
  Decay too fast: decrease D20 and/or P30

- Repeat acquisition

Setup DOSY / Diffusion Experiment

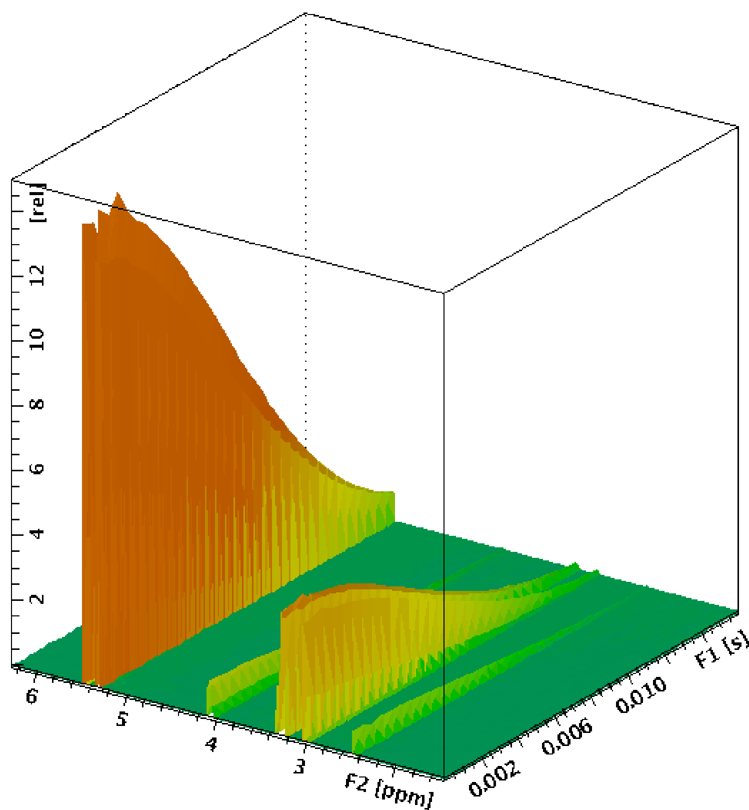
The decay can be easily checked from the stacked view after ‘xf2’

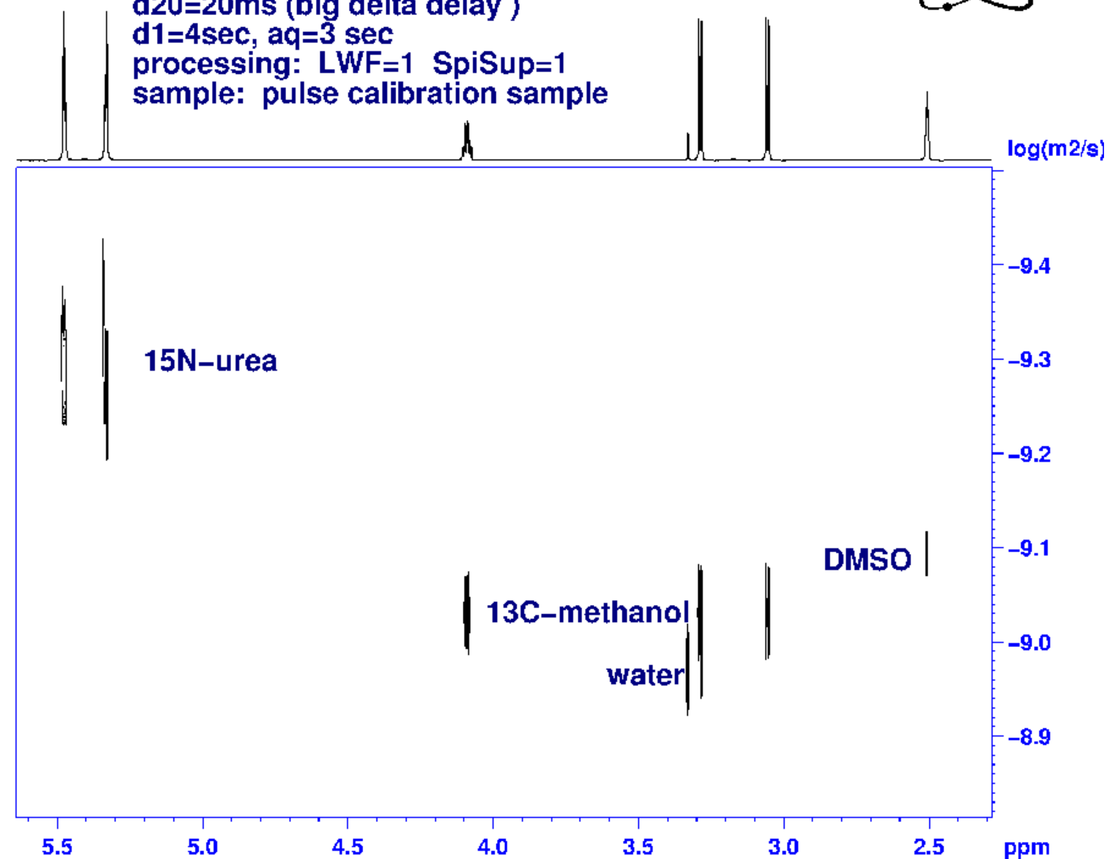
Stacked plot of a diffusion spectrum after F2 transformation. On the F2 axis some of the strong signals of a peptide mixture are visible. The F1 axis shows the decay of the signals with increased gradient strength due to the diffusion of the molecules in solution.



