# CryoProbes at work

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# Outline

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

# Introduction

## *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

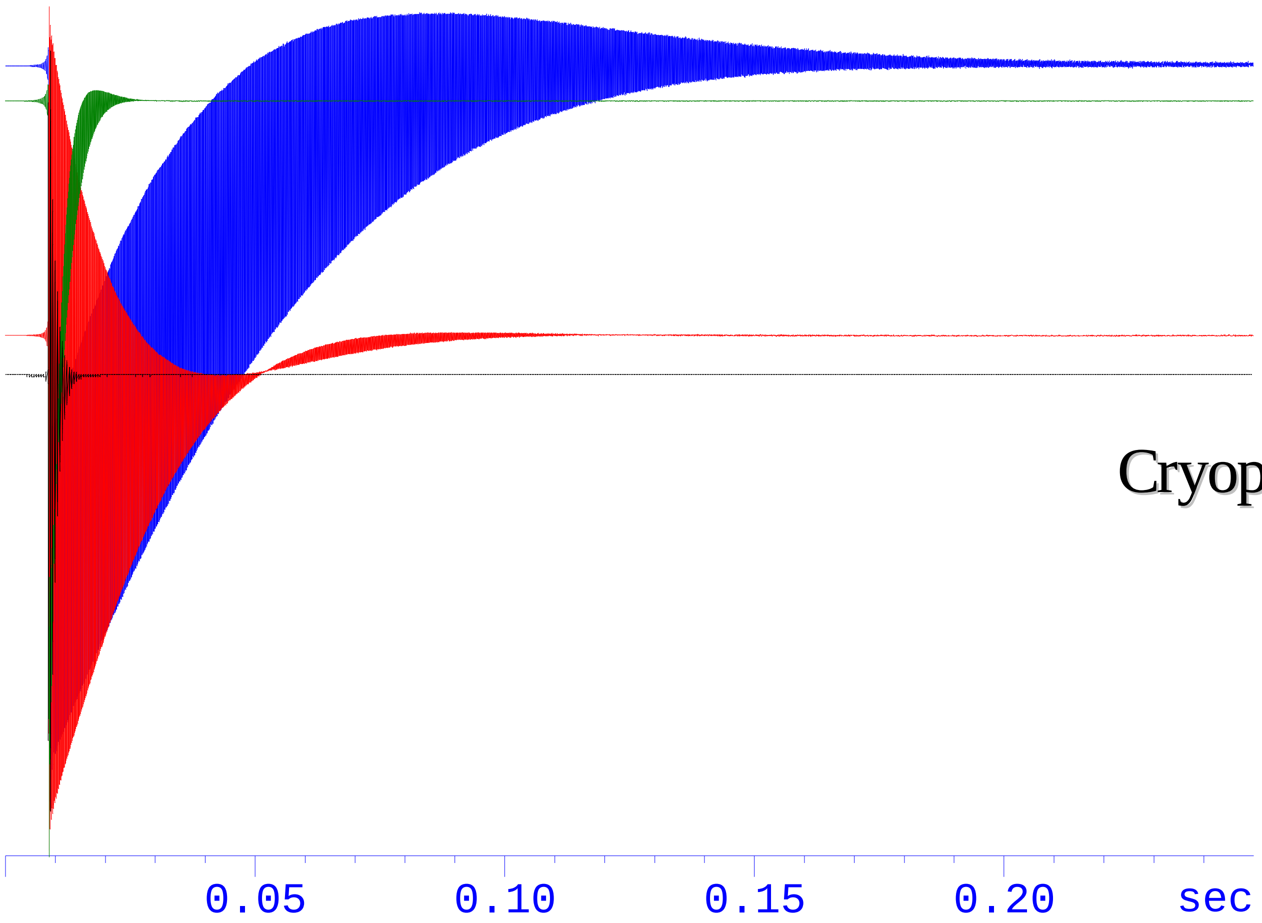
# Radiation Damping: Background

- precessing magnetization induces a voltage in RF coil
- this is our NMR signal…

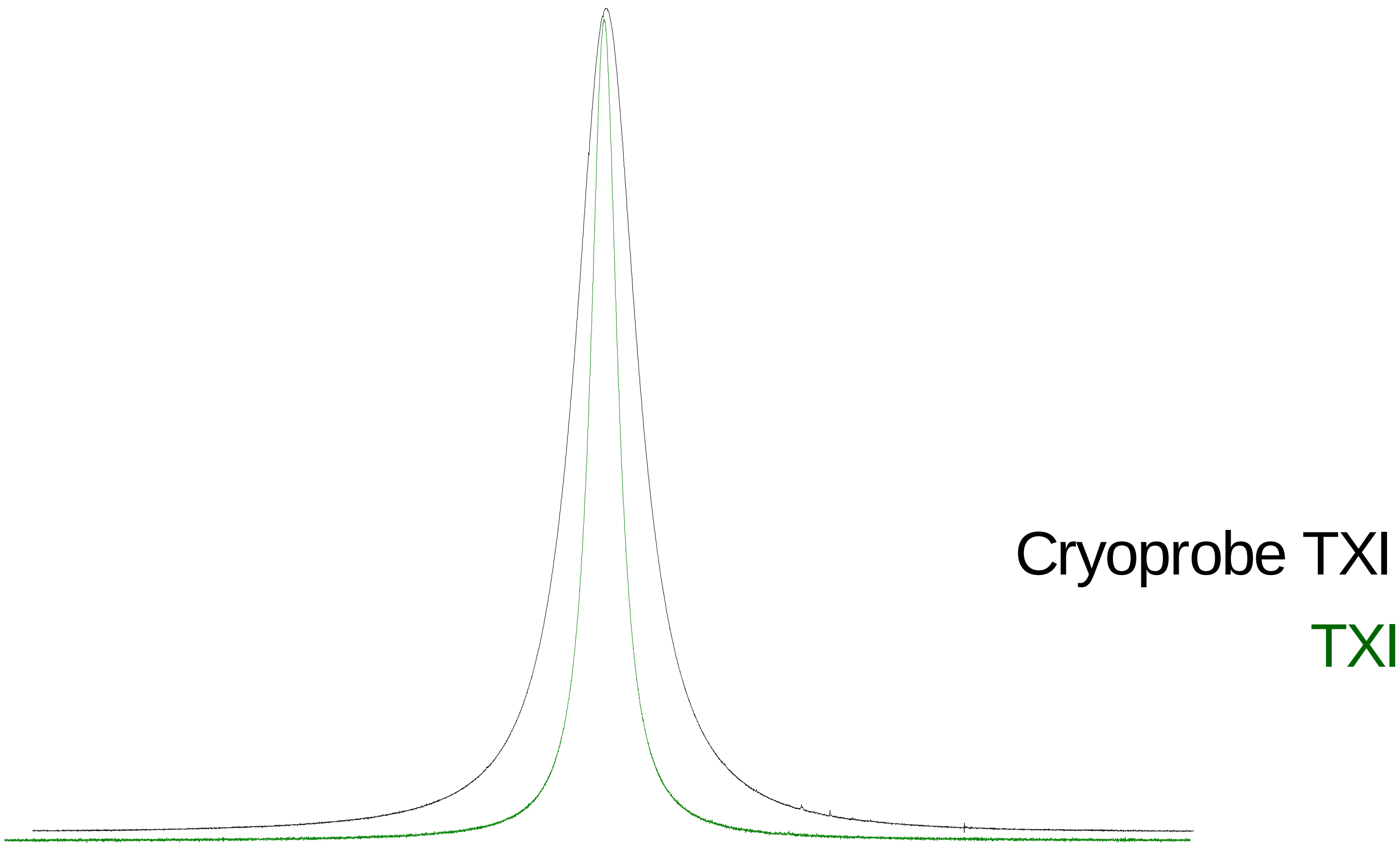
  … but the resulting current in the RF coil is nothing else than a RF pulse!

