

CryoProbes at work

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- Water Suppression / Radiation Damping Effects
- Tips & Tricks for Gradients
- Salt Tolerance

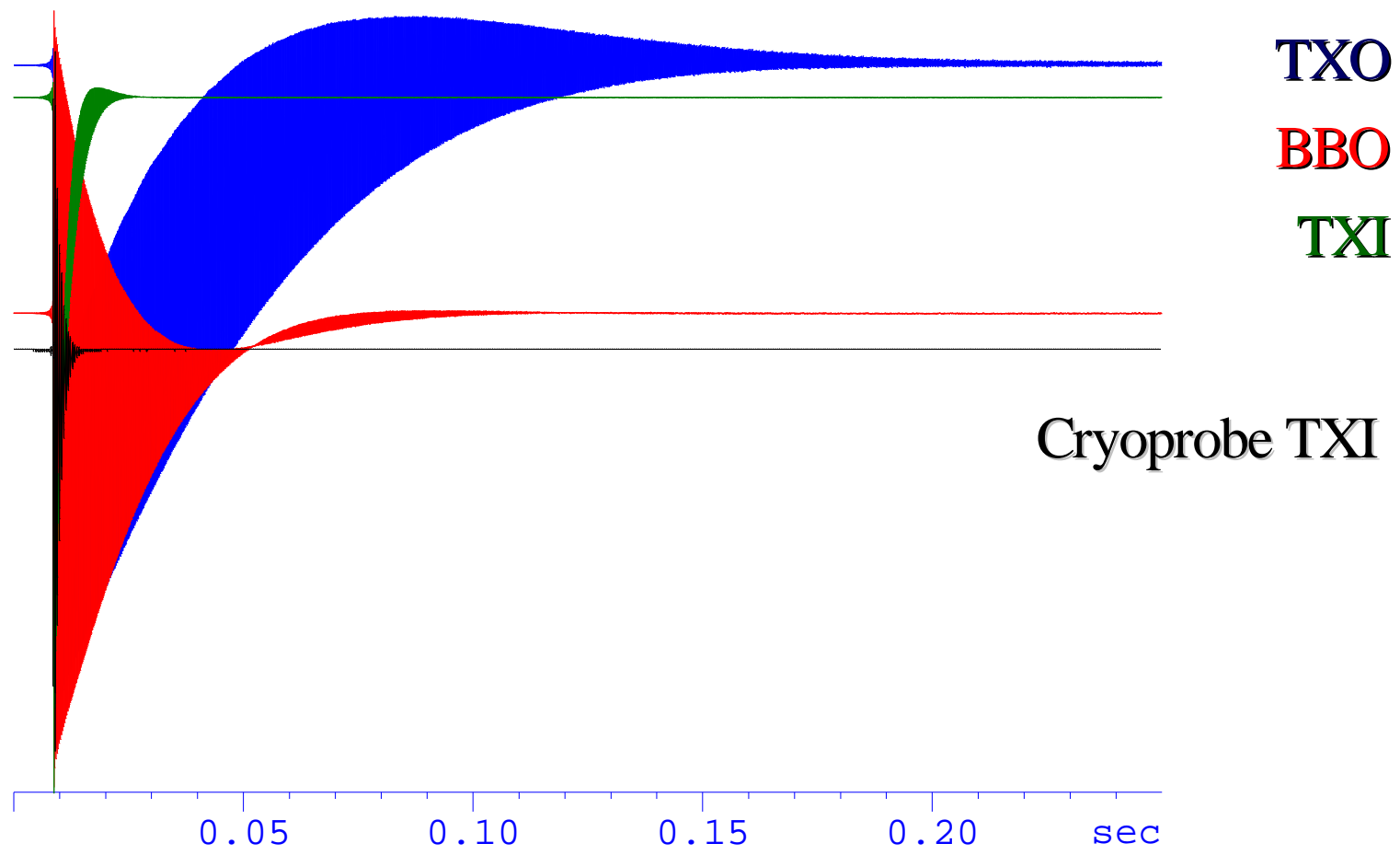
Water Suppression:

- *rules, tricks etc... we know from standard probes also apply*
- *main difference to standard probe:*
 - *enhanced radiation damping*
 - water flipback-pulses need more attention*
 - removing trim pulse of first INEPT-step might give best suppression*
 - *water hump might be broader*
 - use Shigemi tube*
- *Trim pulse 'p28':*
 - *either not needed or best suppression for values of 50-100usec (?)*

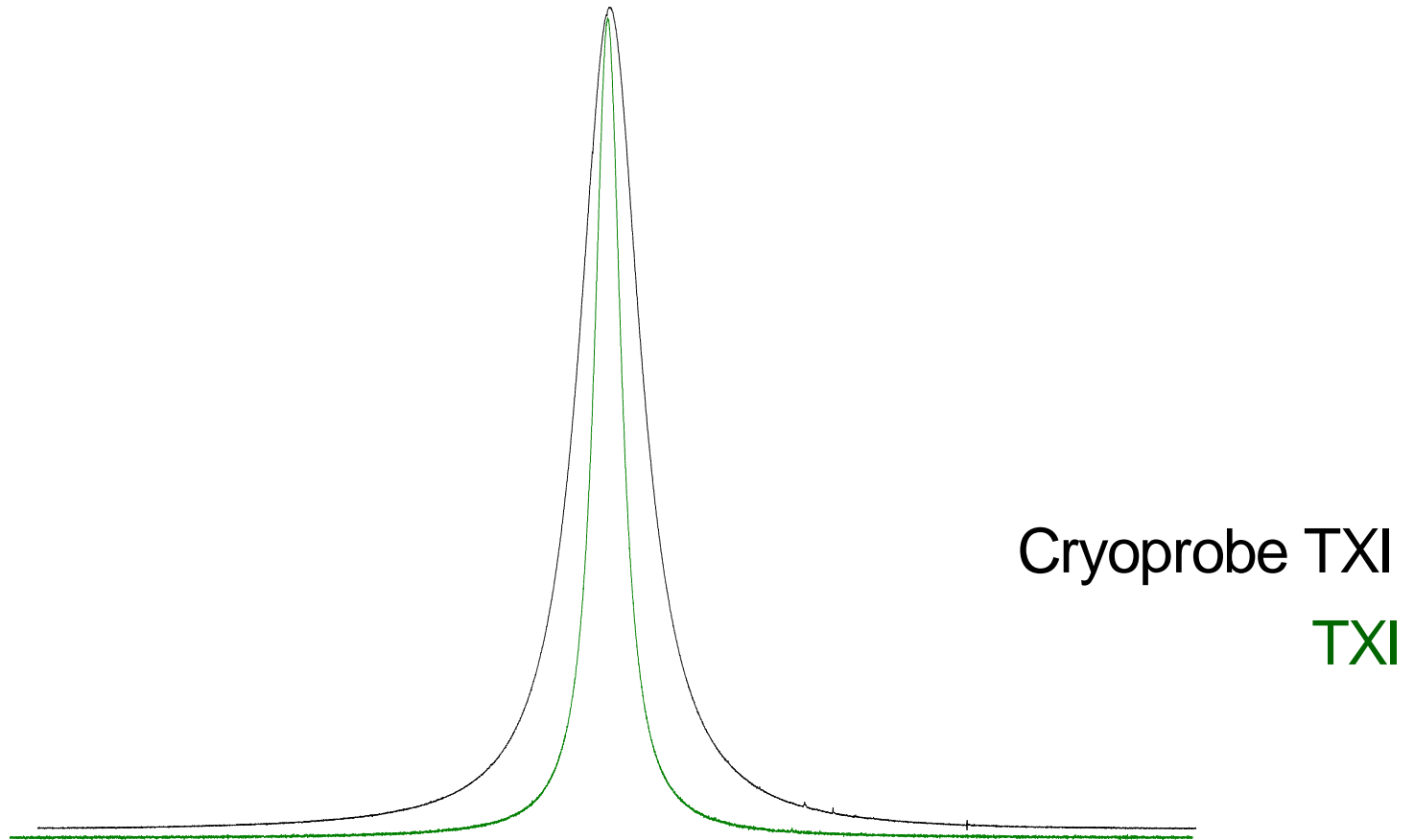
Radiation Damping Effects

- precessing magnetization induces a voltage in RF coil
- this is our NMR signal...
 - ... but the resulting current in the RF coil is nothing else than a **RF pulse!**
- induced pulse has **constant phase relationship** to magnetization:
- induced pulse turns the precessing magnetization back towards +z axis
- intense signals have short apparent T_2 relaxation times