Ex. the pulse calibration sample

optimal decay
d20=100ms

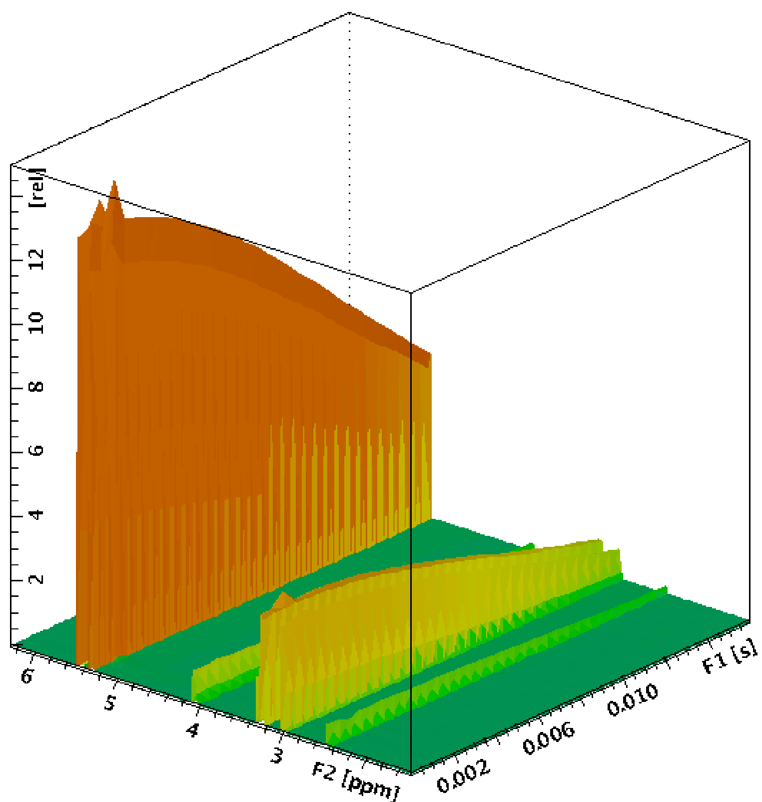




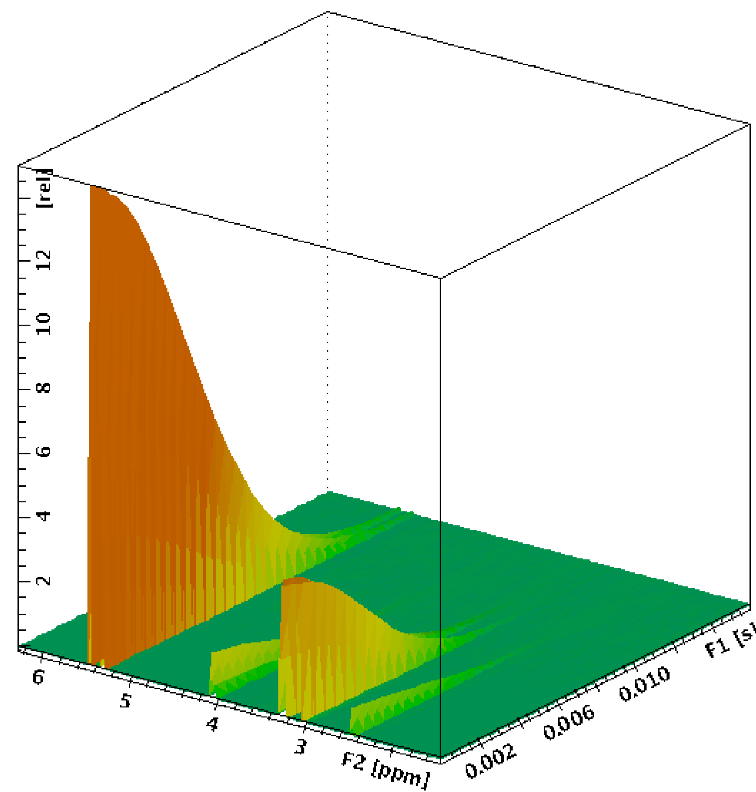
Note: for CryoProbes, max p30=4ms, GPNAM* = SINE.SHAPE

Ex. the pulse calibration sample

decay incomplete
d20=50ms



decay too fast
d20=200ms



DOSY processing

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1. Setup processing parameters:

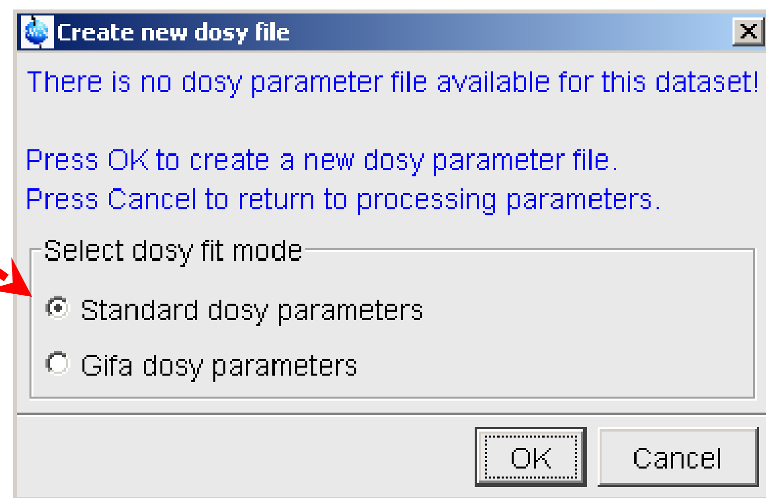
- xau setdiffparm — copy acquisition parameters to DOSY processing parameters (little delta, big DELTA, gamma,..)
- edp — edit parameters for FT in F2-dimension and number of points in the diffusion (F1) dimension

  Note: DOSY processing requires good S/N, therefore window functions like EM with high values for LB, e.g. LB=5 might be required.
- eddosy — edit processing parameters

2. Processing:

- xf2 — F2 Fourier transformation
- Phase correction
- abs2 — F2 automatic base line correction
- dosy2d, dosy3d — calculate the 2D or 3D DOSY spectrum

eddosy parameters



eddosy parameters

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Fid

General
First
Second
Third
Baseline

General

Parameter	Value	Description
FITMODE =	exponential	Type of fit
ExpVar =	Gradient	Variable parameter
Xlist =	difflist	Variable parameter values file name
Nstart =	0	Start of input points
Ndata =	16	Number of input points (TD)
Maxiter =	100	Maximum number of iterations
EPS =	1	Tolerance
Nexp =	1	Number of components to fit
Noise =	63637.00	Noise level (S_DEV)
PC =	4	Noise sensitivity factor
SpiSup =	1	Spike suppression factor
F1mode =	Peaks	F1 output data mode
Imode =	Integral	Fitted intensity meaning
Scale =	Linear	Scaling
LWF =	1	Line width factor
DISPmin =	1e-010	Lower display limit
DISPmax =	1e-008	Upper display limit
Npars =	7	Number of parameters
Nvar =	2	Number of parameters to fit
Gamma [Hz/G] =	4257.64000	Gamma
Grad [G/cm] =	0.00000	Diffusion gradient
Gdist [ms] =	0.00000	Gradient distance, big delta
Glen [ms] =	0.00000	Gradient length, little delta

Parameters will be set with xau dosy (ExpVar, Xlist, Nstart, Ndata)

Parameters will be set with xau setdiffparm (Gamma, Grad, Gdist, Glen)

First component

Parameter	Value	Description
I1vary =	Yes	Fit intensity?
I1 =	1000000000	Intensity
I1min =	-2147483647	Minimum intensity
I1max =	2147483647	Maximum intensity
D1vary =	Yes	Fit diffusion coefficient?
D1 [m²/s] =	1e-009	Diffusion coefficient
D1min [m²/s] =	0	Minimum diffusion coefficient
D1max [m²/s] =	1e-008	Maximum diffusion coefficient

eddosy parameters

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Fid

General
First
Second
Third
Baseline

General			
FITMODE =	exponential	Type of fit	
ExpVar =	Gradient	Variable parameter	
Xlist =	difflist	Variable parameter values file name	
Nstart =	0	Start of input points	
Ndata =	16	Number of input points (TD)	
Maxiter =	100	Maximum number of iterations	
EPS =	1	Tolerance	
Nexp =	1	Number of components to fit	
Noise =	63637.00	Noise level (S_DEV)	
PC =	4	Noise sensitivity factor	*PC: same function as for 'pp'*
SpiSup =	1	Spike suppression factor	*Suppression of 'tails' on peaks*
F1mode =	Peaks	F1 output data mode	
Imode =	Integral	Fitted intensity meaning	
Scale =	Linear	Scaling	
LWF =	1	Line width factor	*Line broadening in diffusion dimension*
DISPmin =	1e-010	Lower display limit	***DOSY peaks will be calculated and displayed***
DISPmax =	1e-008	Upper display limit	***within those values only!!!***
Npars =	7	Number of parameters	
Nvar =	2	Number of parameters to fit	
Gamma [Hz/G] =	4257.64000	Gamma	
Grad [G/cm] =	0.00000	Diffusion gradient	
Gdist [ms] =	0.00000	Gradient distance, big delta	
Glen [ms] =	0.00000	Gradient length, little delta	
First component			
I1vary =	Yes	Fit intensity?	
I1 =	1000000000	Intensity	
I1min =	-2147483647	Minimum intensity	
I1max =	2147483647	Maximum intensity	
D1vary =	Yes	Fit diffusion coefficient?	
D1 [m²/s] =	1e-009	Diffusion coefficient	
D1min [m²/s] =	0	Minimum diffusion coefficient	
D1max [m²/s] =	1e-008	Maximum diffusion coefficient	

eddosy in ProcPars

eddosy setdiffparm xf2 dosy2d

General
First
Second

Copy parameters from experiment (setdiffparm)