- 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

# $H_2O$ FID after 10° pulse @ 700 MHz

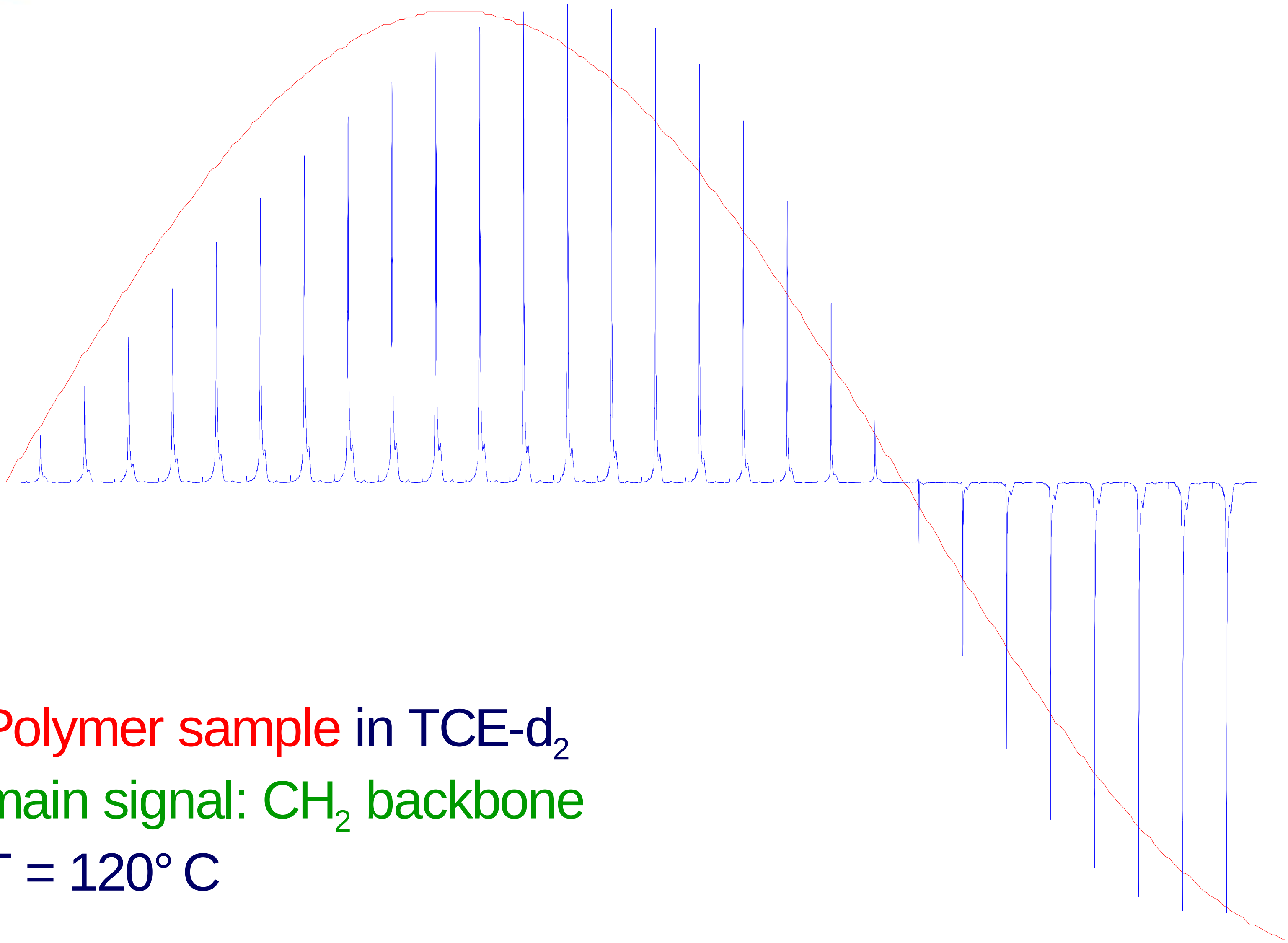


# $H_2O$ signal after 10° pulse @ 700 MHz




conventional TXI versus Cryoprobe TXI

# 600 MHz SEI $^1$H pulse calibration

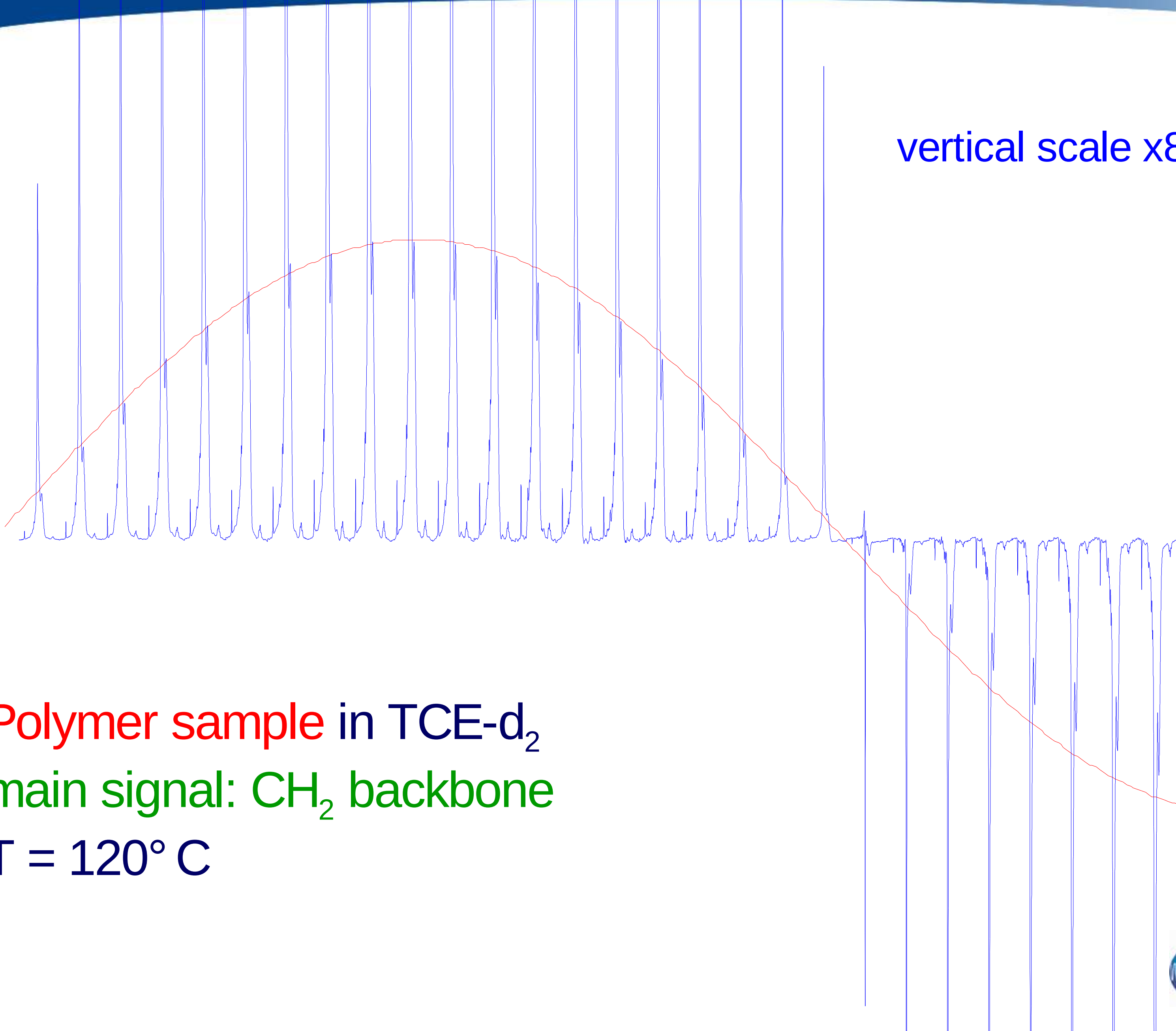


Polymer sample in TCE-$d_2$

main signal: $CH_2$ backbone

T = 120° C

# 600 MHz SEI $^1$H pulse calibration



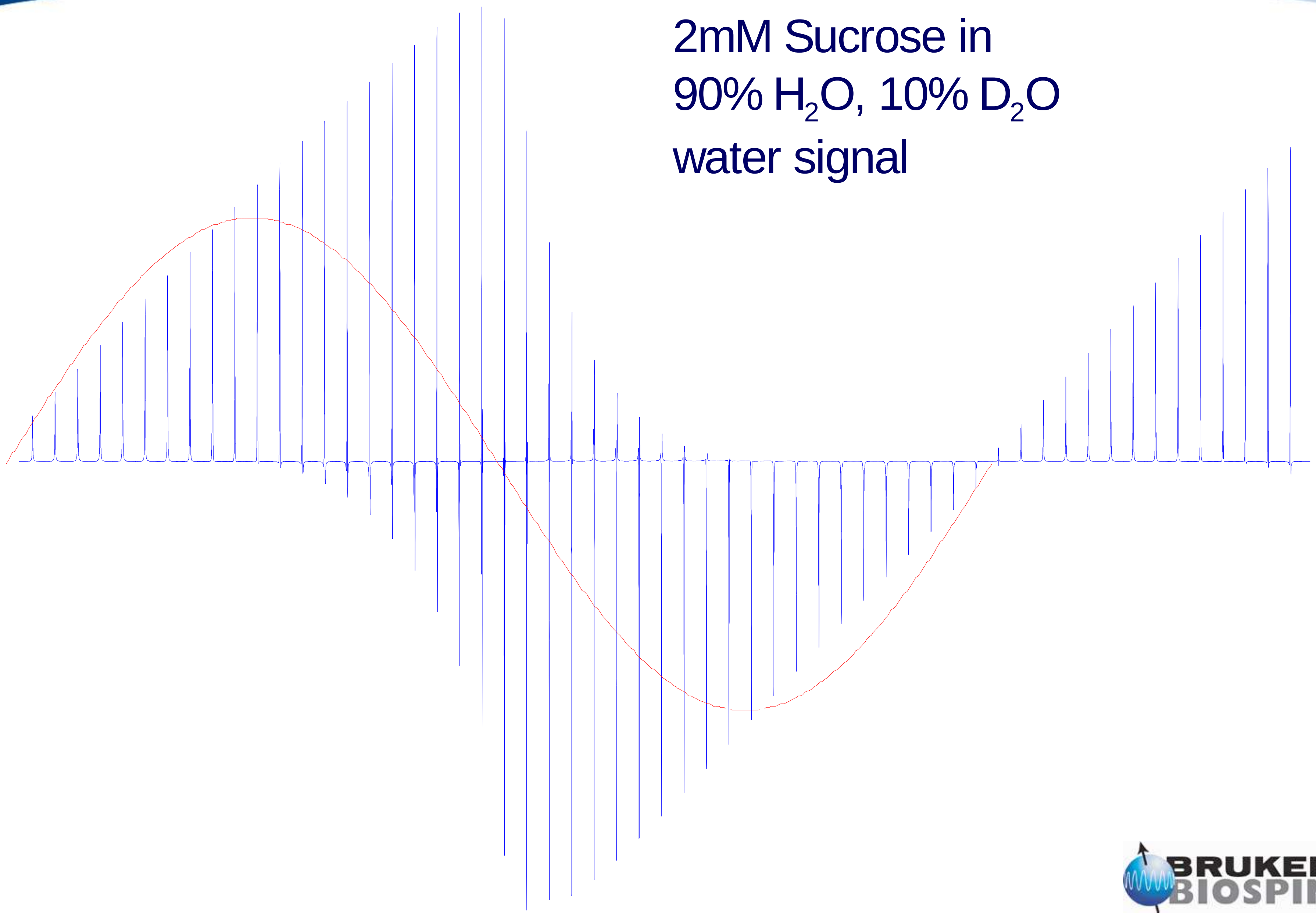

