VnmrJ 3.1

Experiment Guide

Agilent Technologies

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Chapter 1
Introduction

Key points:

- ✓ Content and goals of this manual
- ✓ How this manual is organized
- ✓ Philosophy and benefits of modular experiment setup

Content and Goals

This manual has been created for the benefit of organic chemist/novice NMR spectroscopists to intermediate level NMR spectroscopists.

The purpose of this document is to provide more information about the small-molecule liquids experiments available in the VnmrJ 3.1 experiment selector. Users will find descriptions of the experiments, their utility, guides to parameter selection, processing, and references for further reading.

Organization

The chapters of this manual have been divided into the headings listed on the tabs of the experiment selector found in the middle of the left-hand side of the VnmrJ window:

Experiment Selector

HF_Expts	(HC)Crisis2	Hadamard
Jn(CH)corr	(H)Sel1D	(HC)HetToxys / Sel2D
Service	(HH)Homo2D	JSpectra / J1(CH)corr
Common	Std 1D	(CC)corr / System Studies

PROTON	CARBON
FLUORINE	PHOSPHORUS
(H)PRESAT	(H)wet1D
T1_MEASURE	T2_MEASURE
(C)APT	(C)DEPT
PureShift 1D	(H)HomoDec
CARBONecho	BilevelDec
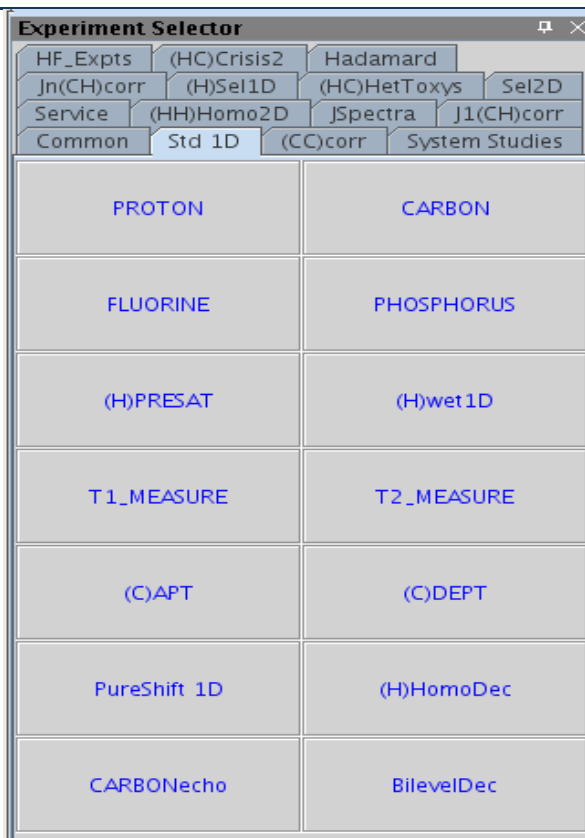
Please note that the DOSY2D and DOSY3D experiments are installed with the Dosy package for VnmrJ, and these tabs will not appear if this package has not been purchased and/or installed. A separate DOSY manual is available under the "Help $\rightarrow$ Online Manuals" menu.

The "Common" tab is a redundant listing of popular routine experiments; similarly, the "System Studies" serve as demonstrations of experiments combined into a single study. Since these experiments are covered under other tabs, these tabs have not been included. Finally, the "Service" tab is intended for the use of system service personnel, not the users of this manual, and has been omitted.

The chapters begin with a general description of the family of experiments and some of the key variants among the experiments. Each experiment in the family is then discussed separately and in depth. The individual experiment descriptions contain a general overview, examples of usage, a pulse sequence diagram, guidance for parameters selection and processing, and literature references. A screenshot of the "Default" panel of the "Acquire" tab, which contains the most pertinent parameters, is shown for each experiment. Pictures of spectra from the experiments are not shown; however, example data sets for most experiments may be found in the directory /vnmr/fidlib on your spectrometer.

Modular Experiment Setup

All the sequences in the experiment selector have been built with a modular design to facilitate parameter setup. By using a "layering" approach where each experiment contributes some

property to the final combination, it is possible to configure hundreds of variations without the need for hundreds of individual entries. Each experiment has a customized "Default" panel under the "Acquire" tab that contains the most important and commonly modified parameters for that experiment. Additional panels are added under the "Acquire" tab for more detailed parameters when necessary. When new experiments are created from existing ones, relevant parameters such as the spectral width are retained in a logical fashion.

To illustrate the modular concept, let's consider a progression of experiments starting with a PROTON and ending with an HSQCADTOXY. A PROTON experiment has been acquired with a customized spectral width (sw). If an HSQC experiment is selected from the proton, this sw will be maintained in the F2 dimension of the HSQC as well as other acquisition dominant parameters such as the 90° pulse width (pw90). Parameters related to the F1 dimension, e.g. number of increments (ni) or the ^{13}C spectral width, need to be set. If the user decides to acquire an HSQCAD instead, these newly set F1 parameters will transfer to the HSQCAD and need not be reset. The necessary adiabatic shaped pulses are calculated automatically from parameters in the probe file. Then, to acquire an HSQCADTOXY from the HSQCAD experiment, only the parameters relating to the TOCSY spinlock (e.g. the mixing time) will need to be set. By contrast, if a TOCSY experiment had been set up first prior to the HSQCADTOXY, then the parameters related to the TOCSY would transfer, and the HSQC-related parameters would need to be set up instead.

Solvent suppression also is much more facile with this modular experiment design. Solvent suppression ("wet", "presat", or "purge" type) is available for nearly all the experiments in the selector. Separate experiments such as "presat COSY" or "wet TOCSY" are not necessary, which greatly simplifies the experiment choices. The desired type of solvent suppression is set by choosing the appropriate (H)PRESAT or (H)wet1D experiment and parameters (PURGE suppression is part of (H)PRESAT). Any experiment that is selected subsequently will automatically contain the same solvent suppression set up.

The focus of this manual is using the "Experiment Selector" buttons and the "Acquire" tab parameter panels to set up experiments. However, the "Experiments" pull-down menu at the top of the VnmrJ window contains some useful functionality for setting up experiments with nuclei other than the typical ^{1}H and/or ^{13}C. Briefly, selecting experiments under the "Convert Current Parameters To Do..." heading retains the currently set transmitter nucleus (tn) and decoupler nucleus (dn). If the HSQCAD experiment is chosen with tn='F19'/dn='P31', the resulting parameters will be for a ^{19}F-^{31}P HSQCAD experiment. Similarly, if the PRESAT experiment is chosen with tn='F19' or 'P31', the result would be a 1D ^{19}F or ^{31}P experiment with presaturation at a selected frequency. This menu allows the facile setup of experiments with "non-standard" nuclei.

One final note is that for all the pulse sequences, necessary shaped pulses are calculated automatically from a combination of parameters in the appropriate module and the probe file. Hence, a probe file containing the relevant calibration information for the experiments is required. Please refer to the VnmrJ Installation and Administration manual, Chapter 9, for information on setting up a probe file.