H₂O FID after 10° pulse @ 700 MHz



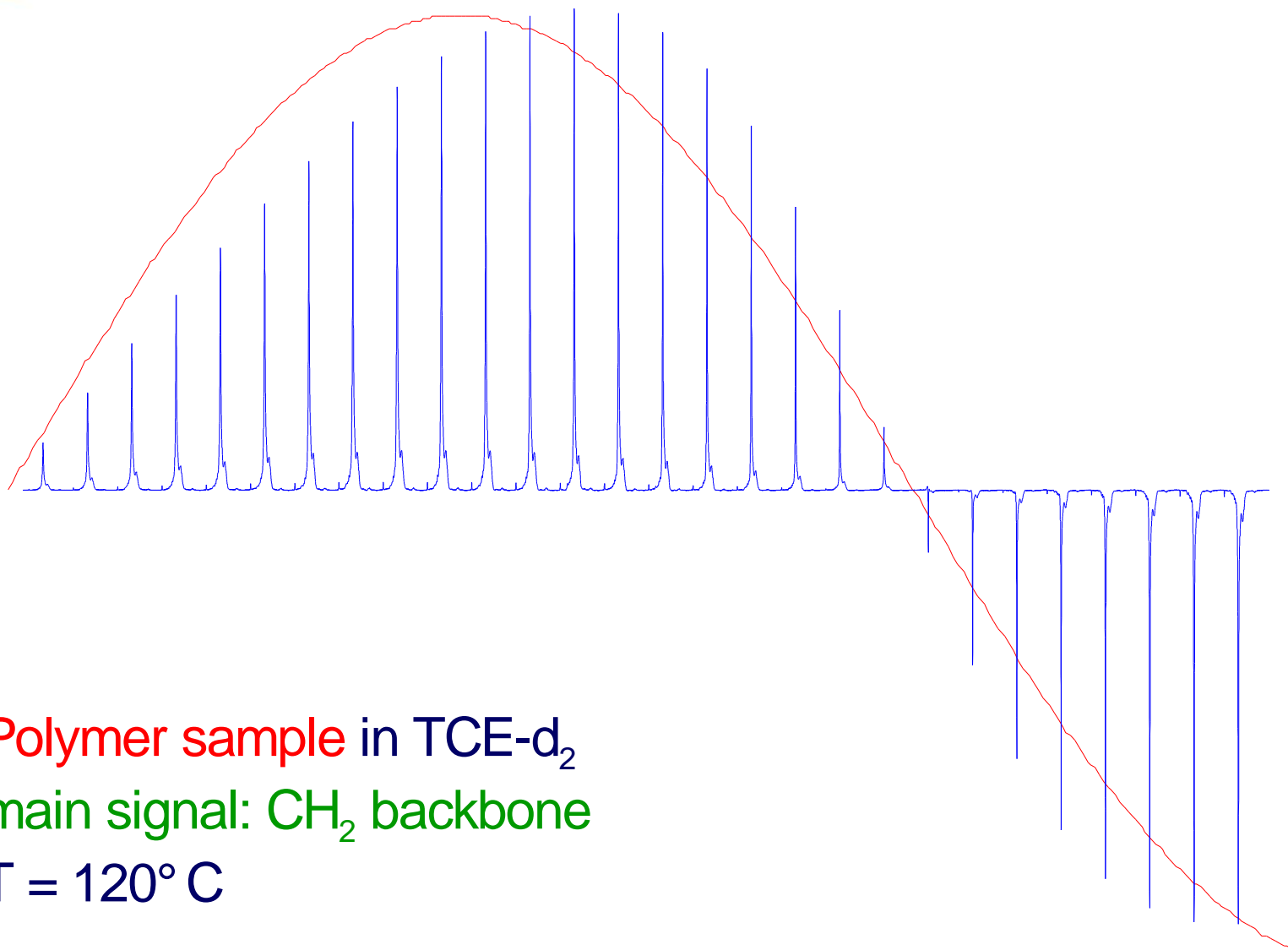
H₂O signal after 10° pulse @ 700 MHz



conventional TXI versus Cryoprobe TXI

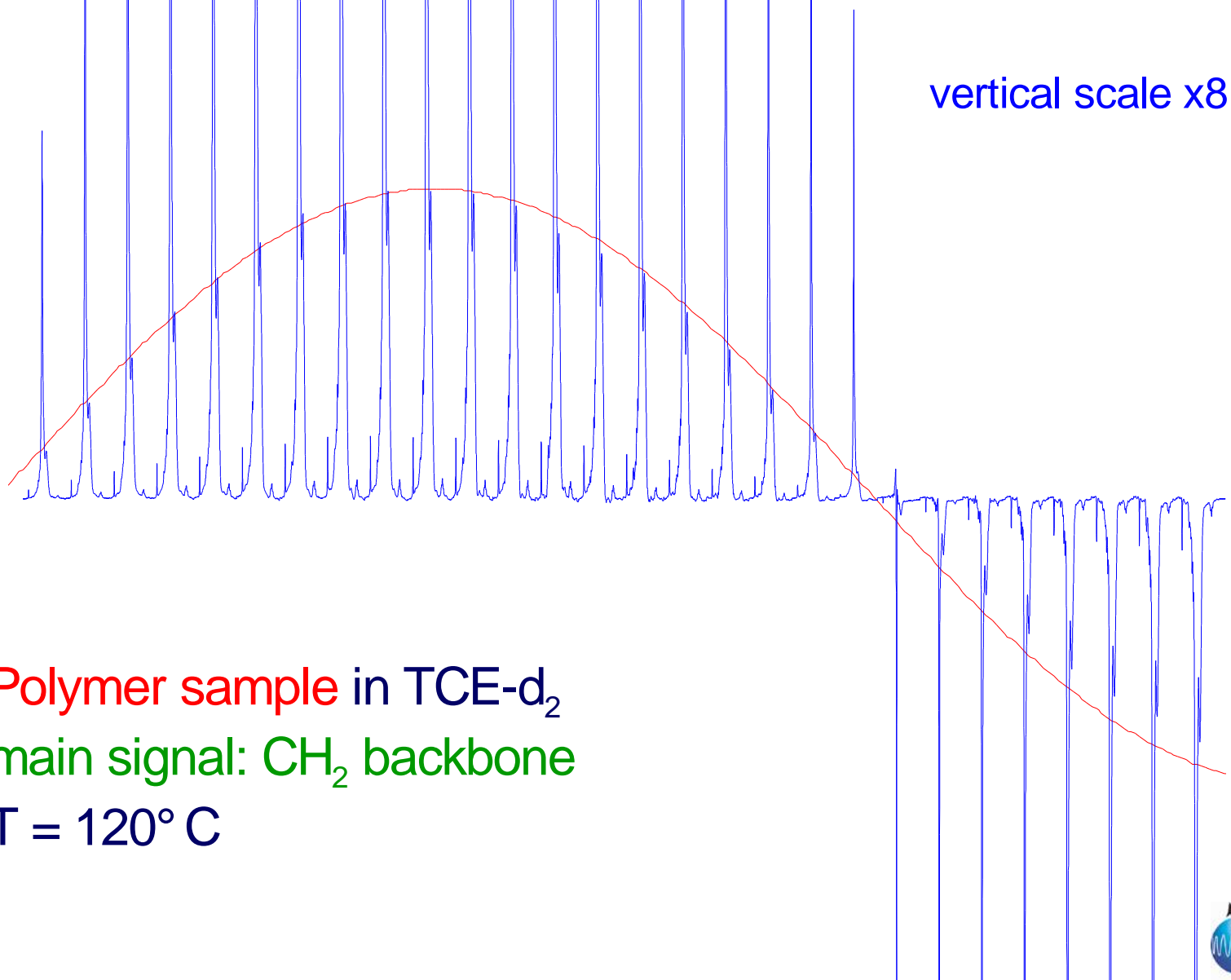


600 MHz SEI ^1H pulse calibration



Polymer sample in TCE-d_2
main signal: CH_2 backbone
 $T = 120^\circ\text{C}$

600 MHz SEI ^1H pulse calibration



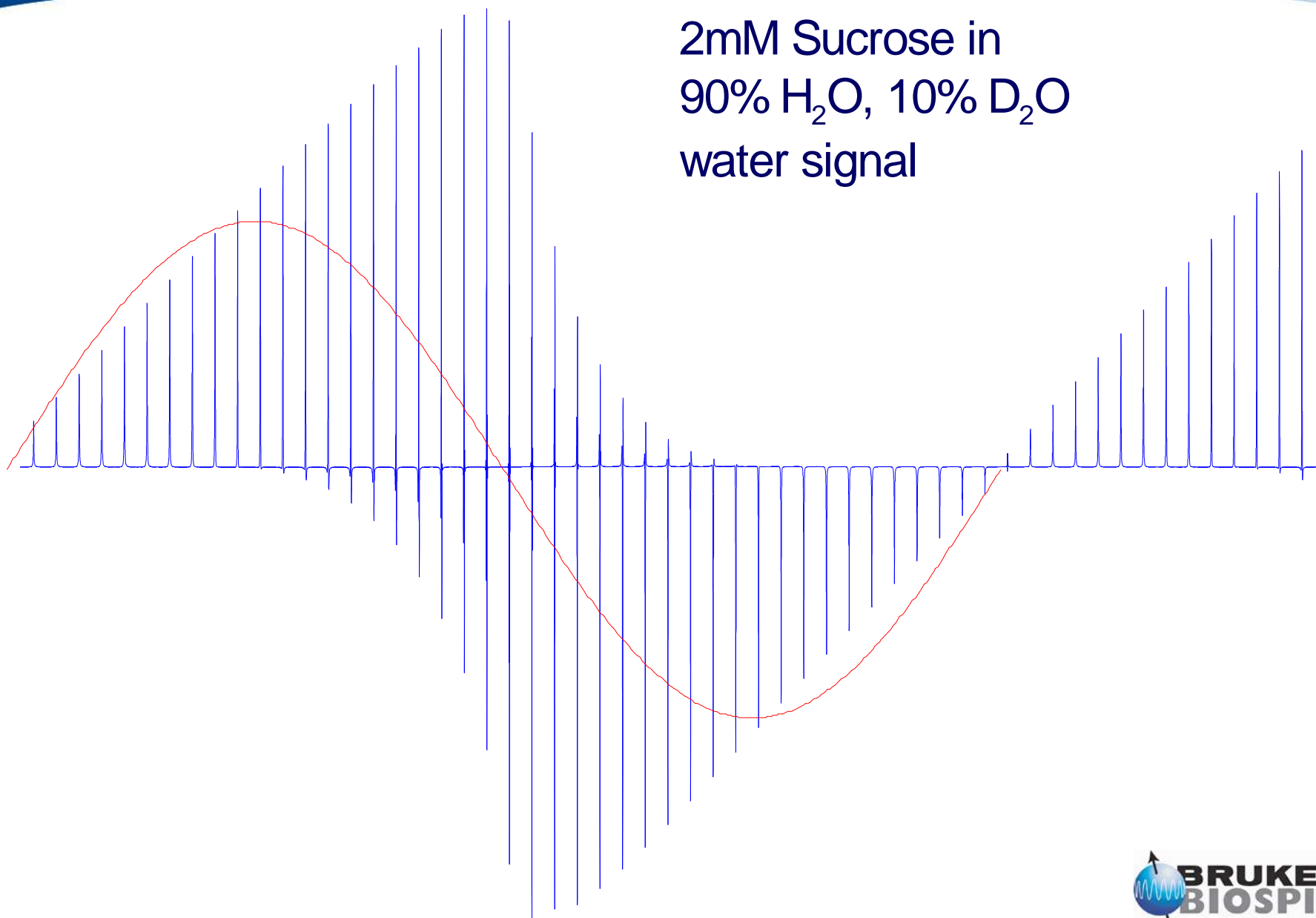
Polymer sample in TCE-d_2
main signal: CH_2 backbone
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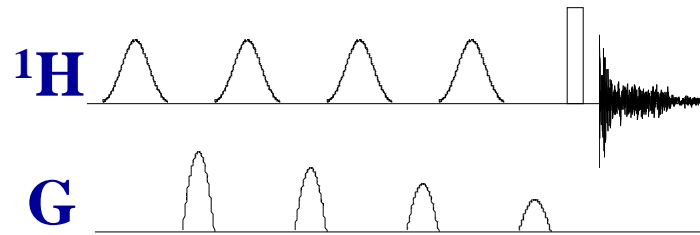
700 MHz TXI ^1H pulse calibration



2mM Sucrose in
90% H_2O , 10% D_2O
water signal

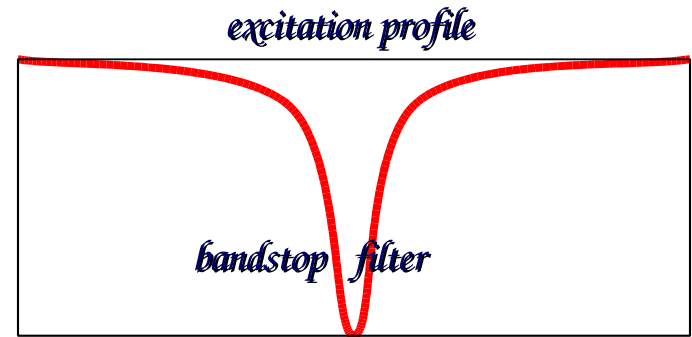
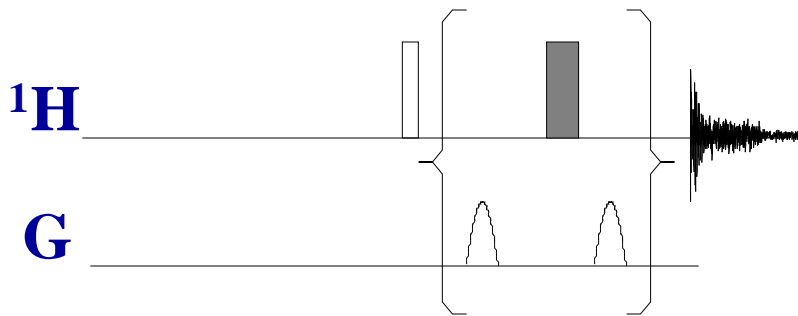


WET - Water suppression



- adjust the pulse power for the first selective pulse to compensate for radiation damping: up to 8dB difference from the theoretical value
- use stronger gradients and / or
- gradient shapes with higher integral than SINE
example: chirp with 10% smoothing

Magnetization destruction based methods: classical & binomial WATERGATE, excitation sculpting

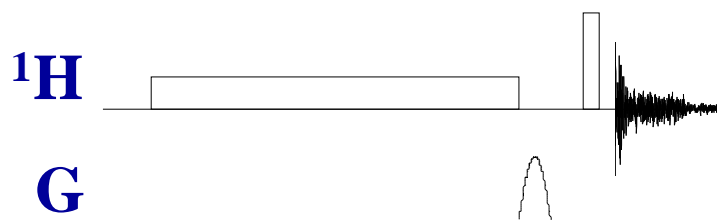


- use stronger gradients and / or
- gradient shapes with higher integral than SINE
example: chirp with 10% smoothing
- for highest suppression capacity: DPFGE
double binomial watergate "w5", zgpgw5
excitation sculpting "es", zgesgp

presaturation:



- use stronger RF irradiation (up to 100 Hz)
- use weak gradient prior to read pulse (3%)



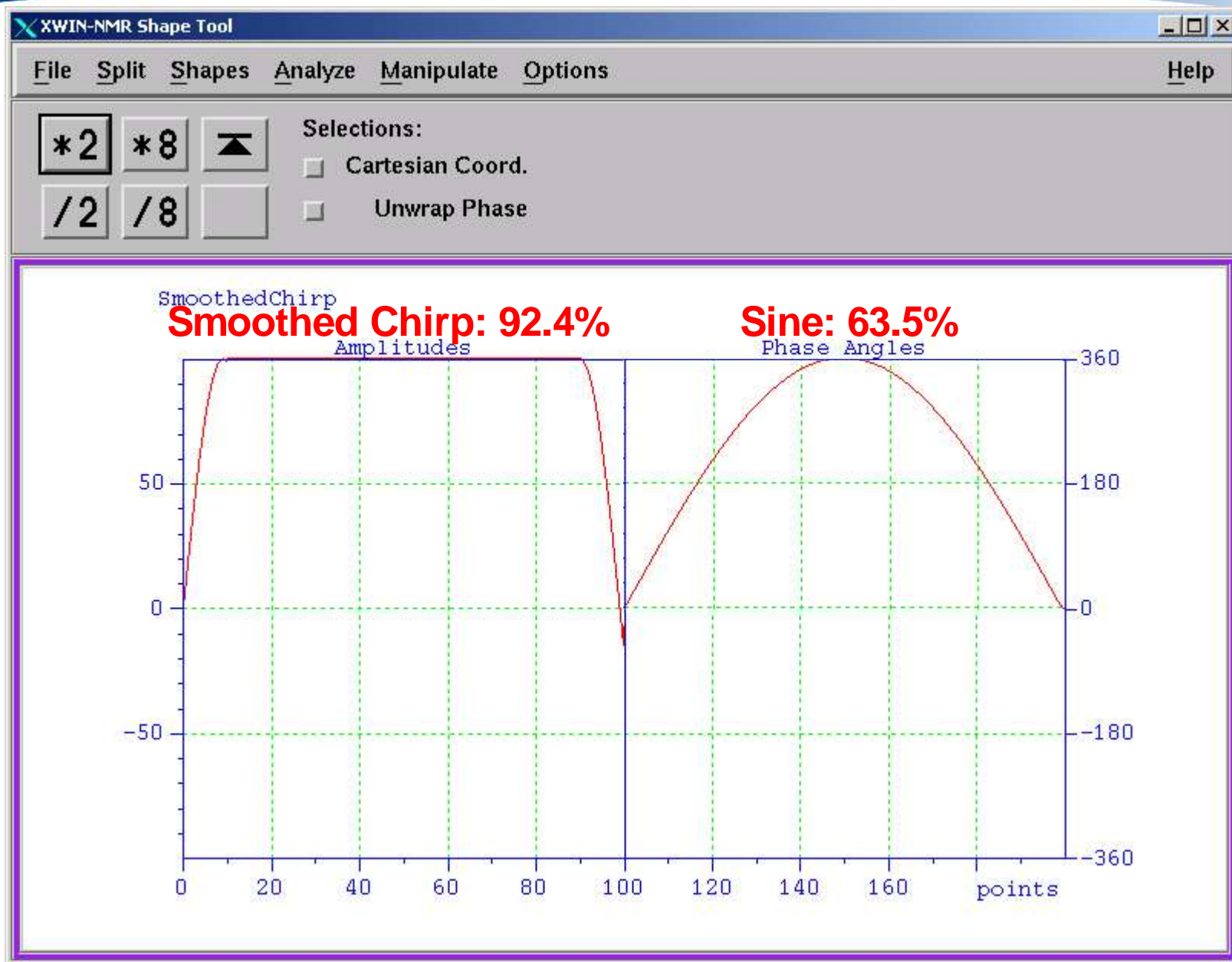
- use volume selection to reduce solvent hump



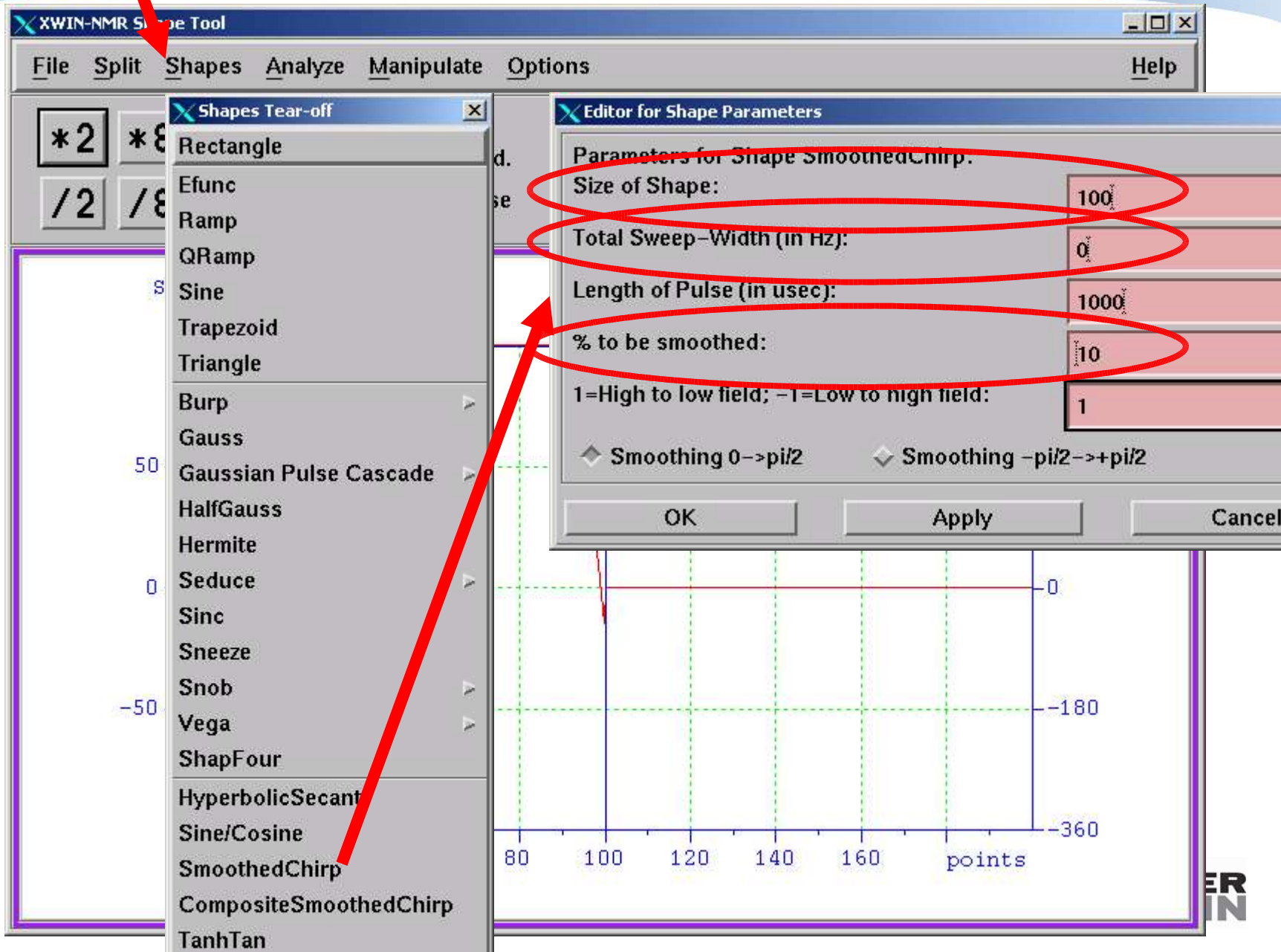
Tips & Tricks for Gradients

- Alternatives for 'SINE.100'
- GRASP: lock phase and artifacts

Tips & tricks for gradients



Tips & tricks for gradients



The screenshot displays the XWIN-NMR software interface. The main window is titled "XWIN-NMR Shape Tool" and features a menu bar with "File", "Split", "Shapes", "Analyze", "Manipulate", "Options", and "Help".