Polymer sample in TCE-$d_2$

main signal: $CH_2$ backbone

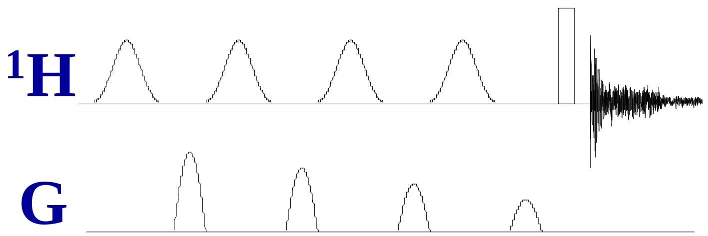
T = 120° C

# 700 MHz TXI $^1H$ pulse calibration

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

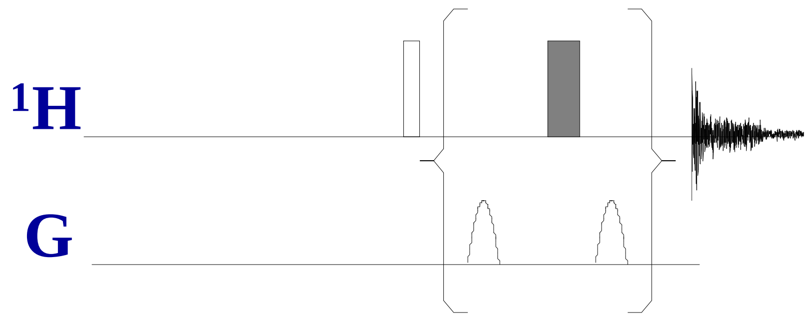


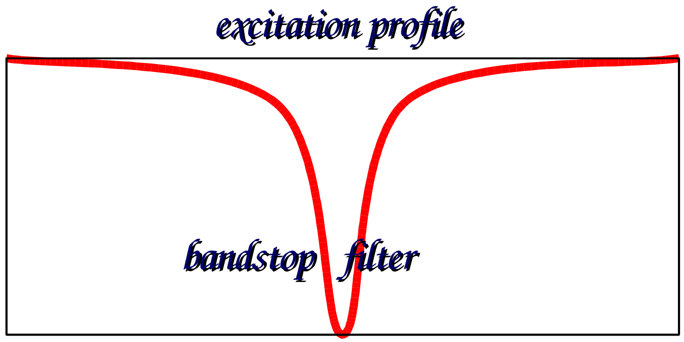
## WET - Water suppression




- 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: DPFGSE

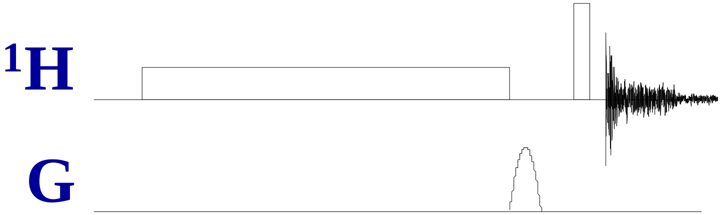
    double binomial watergate "w5", zggpw5