Chapter 2
Standard 1D - Std 1D

Introduction

This tab of the experiment selector includes the set of standard 1D experiments available, including experiments for proton and other nuclei. The experiments commonly used for proton detection include the standard PROTON 1D experiment, experiments with both presaturation and wet-type solvent suppression, T_1 and T_2 relaxation measurements, plus homonuclear and bi-level decoupling experiments as well as an experiment that produces the equivalent of a broad-band decoupled proton spectrum, called Pureshift 1D. Carbon-detected experiments included are the basic CARBON, (C)APT and (C)DEPT multiplicity selection, and a CARBONecho experiment. Finally, there are tabs for [19]F (FLUORINE) and [31]P (PHOSPHORUS) 1D experiments, which are also easily adapted for other X-nuclei.
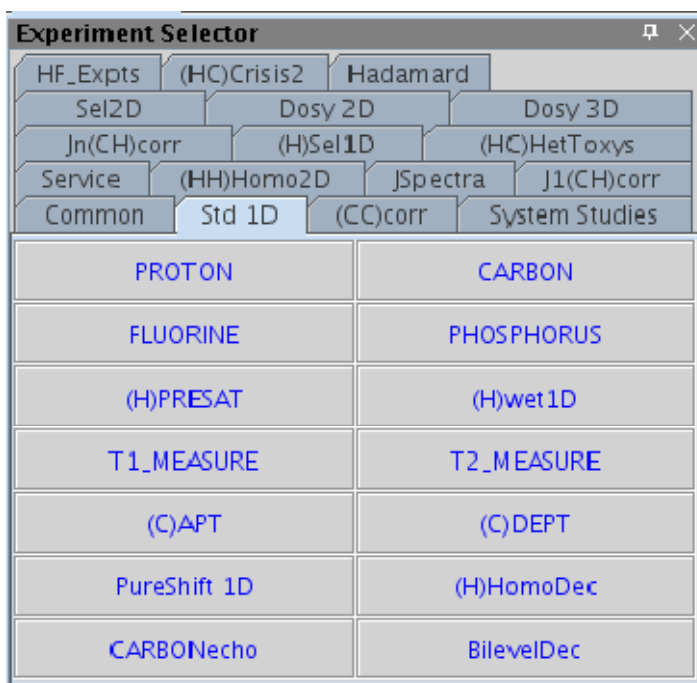
PAGE LEFT BLANK INTENTIONALLY

PROTON
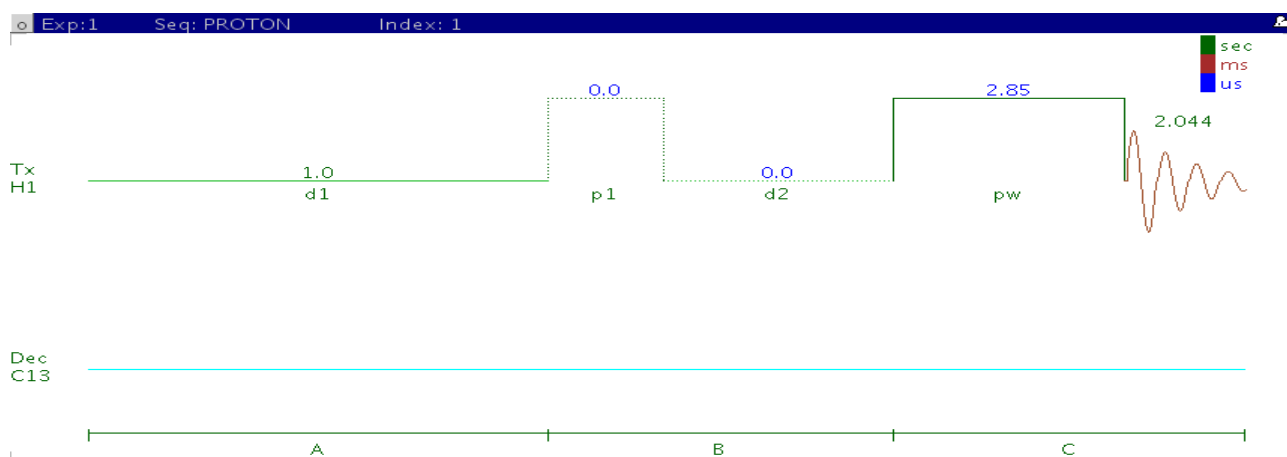
GENERAL DESCRIPTION AND USAGE

Description:
Uses the standard s2pul pulse sequence to acquire a 1D-^{1}H dataset.

Usage:
Just as the PROTON experiment is the first step in a typical small molecule structure elucidation, it is also the basic building block for most of the common liquids NMR experiments. When acquiring under automation, most of the 2D experiments require a 1D proton to be acquired first, and it is helpful to begin with this experiment when acquiring manually as well. Important parameters such as solvent and spectral width will be transferred to further experiments that are setup from the proton dataset. All parameters are set from the "Acquire" tab/"Default H1" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel. Suggested choices for resolution enhancement (at the expense of S/N) are "pseudo echo" and "res-enhance." When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the "Interactive Weighting" button. CAUTION: the spectral integration will be affected with resolution enhancing functions- do not use these types of apodization for quantification.

TIPS, TRICKS AND NEXT STEPS

- The appropriate receiver gain (gain) is most easily set by making sure the Autogain option is checked on right-hand side of the "Acquire" tab. The software will optimize the gain setting prior to data collection.

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the MoveSW button located under the spectrum window and reacquire the data.

- To automatically estimate the 90° pulse for subsequent experiments, click the Est. pw90 button located underneath the spectrum window. This calibration will be used in lieu of the value in the probe file for future experiments on this sample. Calibration is particularly useful with either salty samples or where perfect tuning may not be achievable, both of which can cause longer 90° pulse values.

- The acquisition time (at) which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" panel of the "Acquire" tab. While the default digital resolution that is set from the chosen spectral width is typically much greater than the actual linewidths, you may wish to check that the acquisition time is sufficient when acquiring large spectral widths or when trying to accurately measure linewidths, e.g. for probe lineshape tests.

- A pulse tip angle of 45° is common for PROTON experiments. One should consider increasing to 90° for quantification. Decrease to smaller values (30° and less) in case of very concentrated samples to avoid receiver/ADC overflow.

- Remember that the *total* interscan delay is the sum of the acquisition time and the relaxation delay. When acquiring a multi-scan PROTON experiment for quantification, the interscan delay should be at least 5X the longest proton T_1 value in the molecule (see the T1_MEASURE experiment to measure these values). Typical values for an interscan delay range from 3-5s (d1 of 1-3s with an acquisition time of ~2s), which is not appropriate for quantification; one should consider using ~25s if the T_1 values are not measured. Note that for very precise quantification (<1%) a total relaxation delay of 10 x T_1 is appropriate.

- The PROTON dataset is used as a starting point for a wide range of experiments. Common next steps include setting up solvent suppression, choosing peaks for selective 1D experiments, and homo- and heteronuclear 2D experiments such as TOCSY and HSQC.

Reference(s):

R. J. Abraham, P. Loftus, Proton and Carbon-13 NMR Spectroscopy: An Integrated Approach, Heyden, London, 1978.

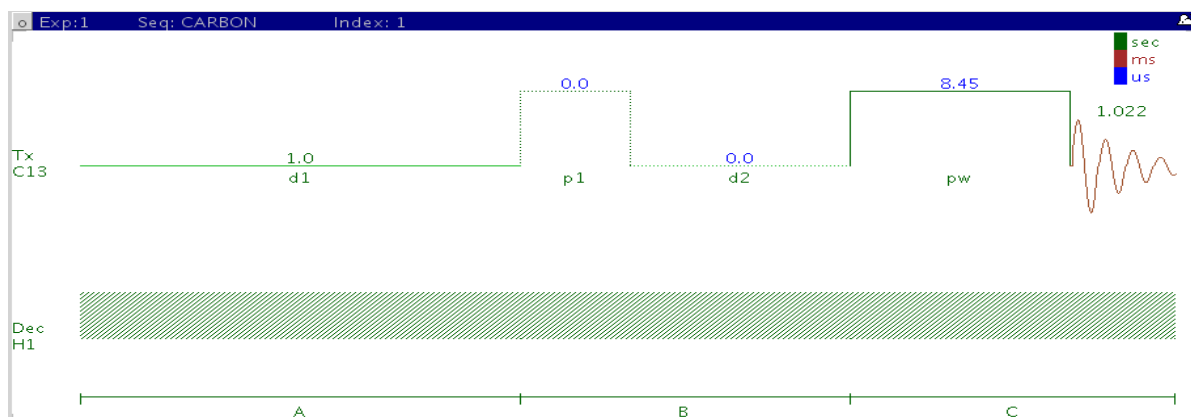D. D. Traficante, Concepts Magn. Reson. 4 (1992) 153-160. (Quantitative Analysis)