On the left, the "Shapes Tear-off" menu is open, listing various pulse shapes: Rectangle, Efunc, Ramp, QRamp, Sine, Trapezoid, Triangle, Burp, Gauss, Gaussian Pulse Cascade, HalfGauss, Hermite, Seduce, Sinc, Sneeze, Snob, Vega, ShapFour, HyperbolicSecant, Sine/Cosine, SmoothedChirp, CompositeSmoothedChirp, and TanhTan. A red arrow points from the title "Tips & tricks for gradients" to the "Shapes" menu item.

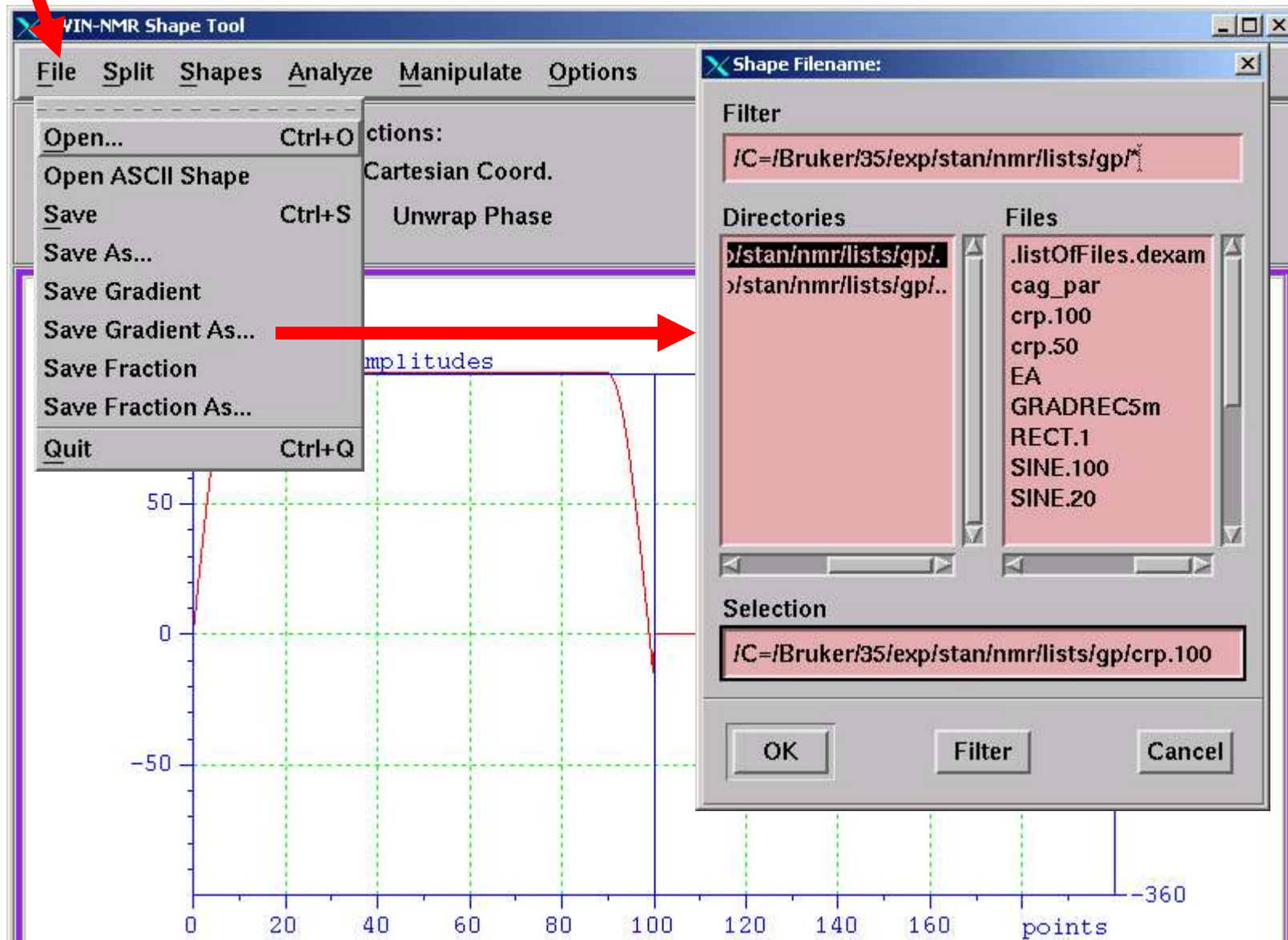
The "Editor for Shape Parameters" dialog box is open, showing parameters for the "SmoothedChirp" shape. The parameters are:

- Size of Shape: 100%
- Total Sweep-Width (in Hz): 0%
- Length of Pulse (in usec): 1000%
- % to be smoothed: 10%
- 1=High to low field; -1=Low to high field: 1

Below the parameters, there are two radio button options: "Smoothing 0->pi/2" and "Smoothing -pi/2->+pi/2". The "OK", "Apply", and "Cancel" buttons are at the bottom of the dialog.

In the background, a plot is visible with the x-axis labeled "points" ranging from 80 to 180 and the y-axis ranging from -50 to 50. A red vertical line is drawn at approximately x=100.

Tips & tricks for gradients



The screenshot shows the WIN-NMR Shape Tool interface. The main window displays a plot of a signal with a red curve and a blue vertical line at approximately 100 points. The x-axis is labeled 'points' and ranges from 0 to 360. The y-axis ranges from -50 to 50. A menu is open over the plot, with 'Save Gradient As...' highlighted. A red arrow points from this menu item to a 'Shape Filename:' dialog box. The dialog box shows a filter path: `/C=/Bruker/35/exp/stan/nmr/lists/gp/`. It lists directories and files. The file `crp.100` is selected in the 'Files' list. The 'Selection' field at the bottom of the dialog shows the full path: `/C=/Bruker/35/exp/stan/nmr/lists/gp/crp.100`. Buttons for 'OK', 'Filter', and 'Cancel' are visible at the bottom of the dialog.

WIN-NMR Shape Tool

File Split Shapes Analyze Manipulate Options

Open... Ctrl+O Options:
Open ASCII Shape Cartesian Coord.
Save Ctrl+S Unwrap Phase
Save As...
Save Gradient
Save Gradient As...
Save Fraction Amplitudes
Save Fraction As...
Quit Ctrl+Q

Shape Filename:

Filter
`/C=/Bruker/35/exp/stan/nmr/lists/gp/`

Directories
`/stan/nmr/lists/gp/`
`/stan/nmr/lists/gp/..`

Files
.listOfFiles.dexam
cag_par
crp.100
crp.50
EA
GRADREC5m
RECT.1
SINE.100
SINE.20