    excitation sculpting "es", zgesgp

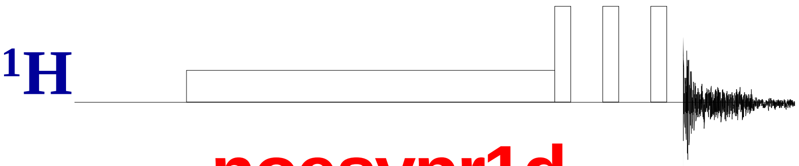
**presaturation:**

zgpr


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




- use volume selection to reduce solvent hump



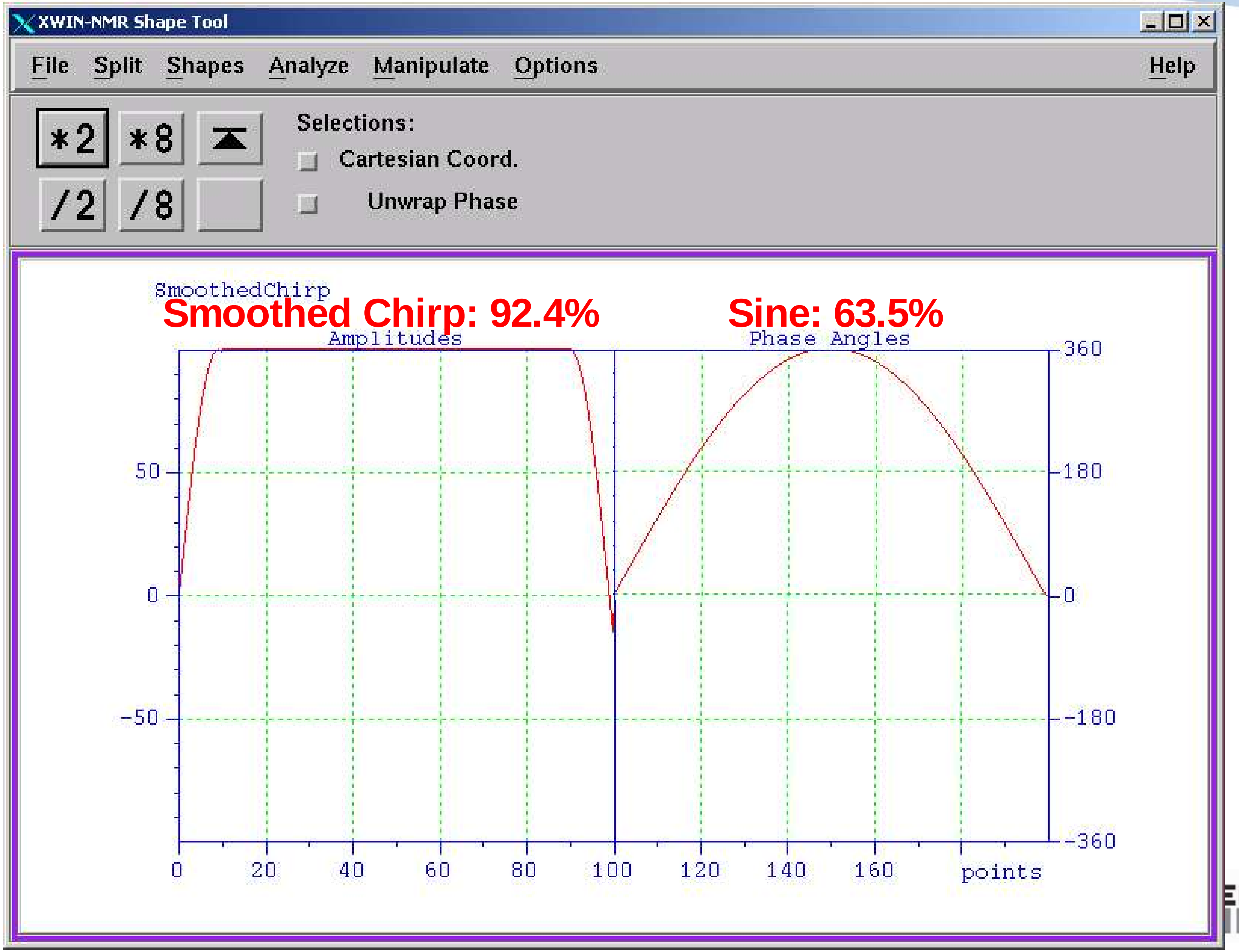

noesypr1d


zgcppr

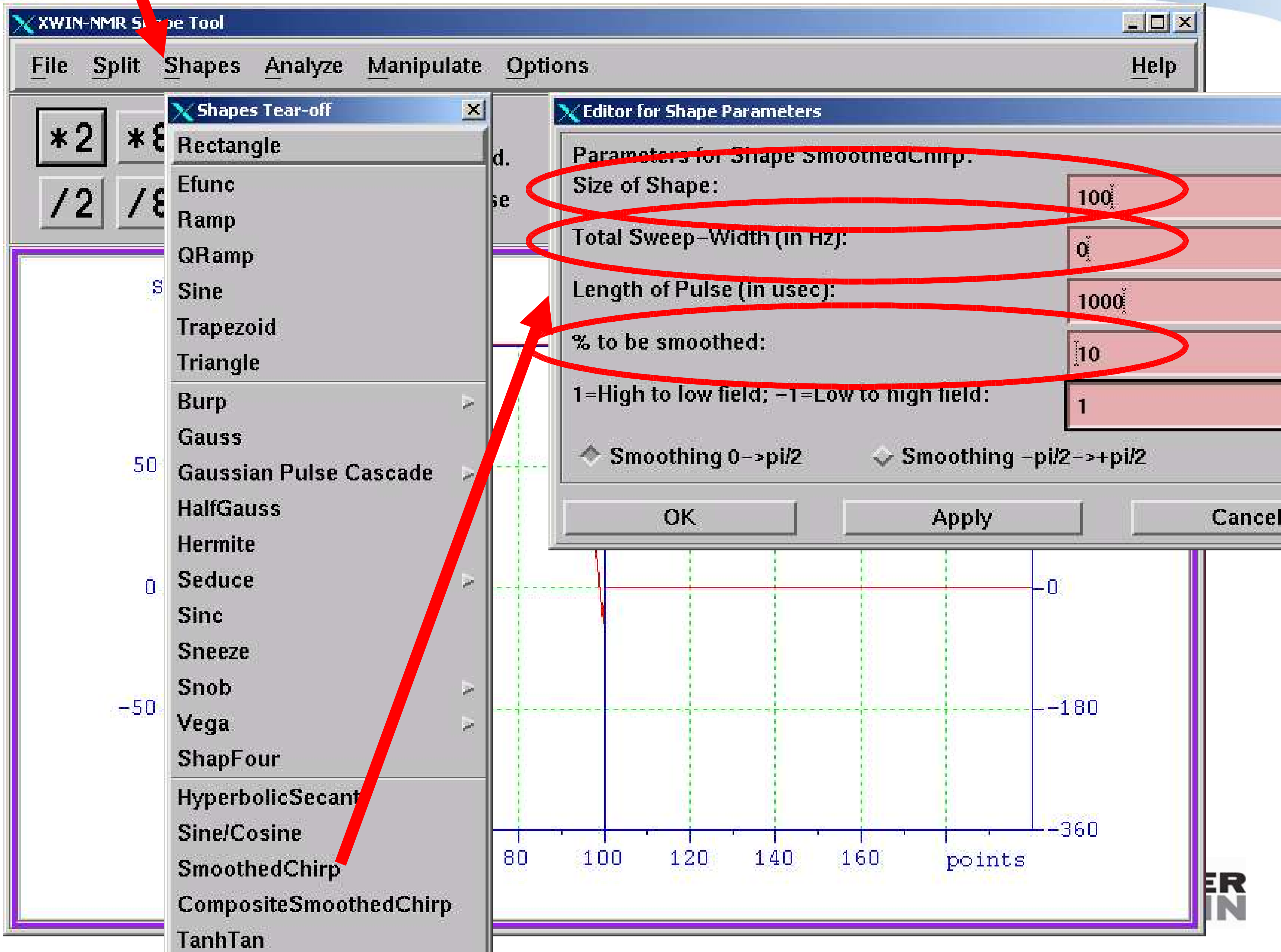