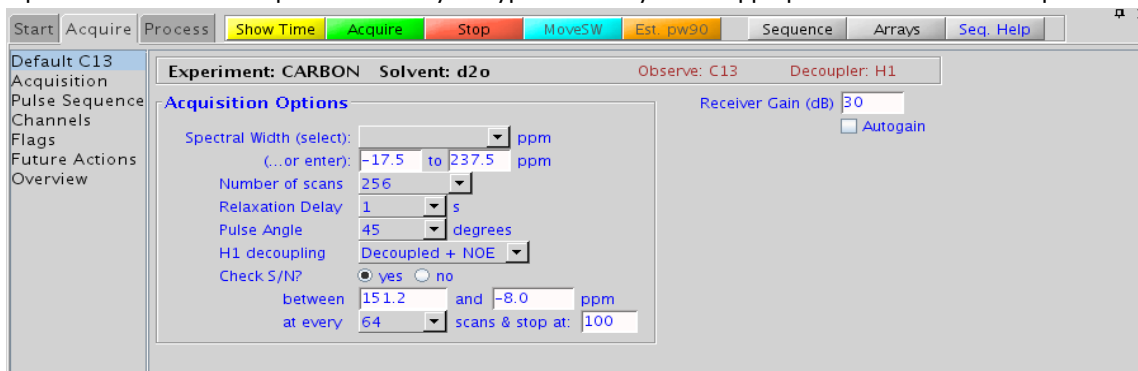# CARBON

GENERAL DESCRIPTION AND USAGE

Description:

Uses the standard s2pul pulse sequence with decoupling to acquire a 1D-^{13}C dataset.

Usage:

The 1D CARBON experiment is a tool commonly used by organic chemists for structure elucidation and confirmation as the carbon chemical shift is highly sensitive to nearby functional groups. Proton decoupling is traditionally used to simplify Carbon spectra to a series of single lines with little or no coupling constant information available. Carbon chemical shifts are also often quite reliably calculated from structural input by prediction software. The CARBON experiment, however, requires a relatively large amount of sample (or spectrometer time) as compared to proton-detected experiments due to the lower relative natural abundance of the ^{13}C nuclide and its reduced receptivity when compared to ^{1}H. All parameters are set from the "Acquire" tab/"Default C13" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 decoupling (dm):	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?:	Select yes or no- acquires acquisition until S/N value between ppm indicated is > specified value
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D carbon spectrum is line broadening, with an lb setting from ~0.5-1 Hz. Carbon spectra will rarely be of high enough S/N for resolution enhancement; however other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" panel of the "Acquire" tab. Because carbon spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small coupling; one should consider lengthening the acquisition time and/or narrowing the spectral width when acquiring coupled carbon spectra.

- The standard proton-decoupled CARBON spectrum is produced by turning on the decoupling during the pulse and acquisition. It is also standard practice to leave the decoupler on during the preacquisition delay (d1), which provides an NOE enhancement to the carbon signals caused by the attached protons. Note that typically the more protons attached, the larger the enhancement. For example, methyl signals will appear larger than quaternary carbon signals. Turn off the NOE enhancement if accurate quantification is desired.

- A common next step for structure elucidation after acquiring the CARBON data is to acquire a series of (C)DEPT experiments to determine the multiplicity of the carbon peaks (e.g. methyl, methylene, and methine).

- Carbon T_2 relaxation times can be <u>very</u> long, particularly for quaternary and aromatic carbons and for degassed samples such as the 10% ethylbenzene standard. Such samples may exhibit the symptom of being difficult or impossible to phase correctly if the spectra are truncated. Increase the acquisition time (at) until the phasing issue is corrected.

- If there is insufficient sample to obtain a CARBON spectrum, one should consider acquiring proton-detected experiments such as HSQC and HMBC, which give similar information and require much less sample. These experiments can also be configured to give multiplicity information.

- The CARBON dataset can be used as a starting point for carbon-detected correlation experiments such as INADEQUATE or HETCOR spectra. Optimized spectral widths will be transferred to the 2D experiment.
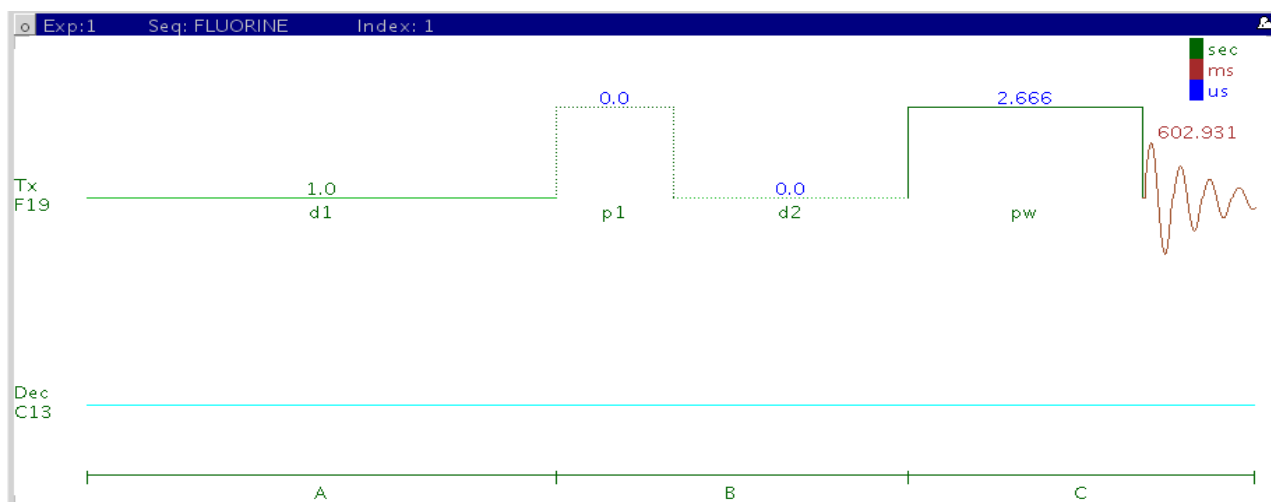
Reference(s):

F. W. Wehrli, T. Wirthlin, Interpretation of Carbon-13 NMR Spectra, Heyden, London, 1976.

R. J. Abraham, P. Loftus, Proton and Carbon-13 NMR Spectroscopy: An Integrated Approach, Heyden, London, 1978.

E. Breitmaier, W. Voelter, Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry, VCH, 1987.

GENERAL DESCRIPTION AND USAGE

Description:

Uses the standard s2pul pulse sequence to acquire a 1D-^{19}F dataset.

Usage:

The 1D FLUORINE experiment is typically used to confirm the presence of fluorine in a molecule. The large fluorine chemical shift range is highly sensitive to nearby functional groups. The experiment is nearly as sensitive as a 1D PROTON experiment. All parameters are set from the "Acquire" tab/"Default F19" panel unless otherwise specified.

KEY PARAMETERS

|	
Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel. Suggested choices for resolution enhancement (at the expense of S/N) are "pseudoecho" and "res-enhance." When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the "Interactive Weighting" button. CAUTION: the spectral integration will be affected with resolution enhancing functions- do not use these types of apodization for quantification.

Many NMR probes contain fluorinated materials, which cause broad signals that can distort the baseline (especially when lying next to or outside the spectral window). The default processing parameters are set to use linear prediction to back predict the first seven points of the spectrum in order to flatten the baseline. These settings may be modified under the "Process" tab on the "More 1D" panel.

TIPS, TRICKS AND NEXT STEPS

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the `MoveSW` button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is very large, once the fluorine signals are located, one should consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.

- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" page of the "Acquire" tab. Because FLUORINE spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small couplings; one should consider lengthening the acquisition time and/or narrowing the spectral width.

- Remember that the *total* interscan delay is the sum of the acquisition time (at) and the relaxation delay (d1). When acquiring a multi-scan FLUORINE experiment for quantification, the interscan delay should be at least 5X the longest fluorine T_1 value of the signals of interest.

- Note that because of the potentially very wide spectral window, determination and use of 90° pulses with FLUORINE NMR experiments can be problematic. The pw90 should be measured with the transmitter offset (tof) positioned near an actual ^{19}F resonance.