FITMODE =	exponential	Type of fit
ExpVar =	Gradient	Variable parameter

Optimizing DOSY processing parameters

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1. No peaks at all in the DOSY spectrum:

- Are the correct values for big / little delta *(D20 / P30)* entered in eddosy?
- Do the display limits *DISPmin / DISPmax* in eddosy correspond to the expected diffusion rates?
- Check peak picking parameter PC

2. Poor separation of peaks:

- Effect not related to processing parameters:

  Difference in diffusion rates is too small

  Poor S/N, record spectrum again with more scans

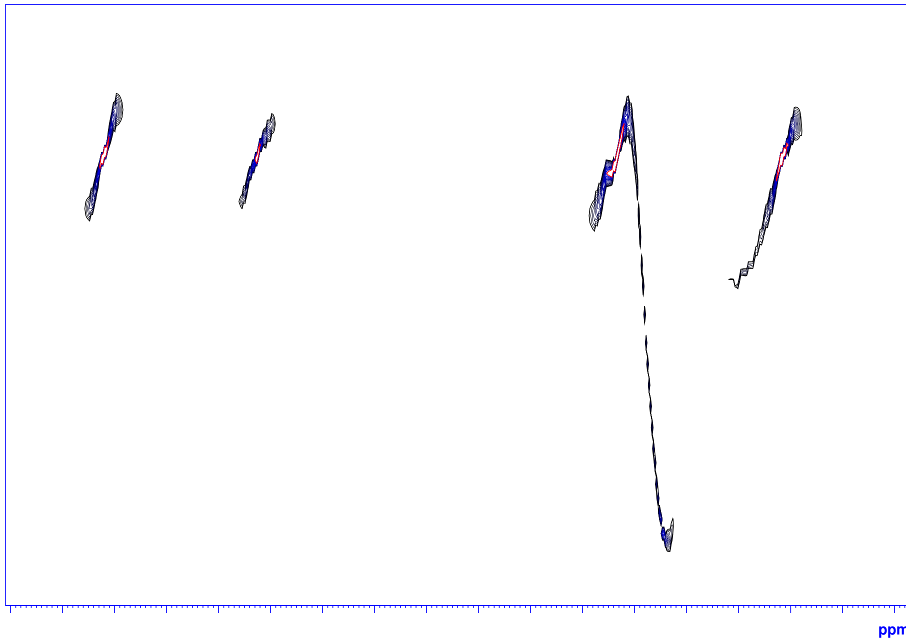
- Change eddosy parameters:

SpiSup	larger values will *reduce tails* on peaks, but separation in F2 dimension will be reduced
LWF	values <1 will result in *better resolution* in diffusion dimension, but truncation artifacts might appear