# Tips & Tricks for Gradients

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

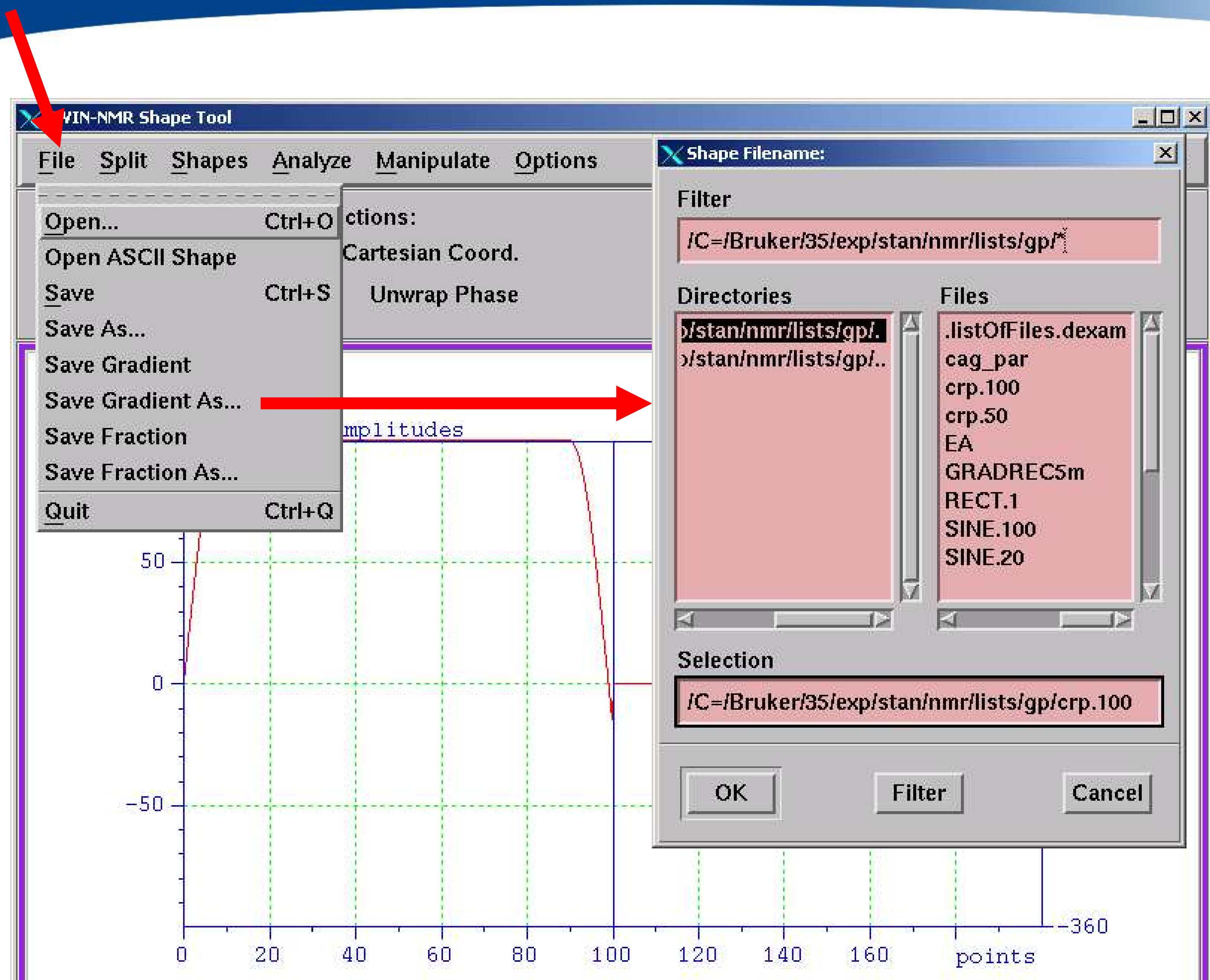
# Tips & tricks for gradients



# Tips & tricks for gradients



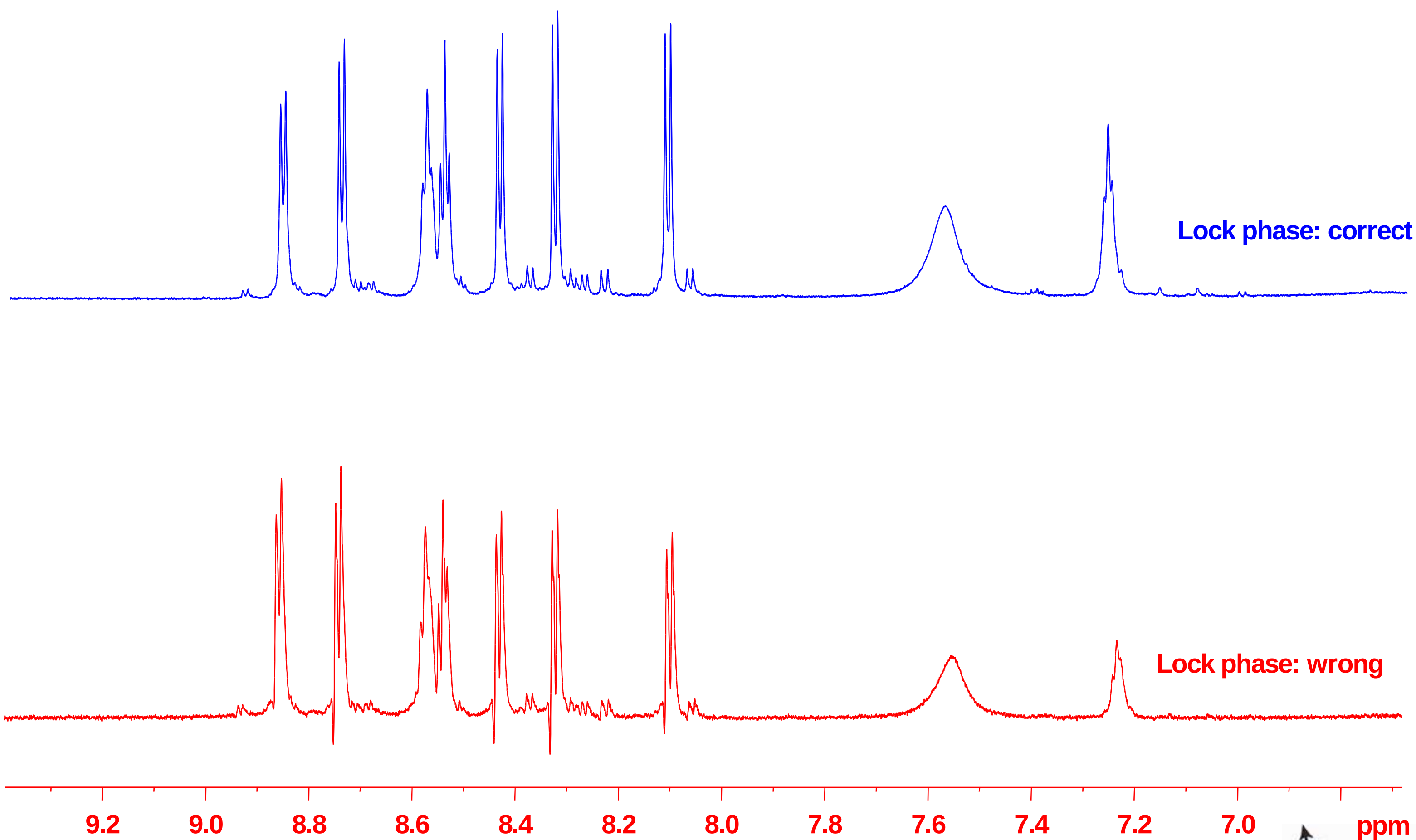
# Tips & tricks for gradients



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

