Some Notes About TOCSY and ROESY

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A. General

1. Spinlock

TOCSY and ROESY sequences have the similarity of using a high power spinlock in the mix portion of each experiment. The spinlock strength is reported in publications in units of Hz, and can be calculated from the pw90 at the spinlock power:

$$\gamma B_{\text{spk}}^{\text{mix}} = \frac{1}{4 \cdot \text{pw90}}$$

where pw90 must be given in units of seconds. Typical tocsy spinlock strengths are 7-10 kHz; I have seen few publications with higher spinlock powers, but don’t go >10kHz without asking me or Monika first. I’ve never seen roesy spinlock >7kHz.

2. Various general parameters

\begin{itemize}
    \item np=4096 or 2048 ; common values, with fn=np
    \item phase=1,2 ; always
    \item ni=400 ; a typical value
    \item proc1='ft' ; start with normal (not linearly predicted) processing
    \item fn1=2*ni ; in this example, start with fn1=1k
    \item d1=1 ; a common setting, probably ok for all TOCSY’s
    \item d1=2 ; d1 must not be too small for ROESY ( \geq 2\times T_1 )
    \item mix=0.08 ; a common setting for TOCSY, shorter for cosy-like, longer for complete mixing (but always \leq 0.2s)
    \item mix=0.1–0.2 ; typical for ROESY (but always \leq 0.5s)
    \item d3=0.00018–0.00030 ; watergate 3-9-19 only: see discussion in next section
\end{itemize}

3. Water Suppression

a) In general, preset (tn- type sequences) provides the best water suppression, but has a serious issue in reducing the intensity of protons that exchange with H2O.

b) Watergate (wg- type sequences) is the most common other type of water suppression used, but can suffer from a broad “notch” of suppression about the water, with reduced intensities for protons close to the water resonance. A variety of watergate sequences are available:

   i. Soft-pulse watergate can provide excellent suppression, but can also be problematic to optimize. See the follow section for notes about this optimization procedure.
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ii. 3-9-19 is a binomial type of watergate sequence with very simple optimization. It’s suppression is often sufficient, and is therefore the most common form of water suppression used on the Madison campus. 3-9-19 requires (as do all high-quality experiments) a calibrated pw90 @ twpr, as well as a correction factor, compH (in BioPack). Good compH values are required, and should match tpwr_cf in the facility probe files (kept at /vnmr/probes/HCN5/HCN5).

The other critical parameter for 3-9-19 is the interpulse delay, d3. This delay works similar to that for jump-return. A pure sinusoidal dependence is imposed on amplitudes as a function of frequency for jump-return: water is at the 0° null. The nulls are at:

$$\text{amplitude nulls } = \frac{1}{d3} \cdot n$$

where $n = 0, \pm 1, \pm 2, \ldots$. Maximum amplitudes then occur at $1/(2 \times d3)$.

For 3-9-19, the amplitude profile is much more square hat, but has these same characteristics with respect to d3:

α. For smaller d3, the nulls move out further from on-resonance, reducing the risk of severe attenuation of upfield methyls, or downfield amide protons, etc.

β. For smaller d3, the notch width for suppression about on-resonance (on H2O) also increases, improving the level of water suppression. But protons close to water can become severely attenuated.

γ. For larger d3, the notch width gets smaller: the suppression will not be as good, but protons close to water will have better intensity.

δ. For larger d3, the ±180° nulls move in toward on-resonance, and at some point will attenuate the protons at the edges of the spectrum.

*d3 = 180 to 300 μs* is common for 3-9-19 watergate water suppression, and depends on field strength, total spread of proton chemical shifts, and nearness of protons to the water resonance.

C) Always properly calibrate pw90 for these sequences.

d) It is important to not run roesy spectra with too small a d1; d1~2xT1 is recommended. This is less critical for tocsy (d1~T1), but too small a d1 will cause serious problems with all 2d experiments.

4. Other setup issues

a) Using a 1H spectrum with integrations set—the regions not integrated will be used for baseline corrections—is best, but unfortunately this is hard to implement with biopack sequences.

b) Always run the first row, phase carefully, and then use calfa. Repeat until the 1st row is phased properly with lp=0.
c) Always properly calibrate \texttt{pw90} for these sequences.

d) It is important to not run roesy spectra with too small a \(d_1\); \(d_1 \sim 2 \times T_1\) is recommended. This is less critical for tocsy (\(d_1 \sim T_1\)), but too small a \(d_1\) will cause serious problems with all 2d experiments.

\section*{B. TOCSY}

We have the following tocsy sequences implemented (\textbf{bolded} are preferred):

- \texttt{tnotocsy} ; an old presat implementation (mlev16)
- \texttt{tntocsy} ; a newer, biopack, presat implementation (mlev16)
- \texttt{wgtocsy} ; biopack watergate implementation, with a flag for switching between 3-9-19, softpulse, and W5 suppression
- \texttt{tocsy} ; an old sequence that should not be used
- \texttt{TOCSY} ; chempack (3.0) sequence that works great, but has poor water suppression
- \texttt{TOCSY1D} ; chempack 1d sequence (poor water suppression)

A primary issue with tocsy is what type of water suppression to use:

- \texttt{presat}
- \texttt{watergate 3-9-19}
- \texttt{watergate softpulse}
- \texttt{watergate W5}

\texttt{presat} provides the best water suppression (typically), but will reduce exchangeable protons to potentially unobservable levels. \texttt{presat} may also present Block-Siegert shifts in the data if the combination of presat power and repetition delay are too high and too fast, respectively.