Selection
`/C=/Bruker/35/exp/stan/nmr/lists/gp/crp.100`

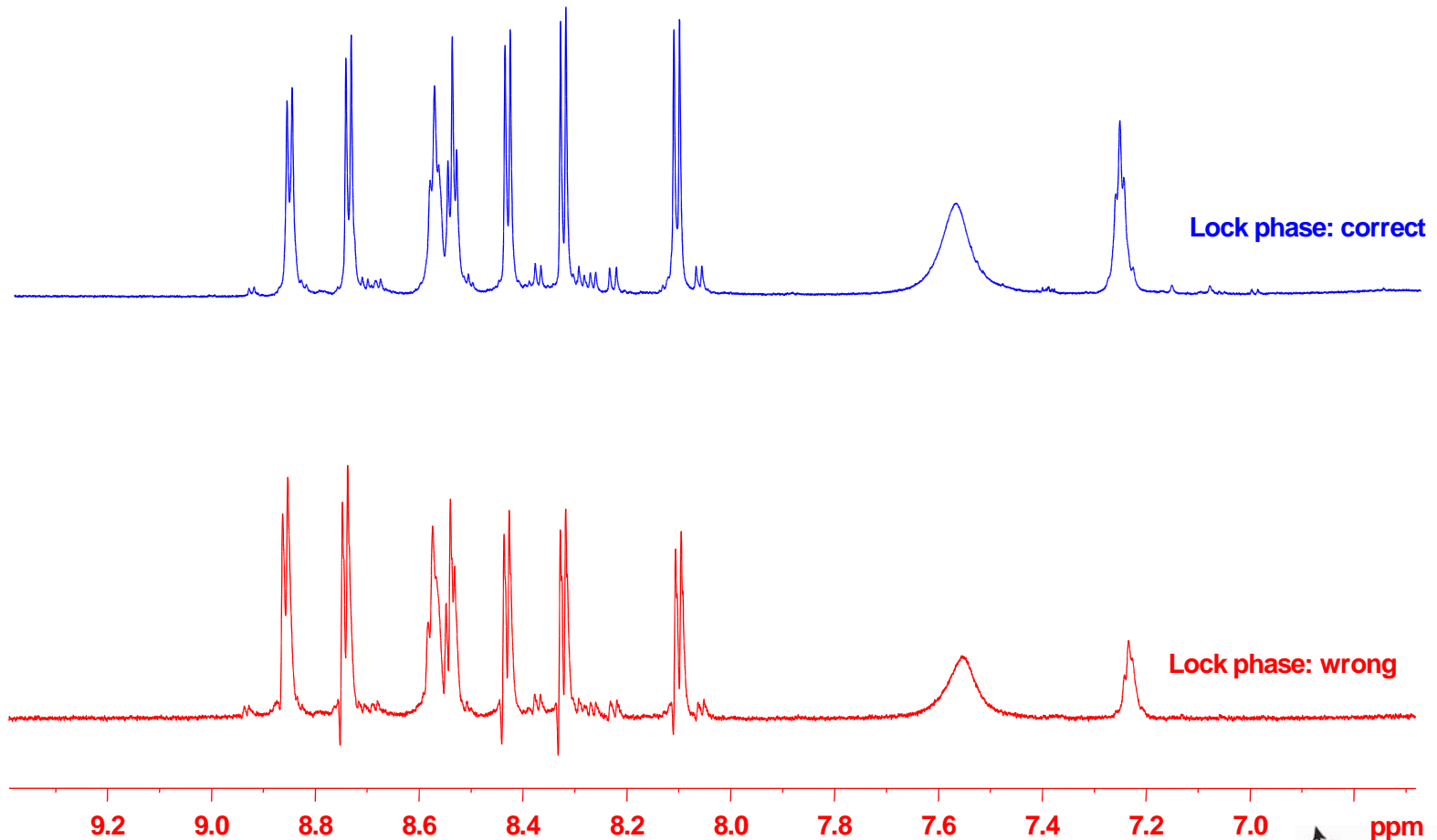
OK Filter Cancel

50
0
-50
0 20 40 60 80 100 120 140 160 points -360

- Alternatives for 'SINE.100'
- **GRASP: lock phase and artifacts**

Artifacts due to wrong lock phase

WATERGATE-experiment



The lock channel can be understood as a ,complete independant spectrometer within the spectrometer“:

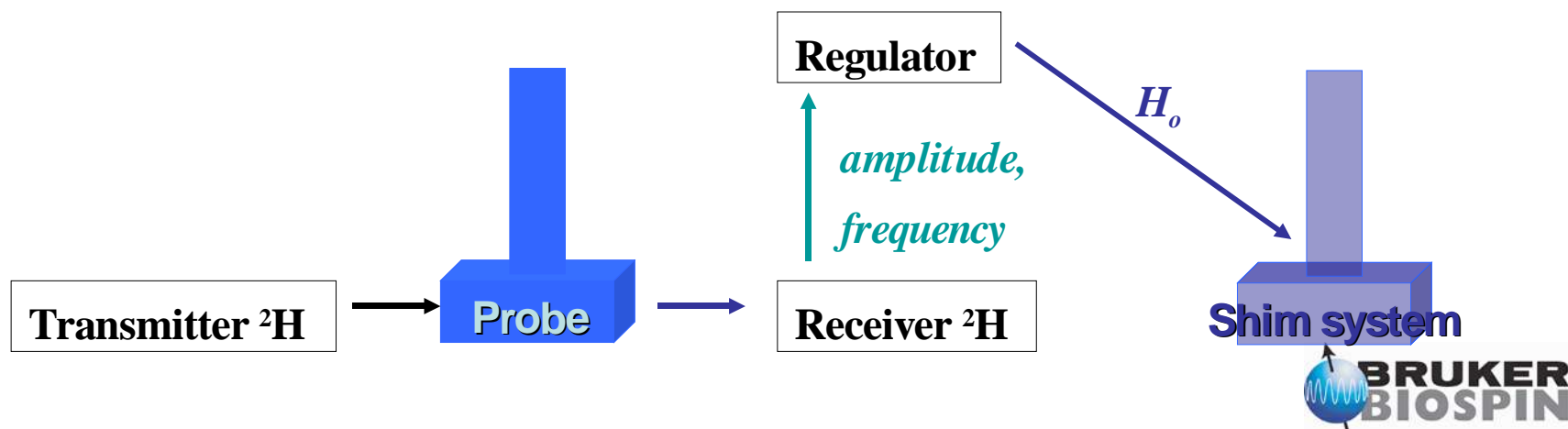
The resonance condition of NMR:

$$\omega = \gamma B_o$$

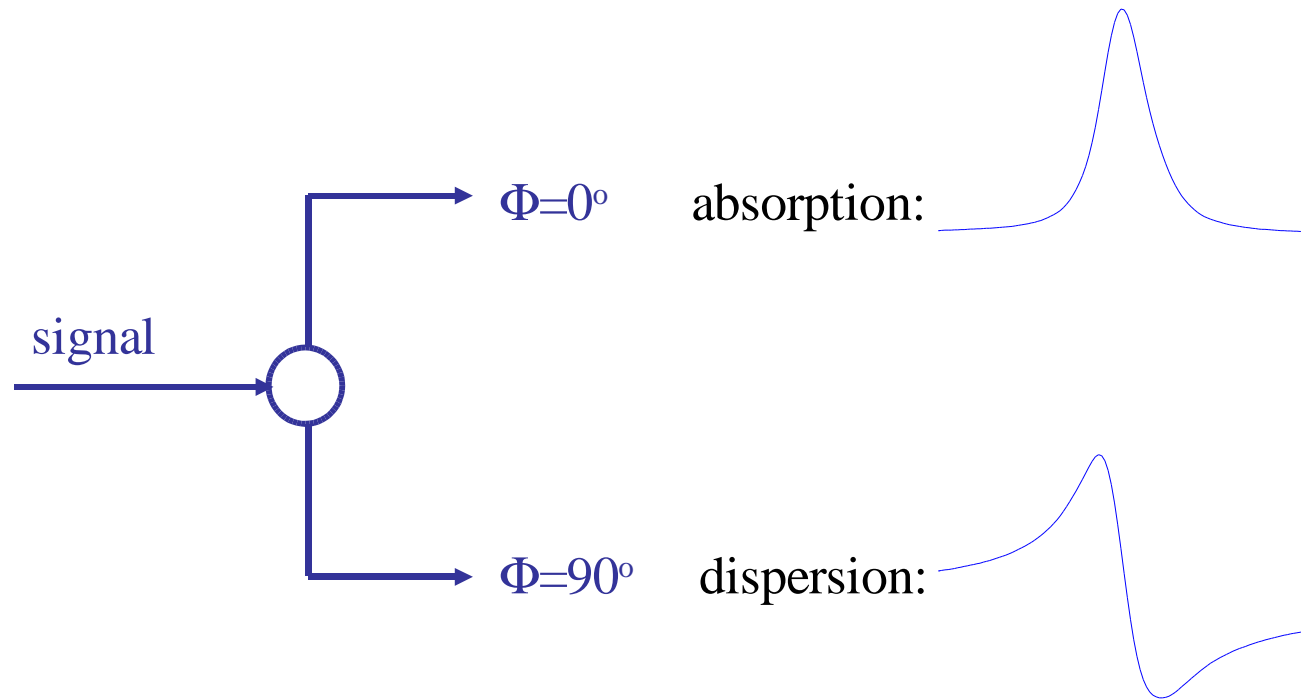
but: B_o is not stable

$$\omega = \gamma (B_o + H_o)$$

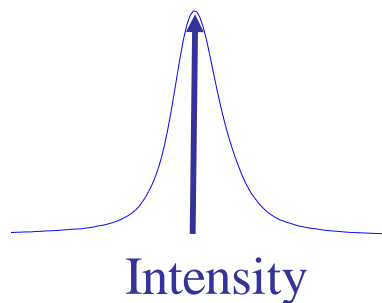
$(B_o + H_o) = \text{const.}$



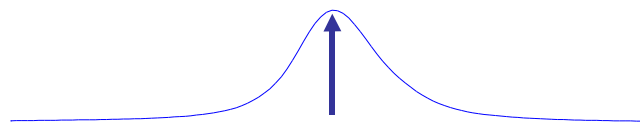
The lock receiver has two quadrature channels:



- The absorption signal is used for field homogenisation
- The signal intensity is a measure for the field homogeneity:



*sharp signal, high
lock level*



*broad signal, low lock
level*

- The dispersion signal is used for field stabilisation
- The position of the zero-crossing of the signal is permanently checked
- Determination of the zero-crossing frequency is more sensitive than determination of the frequency at maximum peak position

- If the lock phase is not adjusted correctly, absorption and dispersion signals will be mixed
- Non-pure phases will result in:
 - imperfect field homogenisation (shimming)
 - imperfect field stabilisation
 - field shifts during experiment using pulsed field gradients

CryoProbe™

Salt Tolerance

$$\frac{S}{N} \sim \frac{1}{\sqrt{R_{Coil} (T_{Coil} + T_{Preamp}) + R_{Sample} (T_{Sample} + T_{Preamp})}}$$

- For $R_{Coil} (T_{Coil} + T_{Preamp}) \gg R_{Sample} T_{Sample}$:



$$\frac{S}{N} \sim \frac{1}{\sqrt{R_{Coil} (T_{Coil} + T_{Preamp})}}$$

- For $R_{Sample} T_{Sample} \gg R_{Coil} T_{Coil}$:



$$\frac{S}{N} \sim \frac{1}{\sqrt{R_{Sample}}}$$

$$R_{Sample} \propto \omega^2 \sigma r^4$$

ω *frequency*
 σ *conductivity*
 b *sample radius*

$$\sigma \propto \sum_i c_i q_i \lambda_i$$

c_i *concentration*
 q_i *charge*
 λ_i *mobility*

• R_s depends on:

• Conductivity = f(salt concentration)
= f(ion mobility)