# Artifacts due to wrong lock phase

## WATERGATE-experiment



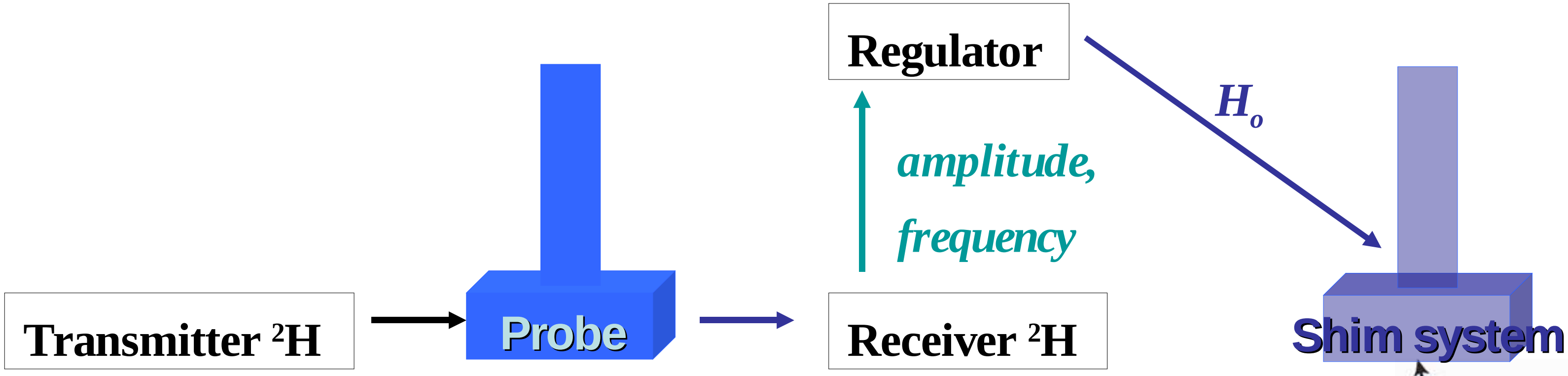
# The lock: details

The lock channel can be understood as a ‚*complete independant spectrometer within the spectrometer‘:*

**The resonance condition of NMR:**

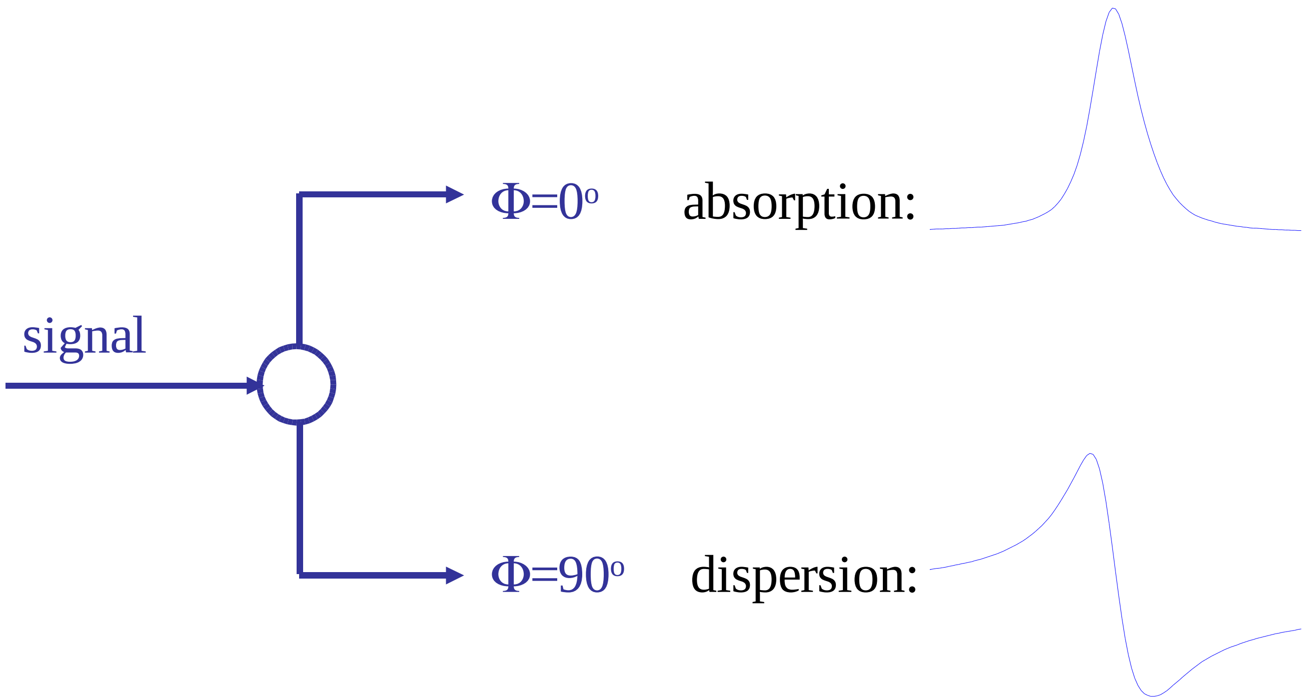
$\omega = \gamma B_o$ — ***but:*** $B_o$ ***is not stable***