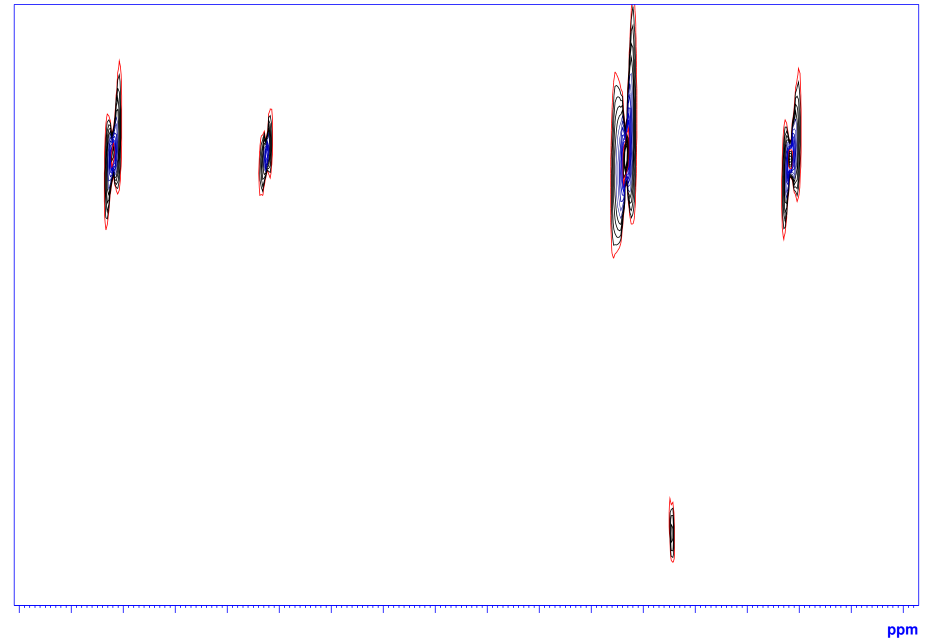
DOSY processing: example for LWF

LWF: broadening in diffusion dimension, reduces truncation artifacts

LWF 0.3, SpiSup 1



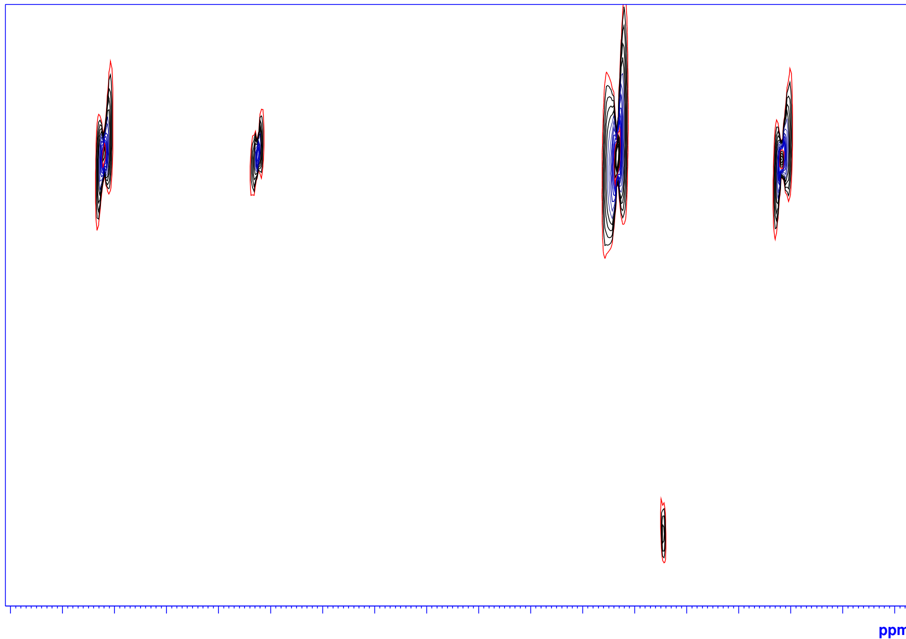
LWF 4, SpiSup 1



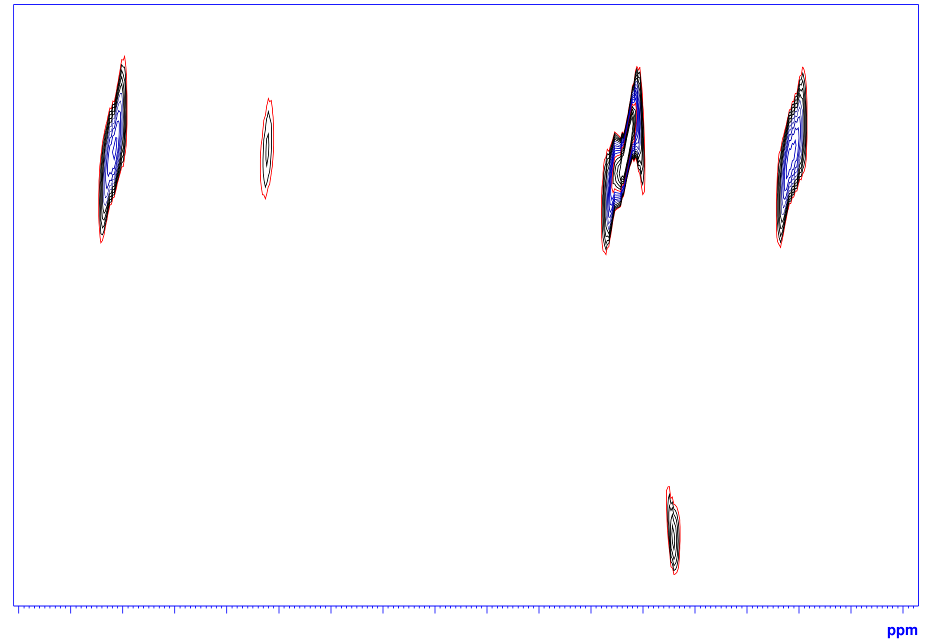
DOSY processing: example for SpiSup

SpiSup: broadening in chemical shift dimension, reduces tails

LWF 4, SpiSup 1



LWF 4, SpiSup 100



DOSY: data

Data can also be analysed with the Topspin T_1/T_2 tool

F1 axis shows diffusion constant:

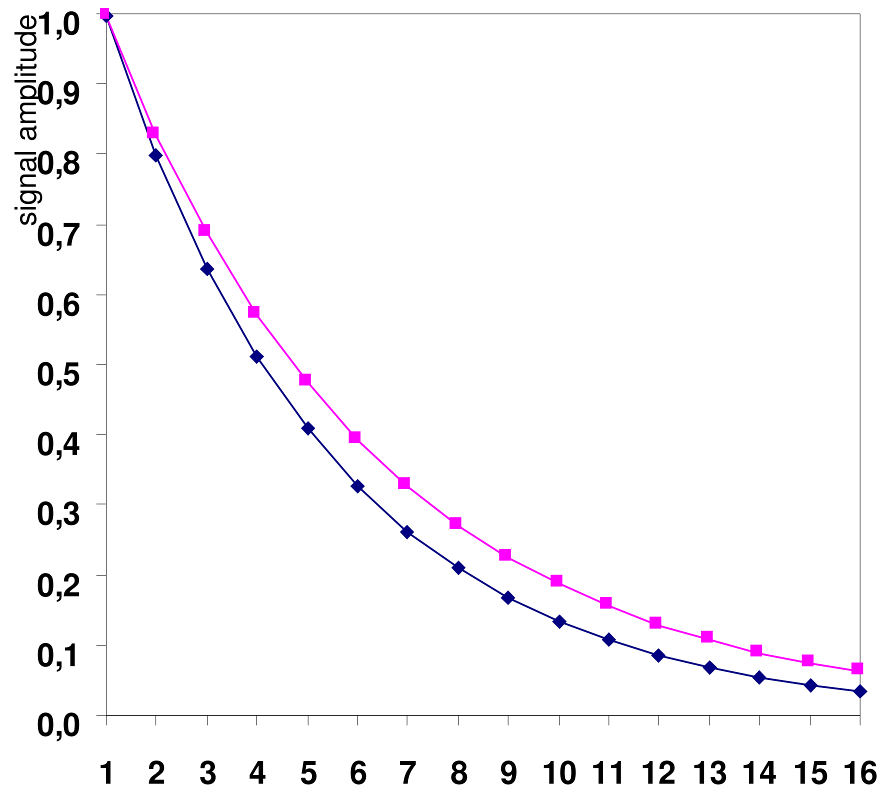
- logarithmic: log D
- linear: D * $1e^9$
- The unit for the diffusion constant is: m^2/sec

DOSY: sources of artifacts

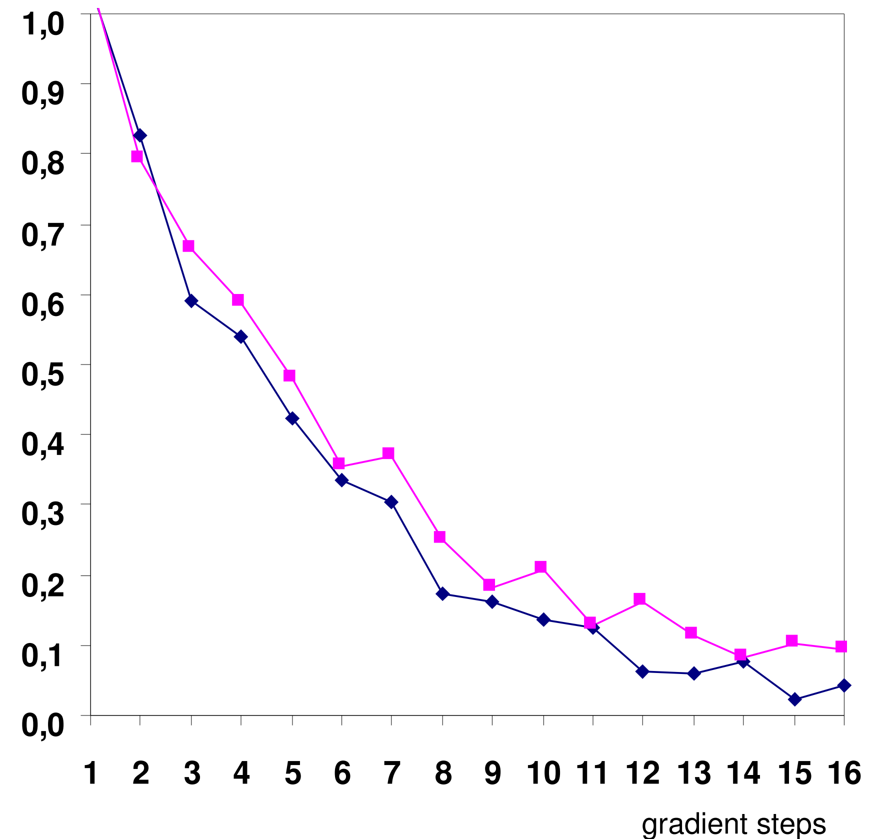
1. Low signal-to-noise ratio

–Poor description of signal decay and bad separation of signals in diffusion dimension

Signal decay: high S/N



Signal decay: low S/N



DOSY: sources of artifacts

2. Convection

self diffusion measurements of a peptide in DMSO at different temperatures and with different pulse sequences. The signal amplitude is plotted versus the square of the gradient strength.

DOSY: effects of convection

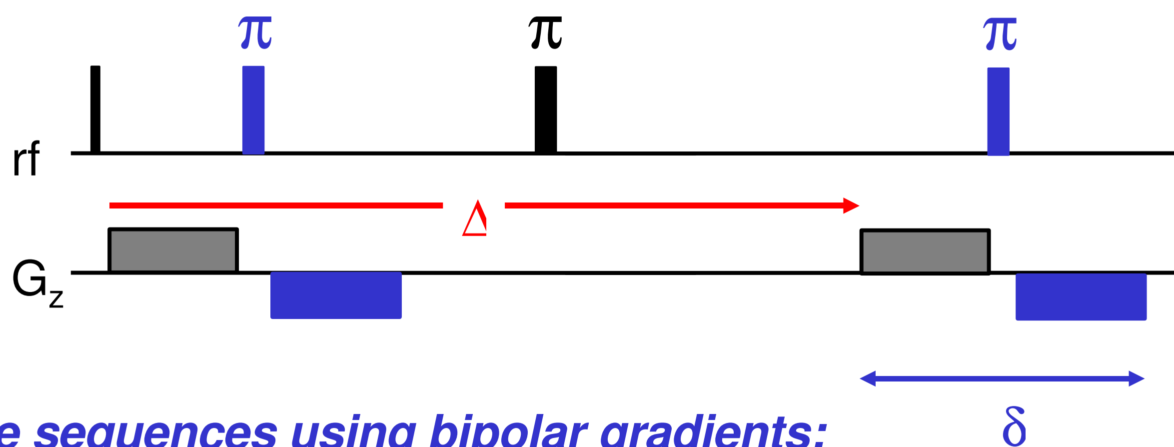
2. Convection

– Reduce convection by:

High flow rates for temperature gas

Capillary tube instead of 5 mm tube

– Use pulse sequences with bipolar gradient to reduce the influence of convection in the DOSY / diffusion experiment



Pulse sequences using bipolar gradients:
stegp1s (ste=stimulated echo)
ledgp2s (led=longitudinal eddy current delay)

Note: for CryoProbes, max p30=4ms, GPNAM* = SINE.SHAPE

Example: two water species

Intra- and inter cellular water

log D
-8.0
-8.4
-8.8
-9.2
-9.6
-10.0

4.85 4.80 4.75 4.70 4.65 4.60 4.55 ppm

Example: mixture of four esters

P-Hydroxybenzoic acid ester
R= -Me, -Et, -Prop, -But

Log D

-8.98
-9.00
-9.02
-9.04
-9.06
-9.08
-9.10
-9.12
-9.14

5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm

Example: mixture of four esters

P-Hydroxybenzoic acid ester
R= -Me, -Et, -Prop, -But

R= -But

Prop

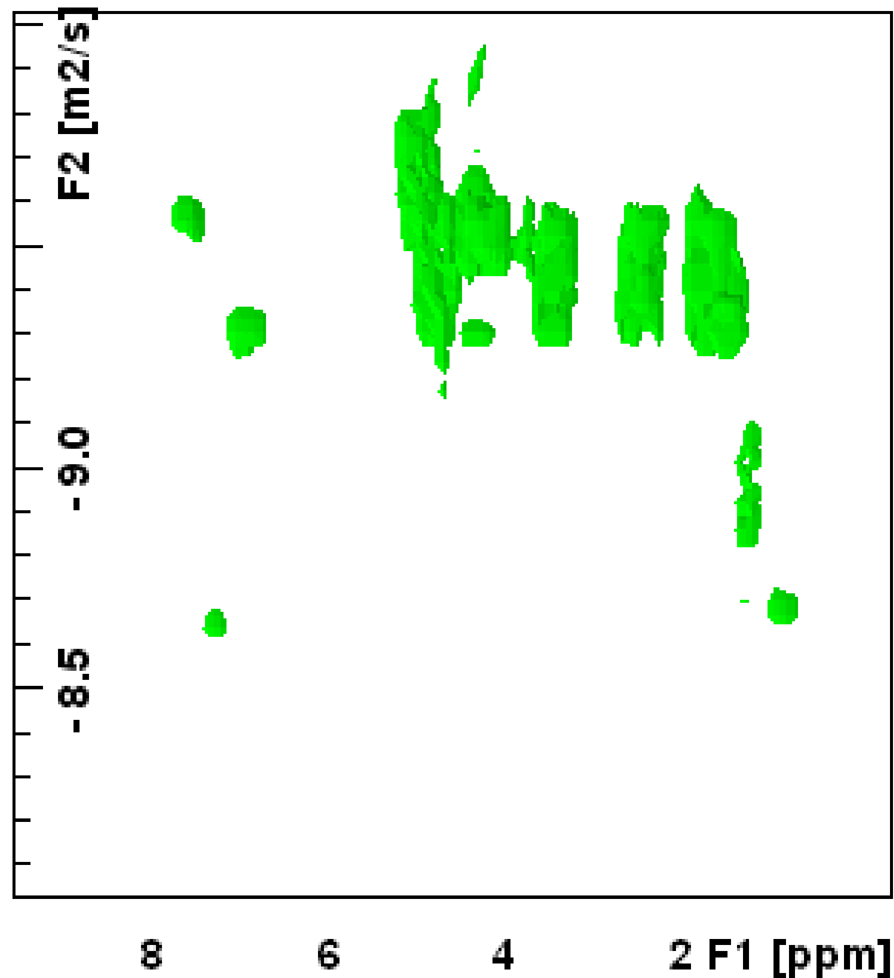
Et

Me

5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm

Example: 3D DOSY-TOCSY

Projection view of the diffusion separation of compounds



Example: 3D DOSY-TOCSY

3D view shows TOCSY correlation peaks

DOSY: pulse and AU programs

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AU Programs

- dosy, setdiffparm

Pulse Programs (1D-versions not shown)

Stimulated Echo Sequences:

- stegp1s, stebpgp1s — bp: bipolar gradients
- stebpgp1s19 — 19: 3919 WATERGATE
- stebpgpin1s — in: INEPT, for X-nuclei

Double Stimulated Echo Sequences:

- dstegp3s

DOSY: pulse and AU programs

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Stimulated Echo plus LED Sequences:

- ledgp2s
- ledbpgp2s — bp: bipolar gradients
- ledbpgpml2s2d — ml: diffusion filtered 2D TOCSY
- ledbpgpml2s192d — 19: additional WATERGATE
- ledbpgpco2s3d — co: 3D DOSY-COSY
- ledbpgpml2s3d — ml: 3D DOSY-TOCSY
- ledbpgpno3s3d — no: 3D DOSY-NOESY

Files used for DOSY and diffusion

Gradient calibration constant is stored in:

XWINNMRHOME/conf/instr/gradient_calib

The gradient ramp (created with *'xau dosy')* is stored in:

XWINNMRHOME/exp/stan/nmr/lists/gp/Difframp

For calculation of diffusion values the following file is used:

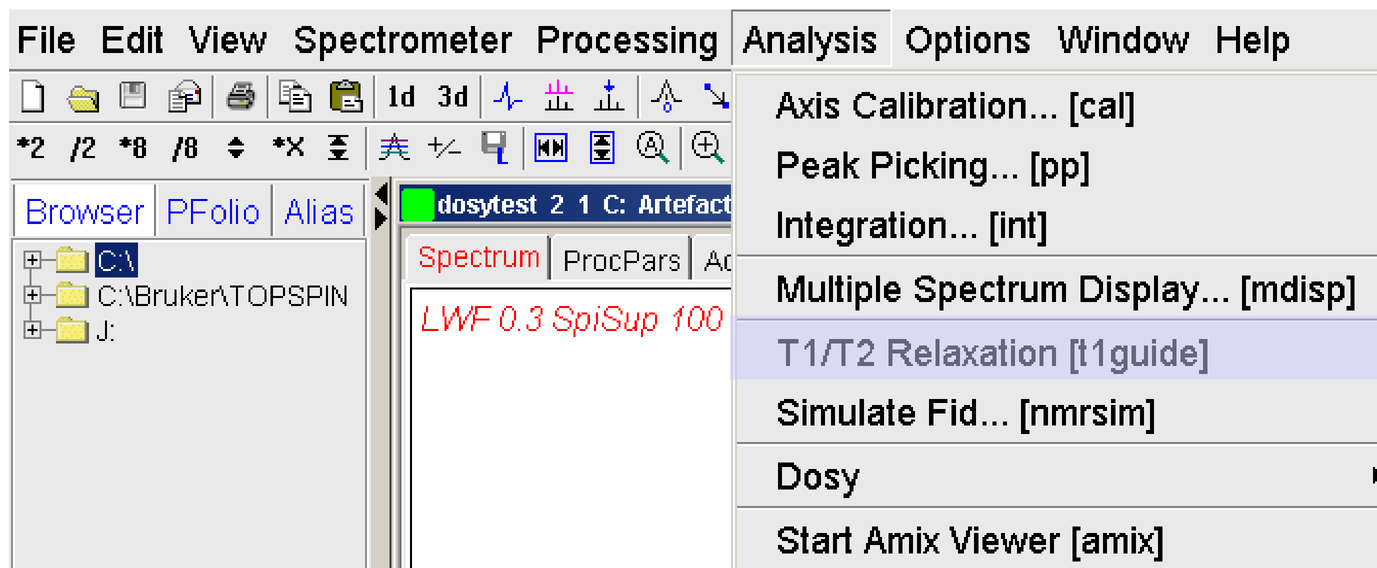
/DISK/data/USER/nmr/NAME/EXPNO/proc/PROCNO/difflist