- This fluorine experiment will produce a proton-coupled ^{19}F spectrum. See the HF experiments (Chapter 11) for a proton-decoupled version of the experiment.

- When acquiring impure samples of fluorinated compounds from a biological origin, one should consider using fluorine spectra in lieu of a more complicated proton spectrum as appropriate.

Reference(s):
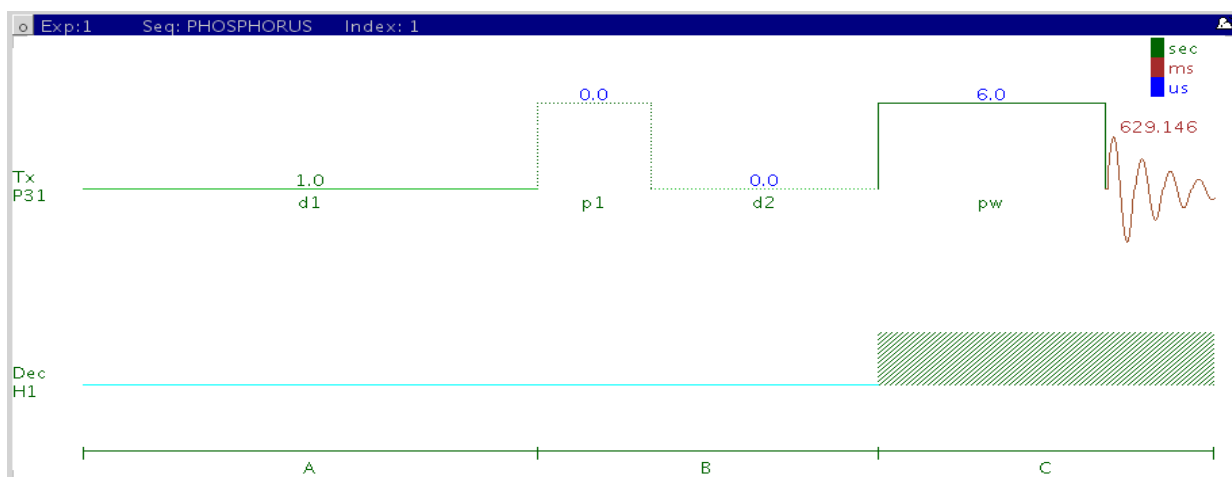
E. G. Brame, Anal. Chem. 34 (1962) 591-592. (Spectra Structure Correlations of F19)

E. F. Mooney, An Introduction to 19F NMR Spectroscopy, Heyden, London, 1970.

S. Berger, S. Braun, H. O. Kalinowski, NMR Spectroscopy of the Non-Metallic Elements, Wiley, New York, (1997) 398-698.

J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23. (Multidimensional ^{19}F NMR)

PHOSPHORUS

GENERAL DESCRIPTION AND USAGE

Description:

Uses the standard s2pul pulse sequence to acquire a 1D-^{31}P dataset.

Usage:

The PHOSPHORUS experiment is typically used to confirm the presence of phosphorus in a molecule. The large phosphorus chemical shift range is highly sensitive to nearby functional groups and the oxidation state of the phosphorus atom. All parameters are set from the "Acquire" tab/"Default P31" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*- controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*- controls the tip angle for the pulse
H1 decoupling (dm):	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?:	Select yes or no- acquires acquisition until S/N value between ppm indicated is > specified value
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

PHOSPHORUS spectra often contain broad signals, which distort the baseline. The default processing parameters are set to use linear prediction to back predict the first five points of the spectrum in order to flatten the baseline. These settings may be modified under the "Process" tab on the "More 1D" panel.

TIPS, TRICKS AND NEXT STEPS

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the [MoveSW] button located under the spectrum window and reacquire the data.

- The appropriate receiver gain (gain) is most easily set by making sure the Autogain option is checked on right-hand side of the "Acquire" tab. The software will optimize the gain setting prior to data collection.

- The acquisition time (at), which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" page of the "Acquire" tab. Because PHOSPHORUS spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see coupling; one should consider lengthening the acquisition time and/or narrowing the spectral width.

- If the sample has been purified using reversed-phase HPLC, remember that many mobile phases commonly used in chromatography contain non-volatile phosphate buffers. Such samples will contain large background phosphorus signals from the buffer; one should consider using a different buffer such as formic acid when anticipating the need of ^{31}P NMR data.

- It is also possible to use presaturation to suppress a large, undesired peak in the phosphorus spectrum. From the PHOSPHORUS parameter set, go to the "Experiments" menu at the top of the VnmrJ window and choose "Convert current parameters to do… Solvent Suppression – Select Peaks => PRESAT submenu". The "Default" panel now displays the appropriate parameters to configure the frequency/frequencies for suppression.

Reference(s):
O. Khul, Phosphorous-31 NMR Spectroscopy, Springer (2008) 1-131.

(H)PRESAT

GENERAL DESCRIPTION AND USAGE

Description:

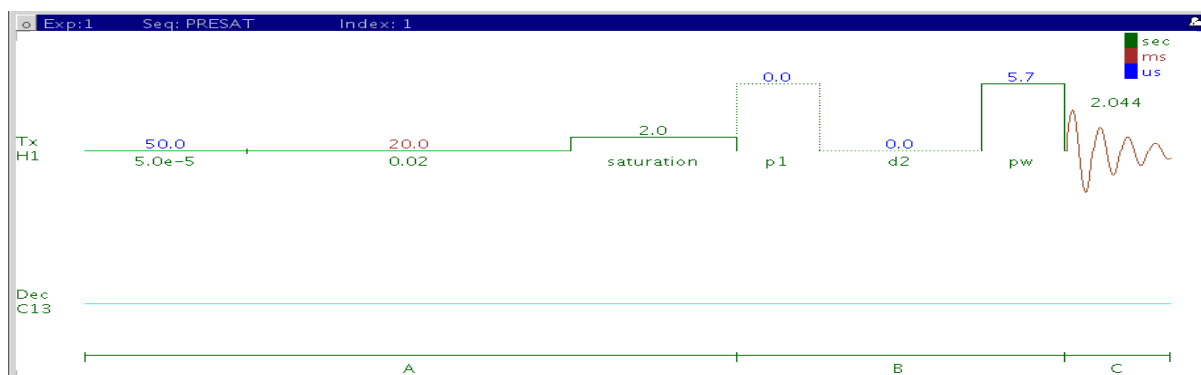A standard s2pul pulse sequence to acquire a 1D-^{1}H dataset with a presaturation option to suppress strong signals. Single peaks are suppressed with a hard pulse, multiple peaks with a shaped pulse.
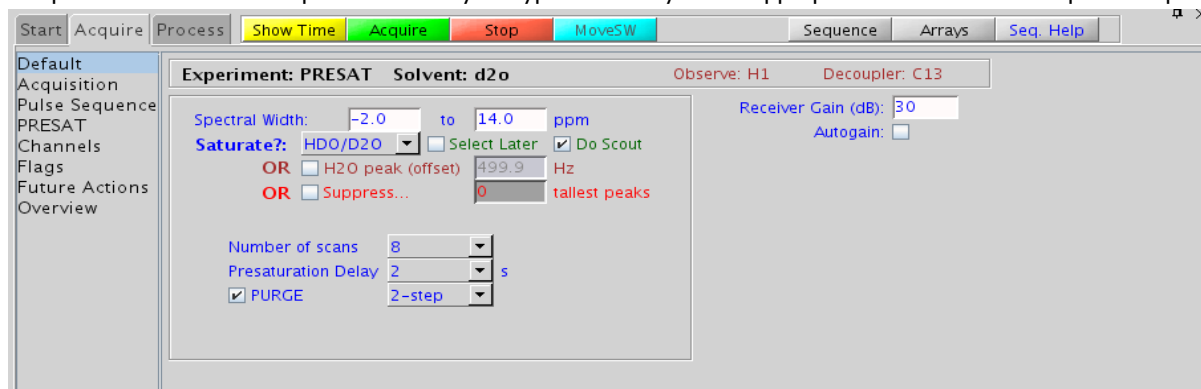
Usage:

Presaturation is a simple method of removing strong signals from a spectrum using irradiation during all or part of the relaxation delay. Common reasons for wanting to suppress signals include: (i) the presence of large solvent signals, (ii) use of non-deuterated solvents, like H_2O, (iii) use of samples with large background impurities or buffers such as urea or acetate, (iv) use of samples of low concentration when the residual proton signal from the deuterated solvent overwhelms the peaks of interest. The most straightforward method is to acquire a standard PROTON spectrum or a scout scan first and then interactively choose the peak(s) to be suppressed from this data. The software also allows the peaks to be chosen automatically via a threshold, or via a pull-down menu with several selections for water. These different setup methods are detailed in the "Tips, Tricks and Next Steps" section. If additional experiments are set up from the (H)PRESAT data set, all the presaturation parameters are transferred to the new experiment. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values (do not change if using previously acquired proton data to select peaks for suppression
Saturate? (satmode):	Choices of water type to suppress in pull-down, or check boxes to select later from scout scan, or type in specific water frequency, or suppression of n tallest peaks
Number of scans (nt):	Select typical choices from pull down menu*
Presaturation Delay (satdly):	Select typical choices from pull down menu*- controls the delay between scans
PURGE (prgflg):	Check box adds PURGE pulse to improve saturation, choose 2-step (default) or 4-step

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
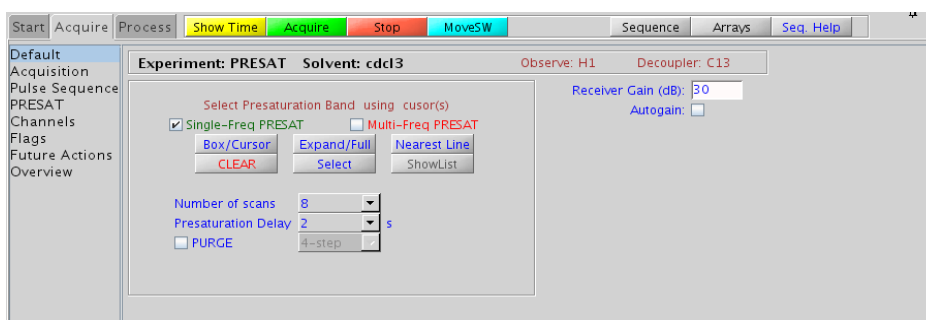
The processing is typically the same as that used for standard PROTON spectrum, except manual phasing may be required due to an unphaseable residual signal from the suppressed peak(s). A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the (H)PRESAT experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) to be suppressed, and the "Default" panel appears differently (see below). Choose single or multi-frequency presat with the check boxes, place the cursor(s) on or around the peak to be suppressed and click [Select]. The width of the peaks chosen will determine the power used for the presaturation. Repeat for additional peaks.

  If a PROTON spectrum has not been previously acquired but the "Select Later" and "Do Scout" options were checked on the "Default" panel shown in the "Key Parameters" sections, the scout scan is acquired and the data displayed, now with the "Default" panel shown above to facilitate the interactive peak selection.

- The appropriate receiver gain (gain) is most easily set by making sure the Autogain option is checked on right-hand side of the "Acquire" tab. The software will optimize the gain setting prior to data collection. Selecting autogain is a good choice in most solvent suppression experiments acquired in automation.

- Clicking the check box to "Suppress… n tallest peaks" and entering in a value for n will result in a scout scan being acquired, and then the software will set up suppression on n peaks as measured from the threshold. This approach works well when acquiring in automation mode.

- The choices for water type in the "Saturate?" pull-down menu control the power of the RF field used for the presaturation. The highest power (50 Hz field) is used for the 90% H_2O, while the other choices (HDO/D_2O, wet DMSO) use lower power. The saturation frequency (satfrq) for the 90% H_2O suppression is automatically optimized for maximum efficiency.

- The power, length, and offset of the presat pulse may be modified manually in the "PRESAT" panel. Remember that presat powers are typically low (typically a small, negative number) and the length usually 2s or less. If additional interscan delay time is needed, the relaxation delay d1 may be set to a larger value than the presat delay.

- Adding the 2-step (or 4-step) purge option may significantly improve the presaturation efficiency, particularly for large water peaks. However the efficiency of the purge option is very dependent upon the pw90 and B1 (in)homogeneity of the probe. The 4-step purge produces the best water suppression. It should be noted, however, that the purge phase cycle is *added* to all subsequent experiments, e.g. the minimum nt for 4-step PURGE is 4.

- Once the solvent suppression has been set up satisfactorily, selecting another experiment type, e.g. zTOCSY, NOESY, etc. will automatically retain suppression parameters for the new experiment. To turn off presaturation, unselect the "Presaturation" check box on the "PRESAT" panel.

- (H)PRESAT will also suppress any exchangeable protons. Use wet-type suppression to preserve these signals

Reference(s):

P. J. Hore, Methods Enzymol. 176 (1989) 64-77. (Solvent Suppression)
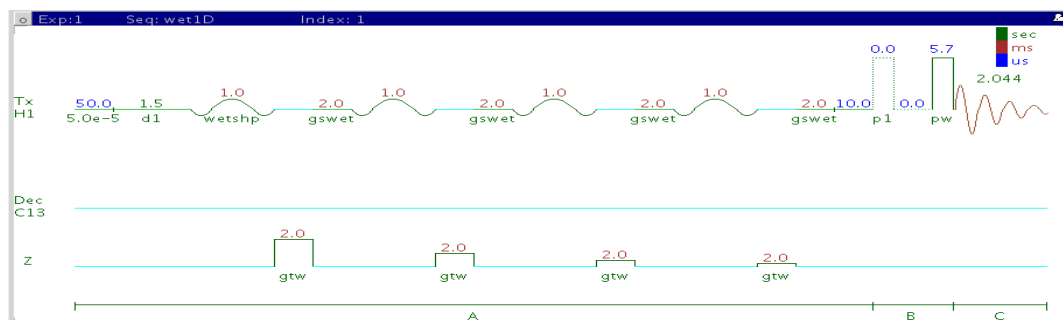
M. Gueron, P. Plateu, M. Decorps, Prog. NMR Spectrosc. 23 (1991) 135-209. (Solvent Signal Suppression)

R. T. MacKay, Ann. Rep. NMR. Spectrosc. 66 (2009) 33-76. (Advances in Solvent Suppression)

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(H)wet1D

Description:

Pulse sequence to acquire a 1D-^{1}H dataset with selective excitation pulses to exclude strong signals.

Usage:

WET solvent suppression removes strong signals from a spectrum by creating a shaped pulse that excites only the solvent resonance(s) followed by the use of gradients to suppress the excited resonance(s). WET suppression has the advantage over other methods (like (H)PRESAT) that the exchangeable protons are often not suppressed as much. It also facilitates the decoupling and hence removal of ^{13}C satellites of organic solvents. However (H)wet1D does not result in the smallest residual H$_2$O signal, and it might also produce baseline artifacts that interfere somewhat with integration. Common reasons for wanting to suppress signals include: (i) the presence of large solvent signals, (ii) use of non-deuterated solvents, e.g. H$_2$O, (iii) use of samples with large background impurities or buffers, such as urea or acetate, or (iv) use of samples of low concentration when the residual proton signal from the deuterated solvent overwhelms the peaks of interest. The most straightforward method is to acquire a standard PROTON spectrum or a scout scan first and from this data, interactively choose the peak(s) to be suppressed. The software also allows the peaks to be chosen automatically via a threshold or a number of common solvent selections. These different setup methods are detailed in the "Tips, Tricks and Next Steps" section. If additional experiments are set up from the (H)wet1D data set, all the presaturation parameters are transferred to the new experiment. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values (do not change if using previously acquired proton data to select peaks for suppression
WET peaks:	Choices of define now (shows list of solvents when checked) or check boxes to select later from scout scan, or suppression of n tallest peaks, or type in specific water frequency
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*- controls the delay between scans
C13 decoupling during WET?:	Turns decoupling on to suppress solvent ^{13}C satellites, e.g. from CH$_3$CN
Optimize CF:	Optimizes the compression factor for the transmitter power used for the wet shape