$\omega = \gamma (B_o + H_o)$ — $(B_o + H_o) = const.$



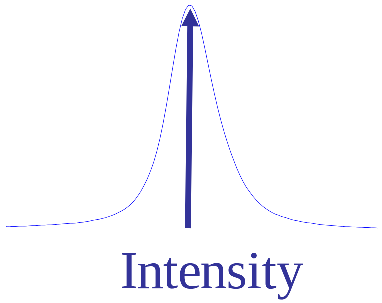
# The lock: lock phase

The lock receiver has two quadrature channels:

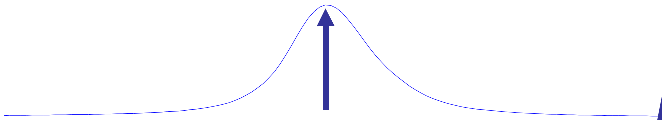


# The lock: field homogenisation

- 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 lock: field stabilisation

- 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

# lock phase

- 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

# Signal-to-noise ratio (S/N) and noise sources

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

- **For $R_{Coil}(T_{Coil} + T_{Preamp}) >> R_{Sample}T_{Sample}$ :**

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

- **For $R_{Sample}T_{Sample} >> R_{Coil}T_{Coil}$:**

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

# Signal-to-noise ratio (S/N) and noise sources

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

| | |
|---|---|
| $\omega$ | *frequency* |
| $\sigma$ | *conductivity* |
| *b* | *sample radius* |

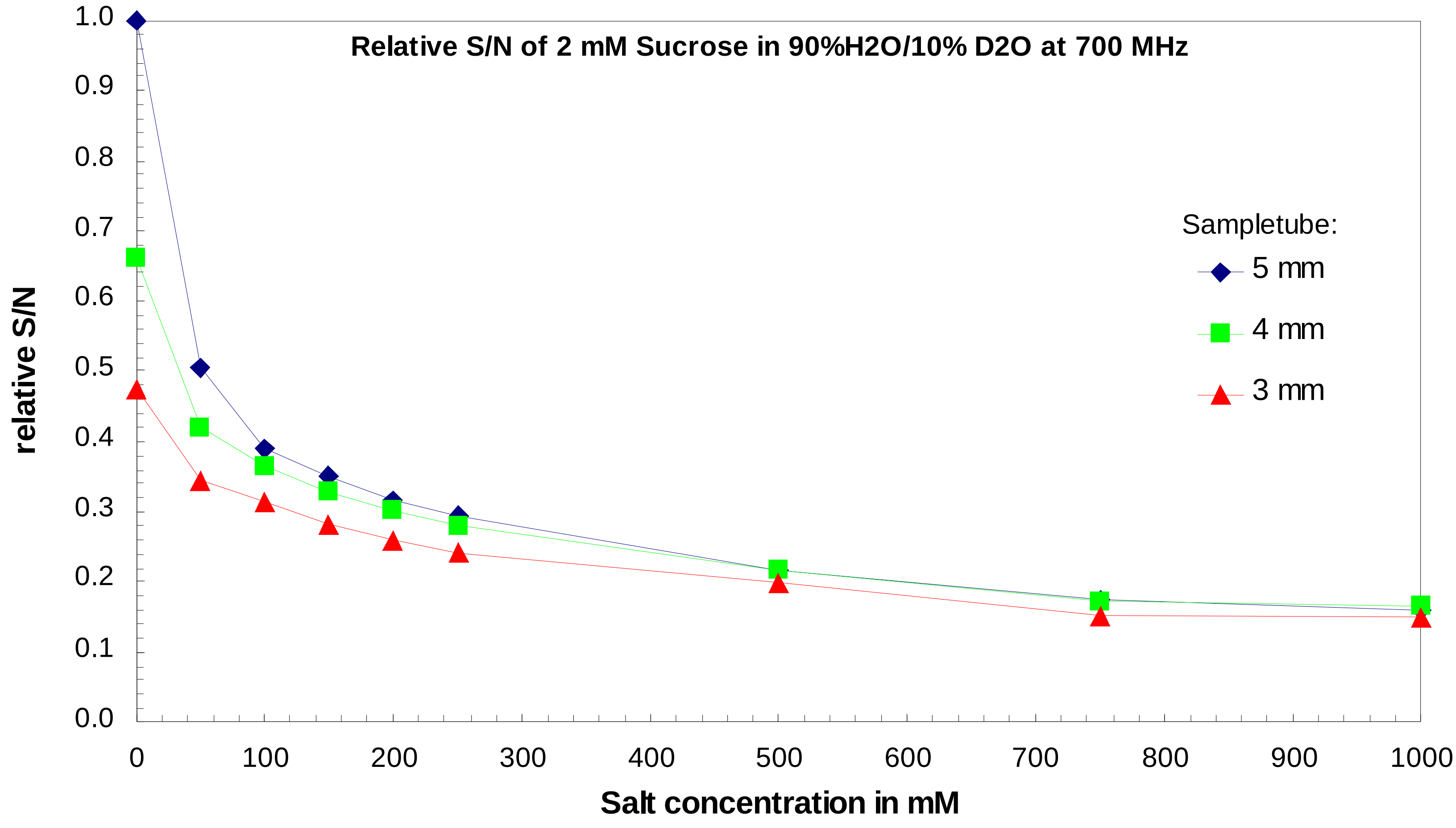
$$\sigma \propto \sum_i c_i q_i \lambda_i$$

| | |
|---|---|
| $c_i$ | *concentration* |
| $q_i$ | *charge* |
| $\lambda_i$ | *mobility* |

# Signal-to-noise ratio (S/N) and noise sources

- ***$R_S$* 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 $$R_{Sample} \propto \varpi^2$$

