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:

$$\gamma B_1^{splk} = \frac{1}{4 \cdot pw90}$$

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

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

3. Water Suppression

a) In general, presat (**tn**– type sequences) provides the best water suppression, but has a serious issue in reducing the intensity of protons that exchange with H_2O.

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.

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:

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

where $n = 0, \pm1, \pm2, \ldots$. Maximum amplitudes then occur at $1/(2\times\mathbf{d3})$.

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 H_2O) 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**; **d1~2×T_1** is recommended. This is less critical for tocsy (**d1~T_1**), 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 **pw90** for these sequences.

d) It is important to not run roesy spectra with too small a **d1**; **d1~2×T_1** is recommended. This is less critical for tocsy (**d1~T_1**), but too small a **d1** will cause serious problems with all 2d experiments.

B. TOCSY

We have the following tocsy sequences implemented (**bolded** are preferred):

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

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

presat
watergate 3-9-19
watergate softpulse
watergate W5

presat provides the best water suppression (typically), but will reduce exchangeable protons to potentially unobservable levels. 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.

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

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

wgtocsy: **3-9-19 watergate** is the simplest of the watergates to set up; it requires only a calibrated 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 **flippw** and **phincr2** (?) and choose values with minimum water signal. See below for more details.

wgtocsy: **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 **nt=1**
av
ni=1
ss=2
phase=1

all other parameters normal.

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

tof	from –205 to –240 (best ~ –220)
flippw	from ~400 to 2300 (best ~1400)
phincr2	from –80 to +80 (best ~ –10)
flippw again	
p180	from 2×**pw90**-2 to 2×**pw90**+2 (best is 2×**pw90**; ?)
phincr1	from –80 to +80 (best ~0)
phincr2 again	
tpwrsf_d	? (I see little variation, so optimizing seems to have little value)
tpwrsf_u	?

At the end, set **nt=8 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:

cw	;oldest and simplest; still preferred by some in the literature
dante	;implemented in tnoroesy; has been badmouthed by some in literature, but data here is competitive to flip-flop and cw
flip-flop	;(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)

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!

tnoroesy ;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 wgtocsy.

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

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

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