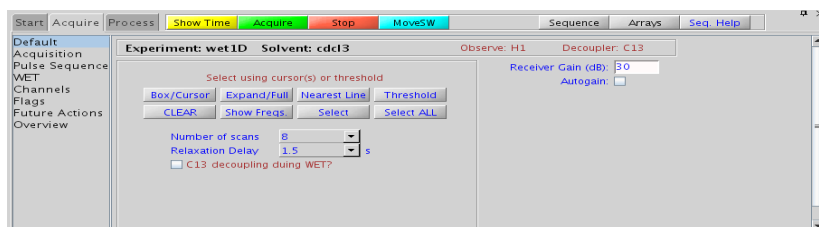*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

The processing is typically the same as that used for standard PROTON spectrum, except manual phasing may be required due to an unphaseable residual signal from the suppressed peak(s). A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the (H)wet1D experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) to be suppressed, and the "Default" panel will appear differently (see below). Choose single or multi-frequency presat with the check boxes. Place the cursor(s) on or around the peak to be suppressed and click   Select . Repeat for additional peaks. A threshold may also be set to select peaks automatically.

If a PROTON spectrum has not been previously acquired but the "Select Later" and "Do Scout" options were checked on the "Default" panel shown in the "Key Parameters" sections, the scout scan is acquired and the data displayed, now with the "Default" panel shown above to facilitate the interactive peak selection.

- The appropriate receiver gain (gain) is most easily set by making sure the Autogain option is checked on right-hand side of the "Acquire" tab. The software will optimize the gain setting prior to data collection. Selecting autogain is a good choice in most solvent suppression experiments acquired in automation.

- Clicking the check box to "Suppress… n tallest peaks" and entering in a value for n will result in a scout scan being acquired, and then the software will set up suppression on n peaks as measured from the threshold. This approach works well when acquiring in automation mode.

- When selecting peaks for suppression via the solvent check boxes, note that the most common choices are displayed, while clicking the "more" button will show additional, less typical choices.

- Try to keep the widths of the selected peak >60 Hz, as peaks that are too narrow will create long, shaped pulse durations.

- If the pw90 is reasonably accurate, the suppression quality using the default parameters should be adequate for most samples. The suppression can be optimized by clicking the "Optimize CF" check box. This will array and attempt to optimize a scaling factor for the shaped pulses tpwr_cf, looking for the value that produces the best suppression. If the desired number of scans is large (>32), this optimization will take a fair amount of time. One should consider lowering nt for the optimization and then increasing after the parameters are set. This optimization may also be done manually from the "WET" panel.

- Once the solvent suppression has been set up satisfactorily, selecting another experiment type, e.g. zTOCSY, NOESY, etc. will automatically   retain suppression parameters for the new experiment. To turn off WET, unselect the "WET" check box on the "WET" panel.

Reference(s):
R. Ogg, P. Kingsley, J. Taylor, J. Magn. Reson. B104 (1994) 1-10. (WET)

S. Smallcombe, S. L. Patt, P. Keifer, J. Magn. Reson. A117 (1995) 295-303. (WET – Applications to LCNMR)

S. L. Patt, J. Magn. Reson. 96 (1992) 94-102. (Shifted Laminar Pulses)

T1_MEASURE
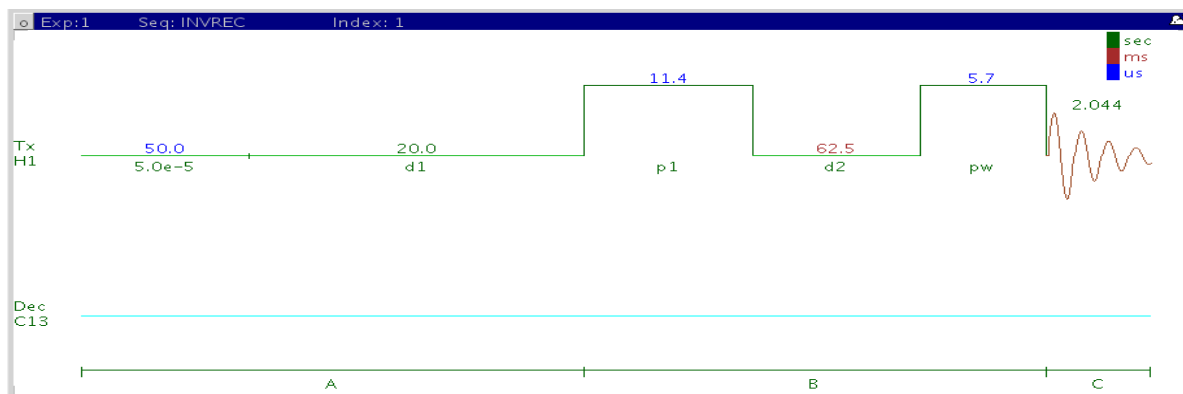
Description:

Uses an inversion-recovery pulse sequence (180°-d2-90°) to measure spin-lattice (T_1 or longitudinal) relaxation rates.

Usage:

Knowledge of the T_1 relaxation rates for the sample is important for multi-scan quantification experiments. Accurate quantification requires an interpulse delay of at least 5x the longest T_1 of interest in the molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*- controls the delay between scans; needs to be longer for accurate T_1 measurements
Decoupling (dm):	Select choice from pull down menu- for X nuclei, choose coupled/uncoupled; +/- NOE
T1 Mode:	Select choice from pull down menu- choose inversion recovery or progressive saturation
Min/Max T1; Total Exp Time:	Use to set the array of values for the T_1 d2 delay- choose a min/max value, select desired total exp time (controls # of steps) and click Array Relaxation Delay

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

The T1_MEASURE experiment, with the inversion recovery option selected, produces an array of 1D spectra, where the peaks heights will slowly progress through zero and then become the opposite phase as the value of d2 increases. To process the entire array, use [Autoprocess], or type "wft". To display a single spectrum in the array, use the buttons to choose a spectrum. Alternatively type ds(#), where # is the number of the spectrum in the array you wish to display. Clicking on the vertical "ArrayedSpectra" panel will display all the spectra in a horizontal array. On the "Process" tab, there is a special panel labeled "T1 Analysis". This panel contains tools to facilitate the T_1 analysis. Click [Display Last Spectrum], expand the spectral region that the T_1 analysis is to be performed for and set the threshold just below the shortest peak to be analyzed. Now click [Do T1 Analysis] to fit the data to T_1 curves for each peak. Use the display and print buttons to view the results.
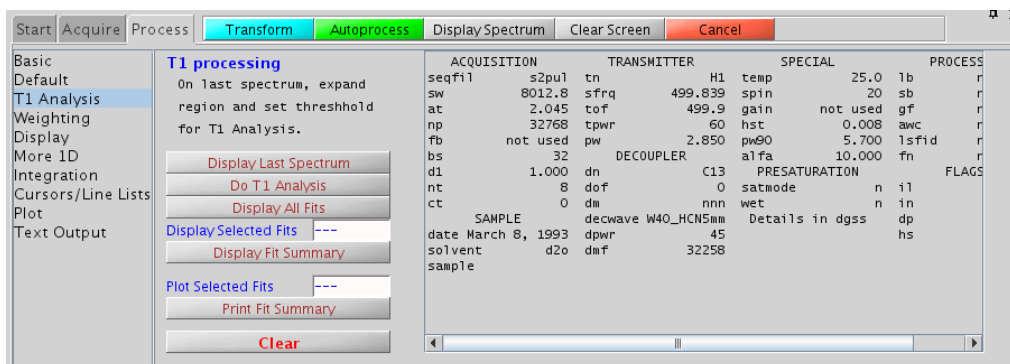
- For the most accurate results, ensure that the relaxation delay is 5x the longest expected T_1- the default value is 20s, which assumes the longest T_1 is < 4s. If you find that your T_1 values of interest are longer than this, lengthen this delay appropriately and reacquire the data.