# Sample diameter for lossy solvents

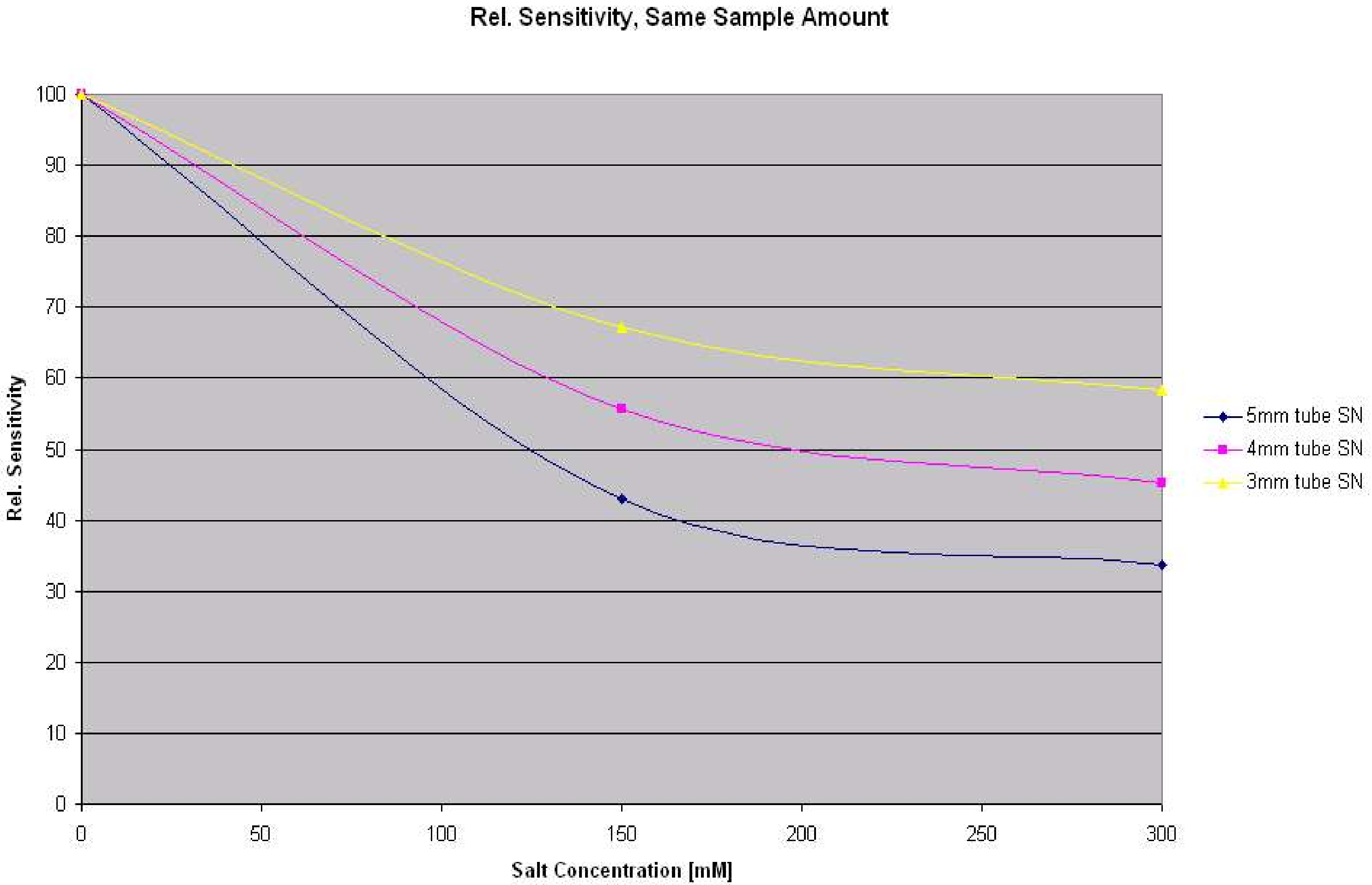


# Signal-to-noise ratio and Sample Diameter

Sensitivity and Salt Dependence as function of sample diameter

Identical Mass in all tubes:

Sucrose in $D_2O$, 600 MHz. TCI CryoProbe



# Sample Diameter and Solvent Volume

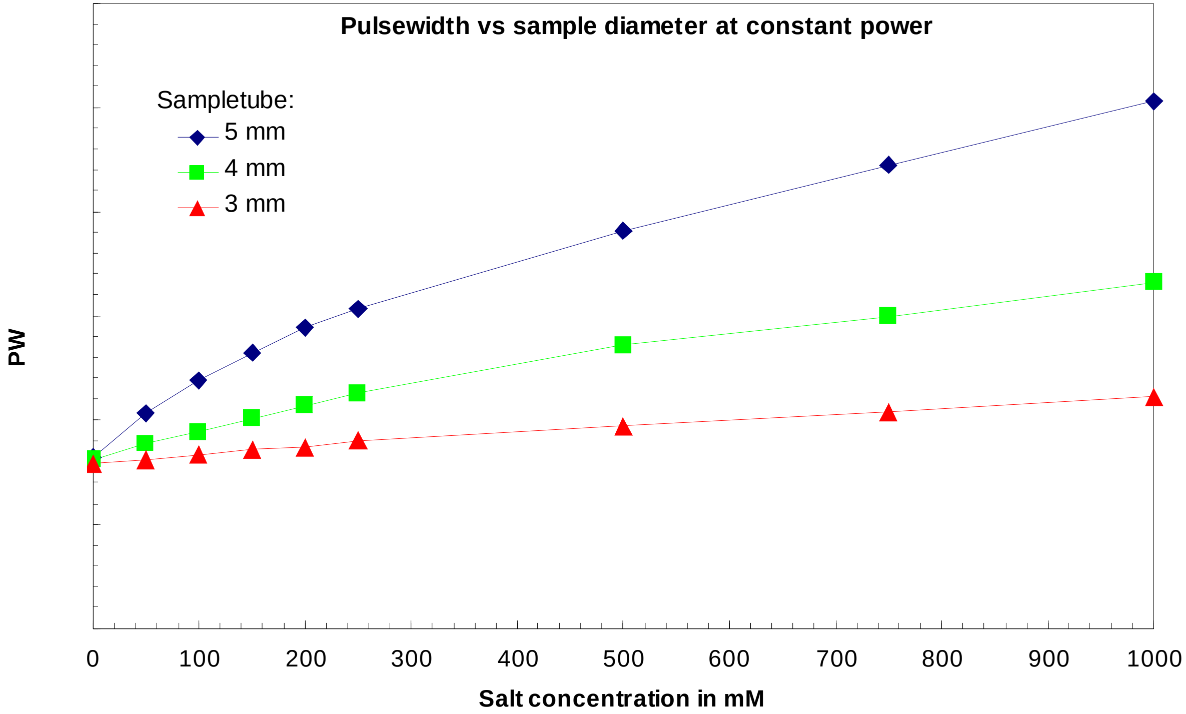
| Sample Diameter | Rel. Volume |
| --- | --- |
| 5.0 mm | 100.0 % |
| 4.0 mm | 63.5 % |
| 3.0 mm | 34.9 % |
| 1.7 mm | 10.7 % |

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

NOTE:

10. this applies only for $R_{Sample}T_{Sample} >> R_{Coil}T_{Coil}$
11. *Constant concentration*

# Sample diameter for lossy solvents




- If sample noise dominates
  - PW shorter with smaller tubes

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

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

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

# Signal-to-noise ratio (S/N) and noise sources

- ***$R_S$* 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 $$R_{Sample} \propto \varpi^2$$

# Signal-to-noise ratio and Conductivity

***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

*Volker Dötsch et al*

# Conclusion

***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