\texttt{tntocsy} should be superior to \texttt{tnotocsy} for \texttt{presat tocsy}, but may not setup as nice as \texttt{tnotocsy}. I heavily modified \texttt{tnotocsy}, whereas \texttt{tntocsy} has a (dumb vanilla) biopack setup. \texttt{tnotocsy}, for example, sets up the spinlock properly to \(\sim 7\text{kHz}\).

The BioPack pulse sequence \texttt{wgtocsy} properly sets up the spinlock parameters based off \texttt{pw90}, \texttt{compH}, \texttt{tpwr}, and \texttt{strength}. The first three are key parameters that must be set in \texttt{ghn_co} and recursively set/implemented via the macro \texttt{BPbiopack2}.

\texttt{wgtocsy}: 3-9-19 watergate is the simplest of the watergates to set up; it requires only a calibrated \texttt{pw90} at normal power. It does give a relatively broad region of nulled data, so it is probably not the best if there are important protons close to water. 3-9-19 is highly preferred in NMRFAM for protein work. It may be that the flipback pulse parameters need to be optimized even for 3-9-19 watergate: array \texttt{flippw} and \texttt{phincr2} (?) and choose values with minimum water signal. See below for more details.
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wgtoesy: softpulse watergate tocsy used to be my favorite, as I got the best water suppression from non-presat types of tocsy, roesy and noesy. It is more complex to optimize (but really not that bad):

Set

\[ \begin{align*}
\text{nt} &= 1 \\
\text{av} & \\
\text{ni} &= 1 \\
\text{ss} &= 2 \\
\text{phase} &= 1
\end{align*} \]

all other parameters normal.

Now array the following parameters one at a time, and choose the value giving minimum water signal:

\[ \begin{align*}
\text{tof} & \text{ from } -205 \text{ to } -240 \text{ (best } \sim -220) \\
\text{flippw} & \text{ from } \sim 400 \text{ to } 2300 \text{ (best } \sim 1400) \\
\text{phincr2} & \text{ from } -80 \text{ to } +80 \text{ (best } \sim -10) \\
\text{flippw again} & \\
\text{p180} & \text{ from } 2\times \text{pw}90 - 2 \text{ to } 2\times \text{pw}90 + 2 \text{ (best is } 2\times \text{pw}90; ?) \\
\text{phincr1} & \text{ from } -80 \text{ to } +80 \text{ (best } \sim 0) \\
\text{phincr2 again} & \\
\text{tpwrsf_d} & \text{ ? (I see little variation, so optimizing seems to have little value)} \\
\text{tpwrsf_u} & \text{ ?}
\end{align*} \]

At the end, set \[ \text{nt}=8 \text{ ph phase}=1,2 \] and observe a normal 1st row. Water suppression should be excellent.

I have much less experience with W5 watergate, so it may be a good choice.

TOCSY spinlocks are either mlev or dipsi, which are not used for ROESY (see below for more info about roesy spinlocks).

C. ROESY

roesy sequences have a big issue with tocsy peaks artifacts. There are three major variations in the type of roesy spinlock, all working to avoid tocsy artifacts, and maximize roe peaks:

\[ \begin{align*}
\text{cw} & \text{ ;oldest and simplest; still preferred by some in the literature} \\
\text{dante} & \text{ ;implemented in tnroesy; has been badmouthed by some in literature, but data here is competitive to flip-flop and cw} \\
\text{flip-flop} & \text{ ;(or tic-toc) newest version greatly decreases tocsy artifacts, but is known to also reduce roe crosspeak intensity, especially as MW increases (so not for proteins; seems to be ok for peptides)}
\end{align*} \]
cw and dante spinlocks = 2-3 kHz
flip-flip spinlocks = 4-6 kHz

Keep in mind that these spinlock versions provide roe’s; we also have to select the form of water suppression to use (presat; watergate 3-9-19, softpulse, W5; and wet).

Versions of roesy are:

- **ROESY**
  - The best implementation by far when in organic solvents (it is the sequence provided in ChemPack 3.0), but unfortunately does not provide the optimal water suppression; can do cw, dante or flip-flop (change via flags); flip-flop, setup by default, is excellent!

- **tnroesy**
  - An old implementation, but is better than tnroesy (not sure why, but is true)

- **tnroesy**
  - Something is wrong with this biopack sequence, so for now, do not use

- **wgroesy**
  - A reasonable watergate roesy sequence (from biopack), although implementation is odd. Uses flip-flop spinlock, and 3-9-19 watergate as defaults. I have modified the wgroesy macro so the spinlock is setup correctly, to ~6kHz, with 3-9-19 watergate water suppression. Other watergate types can be used, and are calibrated as outlined above for wgtoe.

- **wroesy** and **wetroesy**
  - I don’t know much about these. Will have to update later.

**ROESY** properly sets up the spinlock defaulting to flip-flop and ~6kHz. Use this sequence for samples in deuterated organic solvents.

**tnroesy** correctly sets up the dante spinlock to 3kHz, with presat water suppression.