

BEST Pulse Sequences

Introduction: A new group of pulse sequences is now available for the 800 and 900 systems based on the Band-selective Excitation Short-Transient (BEST) model introduced by Schanda, et al. (JACS 2006, **128**, 9042-9043). These sequences allow one to use very short recycle delays ($d1 \sim 0.5$ s) and obtain optimal lineshape due to the fact that magnetization of interest is only selectively excited. This selective excitation means that the bulk of the proton magnetization in the sample remains longitudinal throughout the experiment and does not contribute to the relaxation pathway. Effectively this means that a protonated sample behaves as if it was deuterated, and may allow you to collect high-resolution triple resonance spectra on samples that are only N15 and C13 labeled. As an additional bonus, the short recycle delay means that these experiments can be collected much more rapidly than standard sequences.

Directions: These sequences are included in the current BioPack installation on both the 800 MHz and 900 MHz systems. This means that the parameter libraries and pulse shapes are automatically generated when you select the experiment from the drop-down menus. These sequences are not as well tested as the standard BioPack experiments though, and so some adjustments to the parameters will be necessary before you begin running.

Recycle delay/decoupling: The first parameter that will need to be changed is the recycle delay ($d1$). This parameter does carry with it the ability to do some harm, however, so care should be taken not to make it too short as to exceed the duty-cycle of the probe. On the cold probe this is of particular concern. Start on the safe side with $d1=0.5$ s, and ensure that the N15 decoupling is set to use the standard BioPack “wurst40N” decoupling sequence as this is a much lower power sequence than GARP or WALTZ. You can use GARP to decouple, but if you choose to do so, make sure that you do not exceed 40 for the $dpwr2$ parameter. Unfortunately, TROSY is not an option in the BioPack sequences. Soon we hope to develop a set of TROSY based sequences that will lift this decoupling requirement.

Gradient time $gt1$: For some of the sequences, the default time for $gt1$ is set to 1 ms. Unfortunately, this is not long enough for gradient shaping of the decoding gradient to occur. Depending on which spectrometer/software version you are running, this may or may not cause the sequence to fail. The problem is due to the long selective pulse that occurs after the encoding gradient in the sequence. In order to run the sequence properly you may need to set $gt1$ as low as 700 μ s, and turn off gradient shaping ($gradientshaping='n'$). This may cause issues with the cold probe, so it is important to bear in mind when selecting the sequence you wish to run (standard or BEST).

Other potential issues: So far these sequences are not as well tested as the other sequences we routinely employ. This means that there may be issues with the sequences that are beyond the scope of your ability to fix. In any case, if you fail to see signal with the BEST sequence, having a reference sequence that you know

works to compare to will allow you to quickly determine if your problem is sample or sequence dependent. As we gain confidence in these sequences and develop sequences of our own we should be able to diagnose these problems more quickly, but for now assume that the sequence is wrong and not your sample if you encounter difficulties. You can always revert to the standard pulse sequences if things are not looking correct.

Conclusions: These sequences have the potential to drastically speed up the collection of data on the spectrometers. For example, a standard HSQC experiment which may take an hour to acquire, can be obtained in ~15 minutes using the BEST equivalent sequence. This is a significant time savings, especially if multiple spectra are required, such as in the case of a titration. This potential can also be realized for triple resonance backbone assignment spectra of C13/N15 labeled samples. The BEST sequences are not applicable to NOESY and dynamics experiments, however, so there are limitations. In addition, it may take a little more time to setup the experiment than for the standard sequences. Once we have established a set of “working” parameter sets this time should be less of an issue.

In any case, these experiments should be considered when you use the 800 or 900 MHz spectrometers. If you encounter problems, or are unsure of some parameters in the experiment setup, please do not hesitate to ask for help. As we develop our experience with the BEST sequences we will be able to address these issues more quickly and will all benefit from the discussion.