$$\sigma \propto \sum_i c_i q_i \lambda_i$$

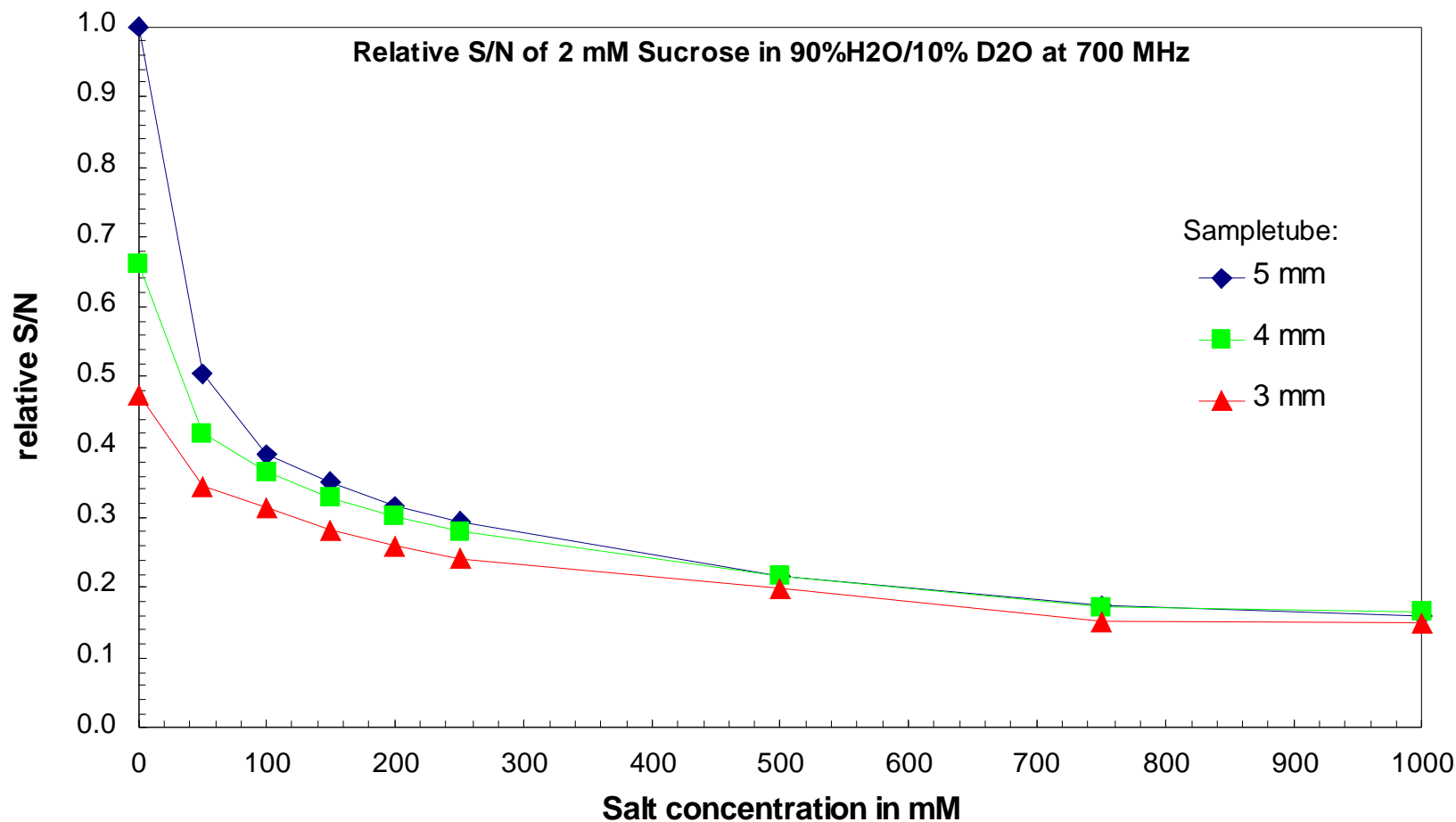
• Sample radius

$$R_{Sample} \propto r^4$$

• Frequency

$$R_{Sample} \propto \omega^2$$

Sample diameter for lossy solvents



Signal-to-noise ratio and Sample Diameter

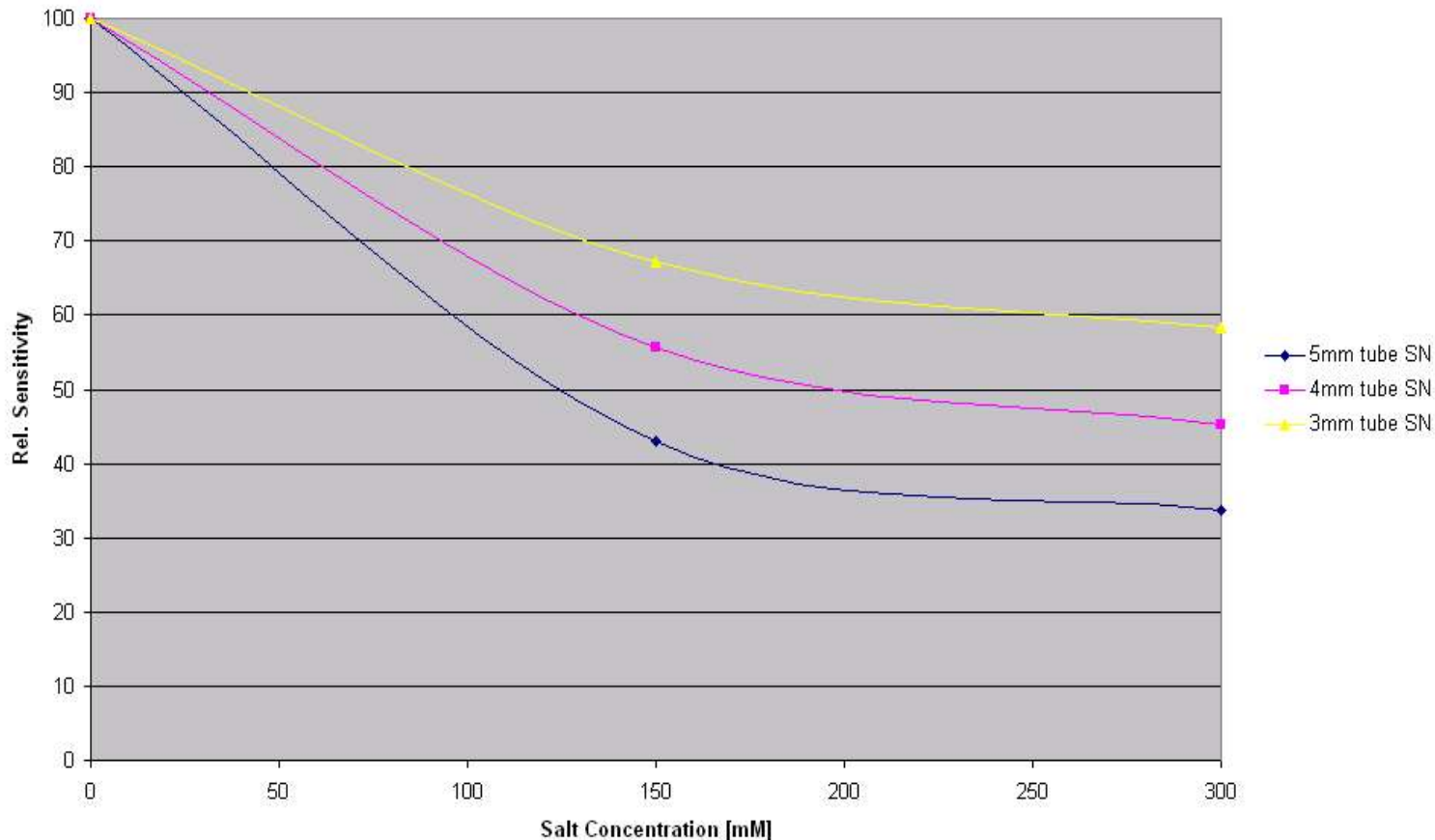


Sensitivity and Salt Dependence as function of sample diameter

Identical **Mass** in all tubes:

Sucrose in D₂O, 600 MHz. TCI CryoProbe

Rel. Sensitivity, Same Sample Amount



<i>Sample Diameter</i>	<i>Rel. Volume</i>
------------------------	--------------------

5.0 mm	100.0 %
4.0 mm	63.5 %
3.0 mm	34.9 %
1.7 mm	10.7 %

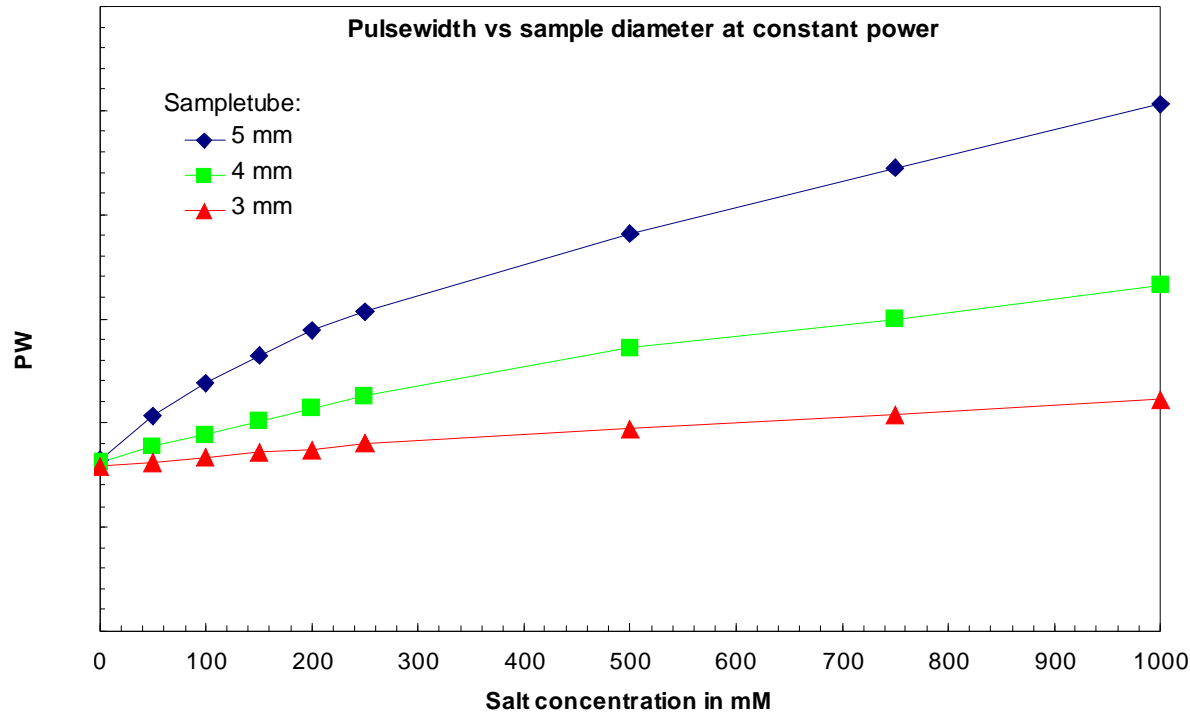
? At high salt concentration the same sensitivity can be achieved with less compound

NOTE:

10. this applies only for $R_{Sample} T_{Sample} \gg R_{Coil} T_{Coil}$

11. *Constant concentration*

Sample diameter for lossy solvents



- **If sample noise dominates**
 - **PW shorter with smaller tubes**

$$PW \sim \sqrt{k_1 R_c} + k_2 R_s$$

$$PW \sim \sqrt{\text{Loss}} \sim r^2$$

For high (> 150 mMol) salt concentration it is better to use smaller diameter tubes

• R_s depends on:

• Conductivity = f(salt concentration)
= f(ion mobility)

$$\sigma \propto \sum_i c_i q_i \lambda_i$$

• Sample radius

$$R_{Sample} \propto r^4$$

• Frequency

$$R_{Sample} \propto \omega^2$$

Low Conductivity Buffers and Sensitivity for Lossy Samples:

4. Buffers with low ion mobility:

- using large organic molecules instead of small inorganic ions

5. For titration both, acid and base, with low ion mobility shall be selected:

- base: *BIS-TRIS propane* acid: *PINES, MOPS*
- base: *TRIS* acid: *bicine*

6. Gain:

- an gain in S/N of up to 50% compared to commonly used buffers

Sample Diameter and Sensitivity for Lossy Samples:

3. Identical Concentration:

- 5 mm tubes have inherent best S/N

4. Identical Mass:

- Best S/N for smallest possible tube diameter (limited only by the solubility)

5. Frequency:

- S/N is always higher for higher frequencies but the sensitivity enhancement becomes a function of the salt concentration

Buffer and Sensitivity for Lossy Samples:

- Try low conductivity buffers