- The appropriate receiver gain (gain) should be set from a PROTON spectrum with a 90° pulse angle. The "Autogain" option is not available for arrayed spectra.

- An accurate 90° pulse measurement is important for this experiment. For best results, measure the 90° pulse for your sample with a standard proton experiment prior to the T_1 analysis.

- To save time, you may wish to perform the analysis initially with a small array containing a broad range of d2 values. Once you know the approximate value of d2 that produces the null, you can perform a second analysis with smaller array steps to target the exact value.

- To view the values in the d2 array, click [Arrays] displayed underneath the spectrum when the "Acquire" tab is selected.

- If you are estimating the T_1 value visually from the null spectrum, remember that $T_1 = d2*1.44$, at the null point, as T_1 relaxation is a first-order rate process.

Reference(s):
A. Derome, Modern NMR Techniques for Chemistry Research, Pergamon Press, New York, 1987.

T2_MEASURE
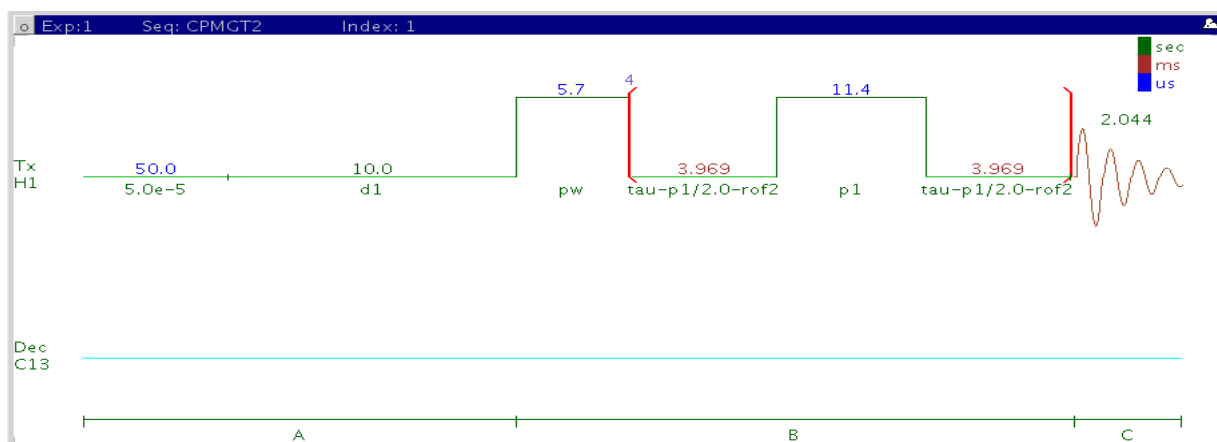
GENERAL DESCRIPTION AND USAGE

Description:

Uses a spin-echo pulse sequence to measure spin-spin (T_2 or transverse) relaxation rates.

Usage:

This experiment allows one to measure the transverse relaxation (T_2) in the absence of magnetic field inhomogeneity, rather than the rate constant for the decay of the FID (T_2*), which is reflected in the 1D linewidths. T_2 relaxation rates are correlated with molecular size, so their measurement can provide insight for small molecules regarding aggregation, binding to large molecules (e.g. proteins), or other non-covalent interactions. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*- controls the delay between scans; needs to be longer for accurate T_2 measurements
Decoupling (dm):	Select choice from pull down menu- for X nuclei, choose coupled/uncoupled; +/- NOE
Min/Max T2; Total Exp Time:	Use to set the array of values for the T_2 delay- choose a min/max value, select desired total exp time (controls # of steps) and click "Array Relaxation Delay" button

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

The T2_MEASURE experiment produces an array of 1D spectra, where the peaks heights will slowly progress to zero as the value of the delay approaches the value for full T_2 relaxation. To process the entire array, use [Autoprocess] or type "wft". To display a single spectrum in the array use the ⬅➡ buttons to choose a spectrum. Alternatively type ds(#), where # is the number of the spectrum in the array you wish to display. Clicking 📊 on the vertical "ArrayedSpectra" panel or typing "dssh" will display all the spectra in a horizontal array. On the "Process" tab, there is a special panel labeled "T_2 Analysis". This panel contains tools to facilitate the T_2 analysis. Click [Display Last Spectrum], expand the spectral region that the T_2 analysis is to be performed for and set the threshold just below the shortest peak to be analyzed. Now click [Do T2 Analysis] to fit the data to T_2 curves for each peak. Use the display and print buttons to view the results.
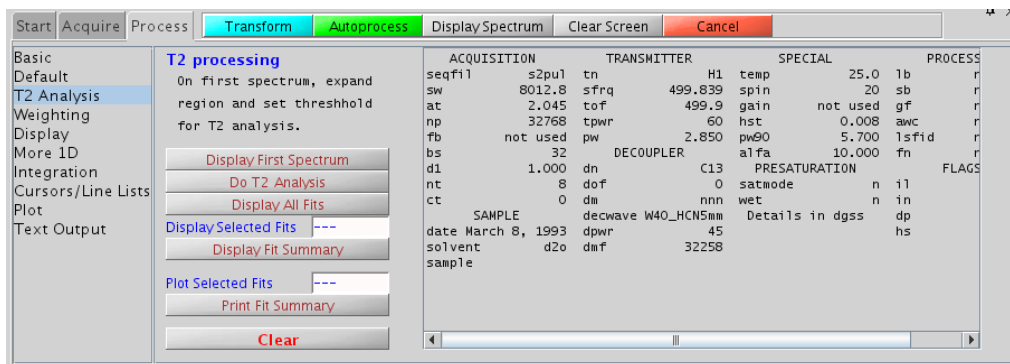
- An accurate 90° pulse measurement is important for this experiment. For best results, measure the 90° pulse for your sample with a standard proton experiment prior to the T_2 analysis.

- The appropriate receiver gain (gain) should be set from a PROTON spectrum with a 90° pulse angle. The "Autogain" option is not available for arrayed spectra.

- To save time, the analysis may be performed initially with a small array containing a broad range of T_2 values. Once the approximate value of T_2 that produces the null is determined a second analysis can be performed with smaller array steps to target the exact value.

- To view the values in the d2 array, click [Arrays] displayed underneath the spectrum when the "Acquire" tab is selected.

- Because broad peaks have short T_2 values, this sequence is sometimes used to remove large, broad, background peaks from spectra and flatten the baseline ("T2 filter"). Select a single, short value (0.1-0.2s) for the T_2 value (bigtau) and acquire the data.

- Strictly speaking, the T2_MEASURE experiment only works well on singlets, e.g. it is good for ^{13}C spectra. For coupled systems, multiplet distortion will occur during the echo, the degree of which depends on the value of T_2 delay.