Processing Diffusion
Experiments

Diffusion experiments: processing

Processing of data from diffusion experiments:

- Diffusion experiments can be performed using exactly the same setup procedure and pulse programs like a 2D DOSY experiment
- Processing will be done using a guided processing tool
- First, process data with *xf2* and phase correction



Diffusion experiments: processing

Bruker TOPSPIN 1.3.b.7 on P1111 as dmo

File Edit View Spectrometer Processing Analysis Options Window Help

dosytest 2 2 C: Artefacte

Spectrum | ProcPars | AcquPars | Title | PulseProg | Peaks | Integrals | Sample | Structure | Fid

DOSY LED experiment

F1 [s]

0.006

0.004

0.002

20 15 10 5 F2 [ppm]

NMR Relaxation Guide

Close

Extract Fid

Transform/Phase

Define Ranges

Relaxation Window

Fitting Function

Start Calculation

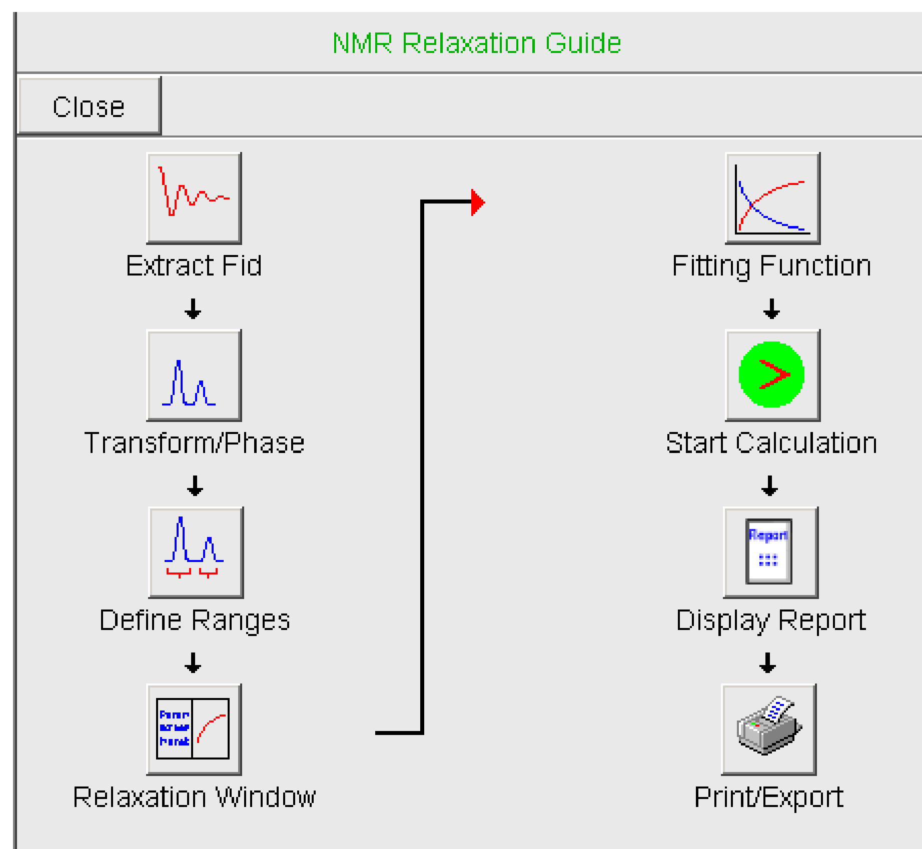
Display Report

Print/Export

Start | Inbox - Microsoft Outlook | TOPSPIN 1.3.b | Bruker TOPSPIN 1.3.b.... | 10:39

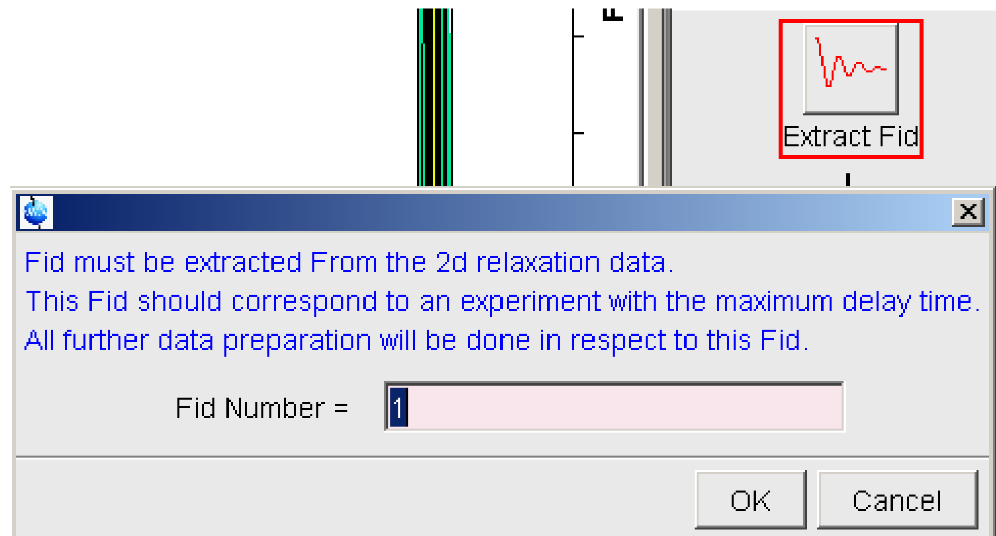
Diffusion experiments: processing

Go through the individual points step-by-step:



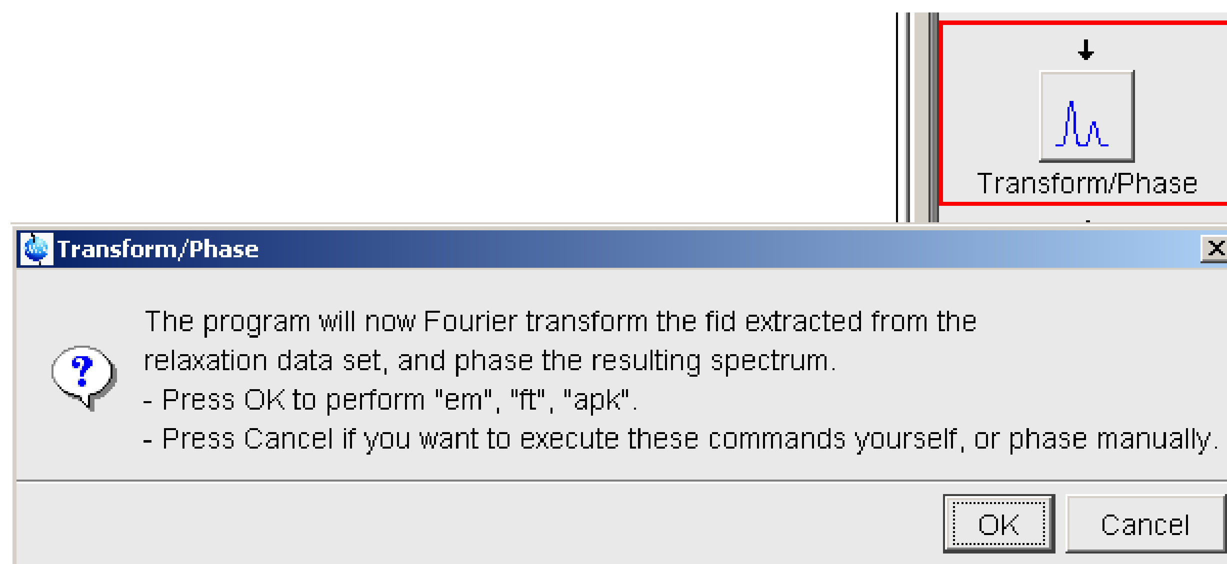
Diffusion experiments: processing

Extract FID:



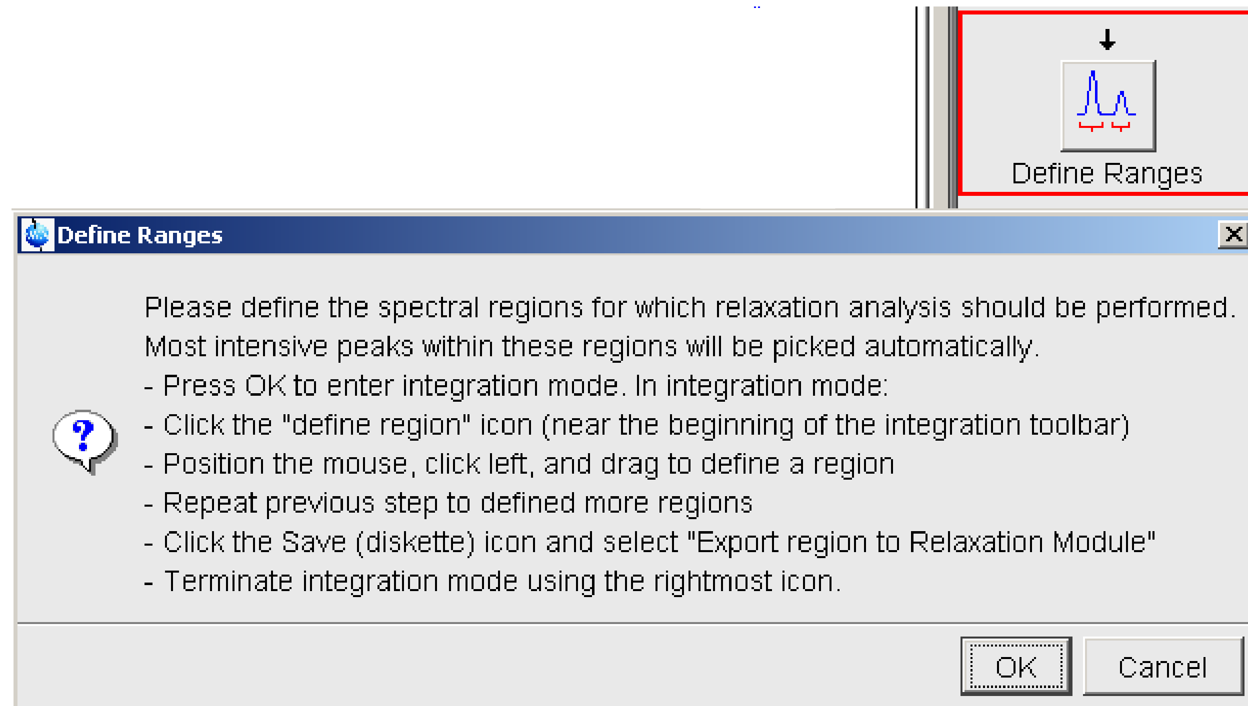
Diffusion experiments: processing

FT and phase correction:



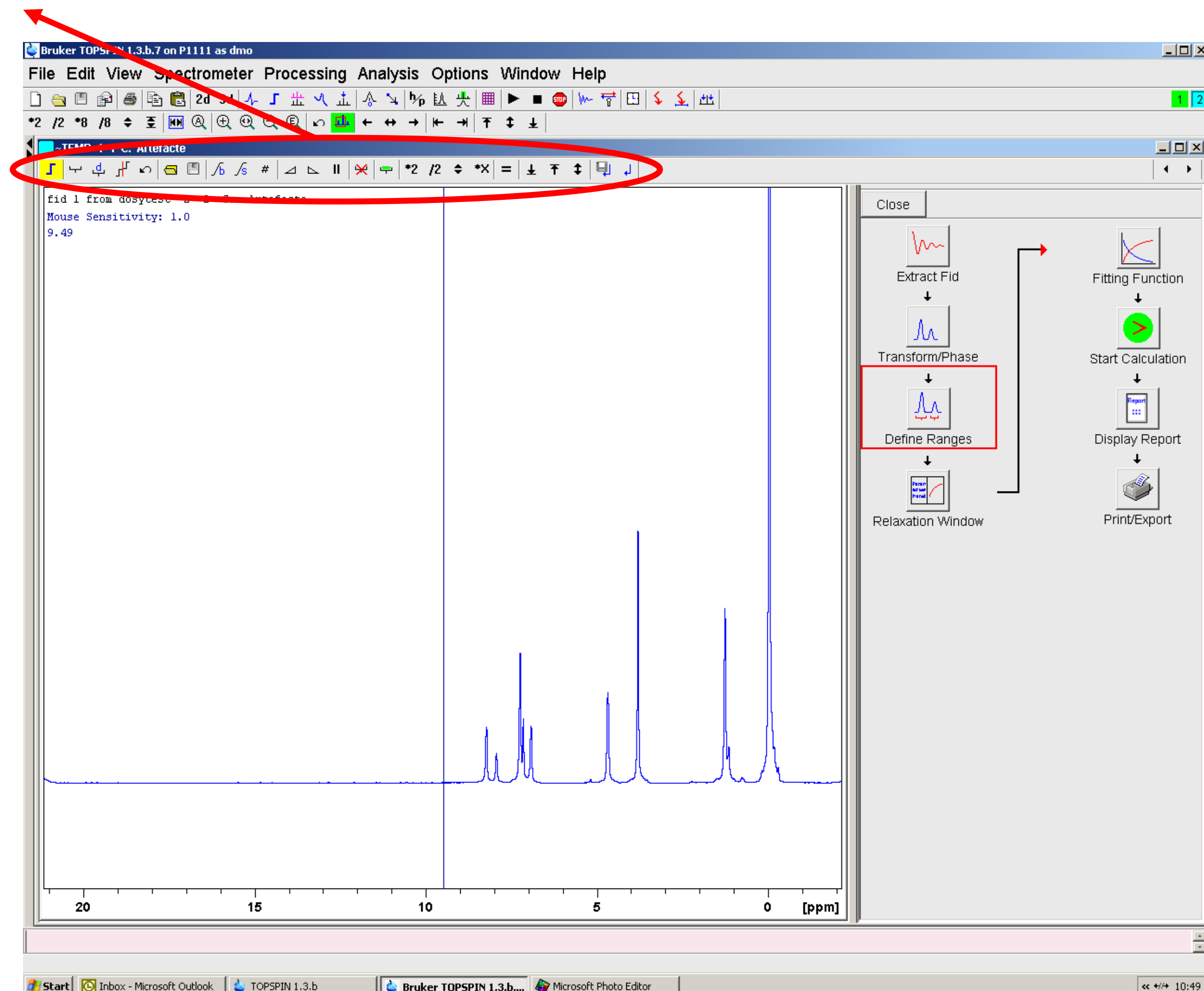
Diffusion experiments: processing

Define peaks to be analyses using integral ranges:



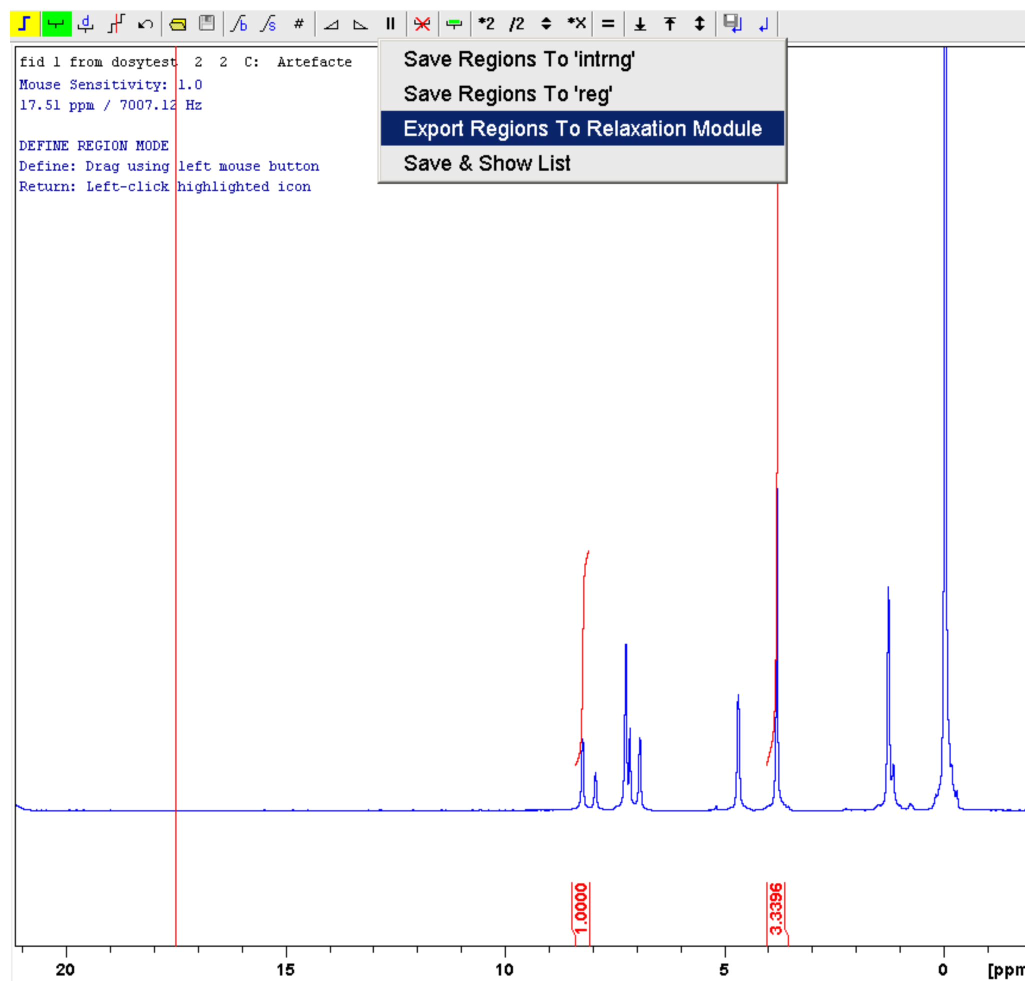
Diffusion experiments: processing

Define peaks to be analyses using integral ranges:



Diffusion experiments: processing

Define peaks to be analyses using integral ranges:



Diffusion experiments: processing

Define parameters for fitting:

1.

Relaxation parameters

General Parameters

1	FID # for phase determination
1000.0	Left limit for baseline correction
-1000.0	Right limit for baseline correction
5	Number of drift points
1.0E-5	Convergence limit
16	Number of points
1	First slice
1	Slice increment

Fitting Function

vargrad — Function Type

expdec
gaussdec
lorgauss
linear
varbigdel
varlitdel
vargrad
raddamp

Number of components

List file name

Increment (auto)

meters

Setup   Reset

Additional Parameters

10000.0	GAMMA(Hz/G)
10.0	LITDEL(msec)
100.0	BIGDEL(msec)
1.0	GRADIEN(G/cm)

OK   Cancel

Fitting Function → Start Calculation → Display Report → Print/Export

2.

Relaxation parameters

General Parameters

1	FID # for phase determination
1000.0	Left limit for baseline correction
-1000.0	Right limit for baseline correction
5	Number of drift points
1.0E-5	Convergence limit
16	Number of points
1	First slice
1	Slice increment

Fitting Function

vargrad	Function Type
1	Number of components
vdlist	List file name
	Increment (auto)

vdlist
auto
vplist
vclist
difflist
dw

meters

Reset

10000.0	GAMMA(Hz/G)
10.0	LITDEL(msec)
100.0	BIGDEL(msec)
1.0	GRADIEN(G/cm)

OK   Cancel

Fitting Function → Start Calculation → Display Report → Print/Export

Diffusion experiments: processing

Start the calculation

Fitting type
Intensity
Area
Current Peak
1 of 2
Brief Report
Data point 1 at 8.216 p
I[0] = 2.224e-00
Diff Con. = 2.433e-
RSS = 9.931e-007
SD = 3.523e-004

Diffusion : Variable Gradient
*I=I[0]*exp(-D*SQR(2*PI*gamma*Gi*LD)*(BD-LD/3)*1e4)*
Current Peak=1 Diff.Con.=2.433E-11m2/S

1 4 9 16 [G/cm]

NMR Relaxation Guide
Close
Extract Fid
Transform/Phase
Define Ranges
Relaxation Window
Fitting Function
Start Calculation
Display Report
Print/Export

Gradient Calibration for
Diffusion Experiments

Gradient Calibration

1. Calibrate the temperature:

– Record a ^{1}H-spectrum of methanol or glycol:

Methanol: Low temperature 175 to 330 K (-98 to +57 ^{o}C)
Glycol: High temperature 310 to 410 K (+37 to +137 ^{o}C)

– Define plot region and use *xau calctemp* to calculate the temperature
– Methanol sample: 4% MeOH in Methanol-d4
– Glycol sample: 80% Glycol in DMSO-d6

2. Gradient calibration:

1. A diffusion experiment will be done for a sample with known diffusion value

Sample	Temperature [C]	D [$10^{-9}m^2/s$]
H_2O	20	2.031
H_2O	25	2.299
D_2O	25	1.872

Data by courtesy of Dr. M. Holz (Inst. of. Phys. Chem., Uni Karlsruhe, FRG)

Gradient Calibration

2. Gradient calibration:

2. From the difference $D_{measured}$ and $D_{literature}$ the gradient calibration constant ***GCC*** will be calculated:

$$GCC_{New} = GCC_{old} * \sqrt{D_{measured} / D_{literature}}$$

3. The new gradient calibration constant has to be entered using the TopSpin command *setpre*:

File Edit Channel Enable Disable Help

Grad. calib. const. [Hz/cm]
Grad. calib. const. [G/mm]
Gradient scaling factor
Rate to measure temperature
Rate to check error status
Clear all preemphasis values

9.998 0.100
0.00 1.00
0.9998 0.0100
0.00 1.00

Fast Base Preemp. Z
0.2 ms
Time [ms] 0.09998 0.00100
Gain [%] 0.00 1.00

Store Recall Exchange Undo Clear

Gradient Calibration

Note:

The gradient calibration has to be done BEFORE recording diffusion experiments, as he file *difflist* stored with the data set is calculated from the gradient calibration constant and the entries of Difframp.

Further information:

TopSpin -> Help menu -> Software and Application Manuals

1D and 2D Step-by-Step Advanced

Dosy

DOSY CONTIN/DECRA

Diffusion