Reference(s):

H. Y. Carr, E. M. Purcell, Phys. Rev. 94 (1954) 630-638. (T2)

S. Meiboom, D. Gill, Rev. Sci. Instrum. 29 (1958) 688-691. (T2)

A. Derome, Modern NMR Techniques for Chemistry Research, Pergamon Press, New York, 1987.

(C)APT
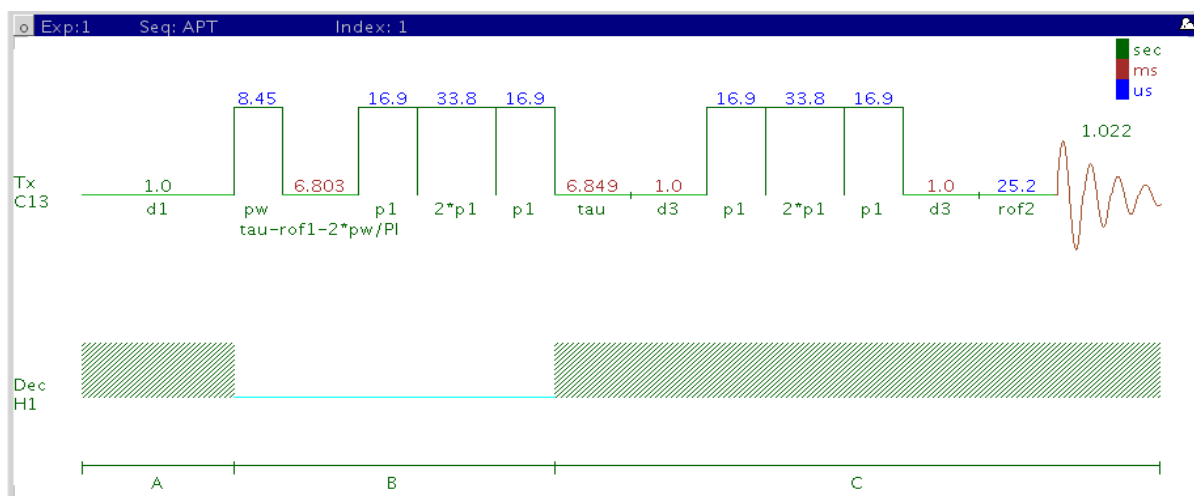
GENERAL DESCRIPTION AND USAGE

Description:

Attached proton test experiment to determine carbon multiplicity.

Usage:

The (C)APT experiment is used as an adjunct or a replacement for a 1D CARBON spectrum. The default parameters should result in a spectrum with methyl and methine-type carbon signals having opposite phase with respect to that of methylenes and quaternary-type carbons. Quaternary carbon lines will typically be smaller than methylene signals, and can often be distinguished in this manner. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Select typical choices from the pull down menu or type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*- controls the delay between scans
Check S/N?:	Select yes or no- acquires data until S/N value between ppm indicated is > specified value

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel. Manual phasing may be necessary as the spectra often contain both positive and negative peaks. Proper phasing would typically be with the solvent resonance "up" which will result in quaternary and CH_2 carbons also pointing up and CH/CH_3 carbons pointing down.

TIPS, TRICKS AND NEXT STEPS

- Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The (C)DEPT experiment can produce edited carbon sub-spectra containing a single type of carbon multiplicity for easy interpretation. However the (C)APT is a single experiment, compared to multiple experiments for the full (C)DEPT series. Protonated carbons are also enhanced in DEPT by magnetization transfer from the protons as opposed to APT.

- If there is insufficient sample to obtain an (C)APT spectrum, one should consider acquiring proton-detected experiments such as gHSQC, which gives similar information and requires much less sample. The gHSQC experiment can also be acquired with multiplicity editing to give the same type of information as the (C)APT experiment.

Reference(s):

S. L. Patt, J.N. Shoolery, J. Magn. Reson. 46 (1982) 535-539. (APT)

(C)DEPT
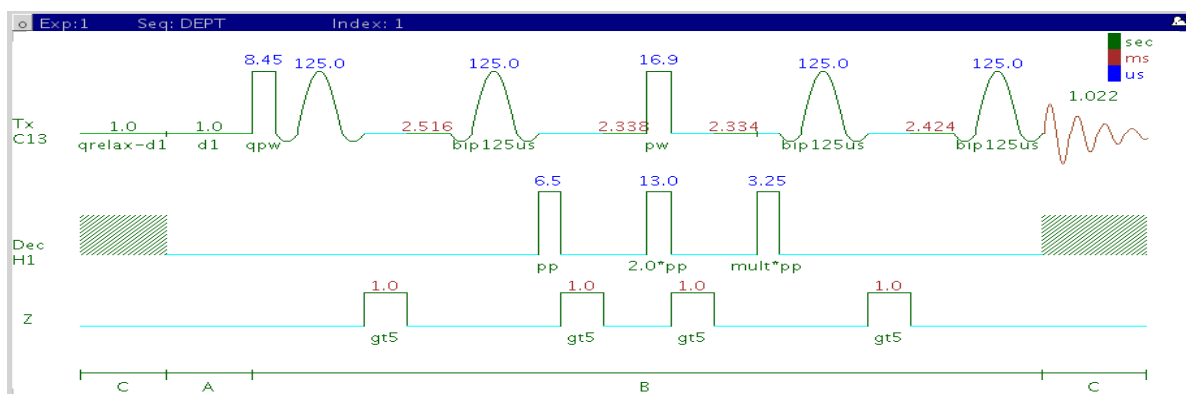
GENERAL DESCRIPTION AND USAGE

Description:

Experiment to determine carbon multiplicity.

Usage:

(C)DEPT experiments are used to produce edited subspectra of 1D carbon data that contain only carbons of a certain multiplicity type. Choices include (1) methines and methyls peaks positive, methylenes negative, (2) methines only, or (3) methyls, methylenes, and methines (no quaternary carbons). Mathematical combinations of the three choices can be used to produce spectra that contain only a specific type of carbon, e.g. methylenes only can be produced by the linear combination of choices 1-3. Additionally, quaternary carbons can be selected (DEPTQ) to be in-phase or anti-phase with respect to the methyls or not to be present at all (traditional DEPT). With full DEPTQ editing (8 spectra), quaternary carbons can also be separated out. Individual subspectra for each carbon are generated with the "Full Edit" choice of the "XH Multiplicity editing" pull down. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent:	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Select typical choices from the pull down menu or type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*
XH Multiplicity editing (mult):	Select choice of (C)DEPT experiment from pull down menu
Quaternary Carbons (qphase):	Select choice of DEPTQ- allows for the addition of antiphase or in-phase quat. carbons
Quat. ^{13}C relax delay (qrelax):	Allows for an additional delay to be included for subspectra containing quat. carbons in the sequence

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
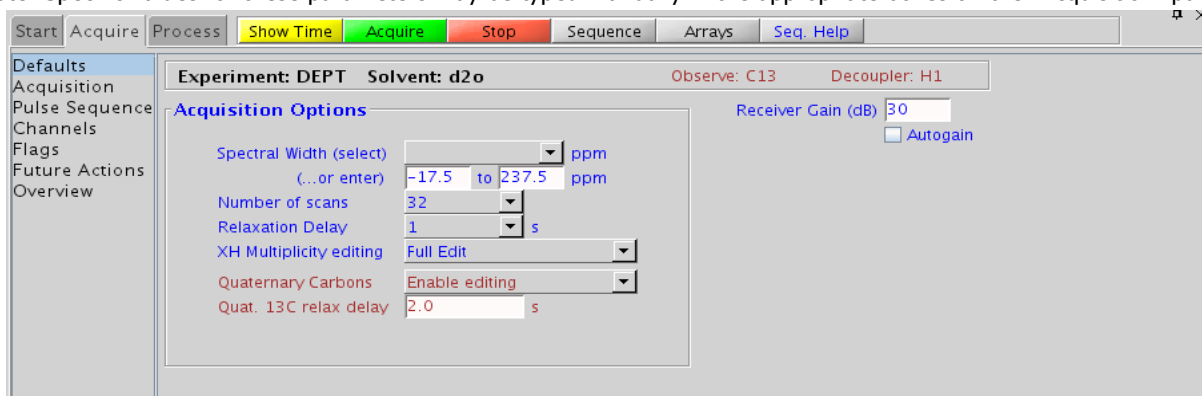
PROCESSING

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~0.5-1 Hz. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel. Manual phasing may be necessary as the spectra may contain both positive and negative peaks.

TIPS, TRICKS AND NEXT STEPS

- Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The (C)DEPT experiment can produce edited carbon sub-spectra containing a single type of carbon multiplicity for easy interpretation. Protonated carbons are also enhanced in DEPT by magnetization transfer from the protons as opposed to APT.

- A single DEPT experiment with the "XH/XH3 up & XH2 down" option along with the Quaternary carbon "Antiphase" option is a good alternative to APT, as it provides the same information, but with enhanced sensitivity for protonated carbons.

- If there is insufficient sample to obtain a (C)DEPT spectrum, one should consider acquiring proton-detected experiments such as gHSQC, which gives similar information and requires much less sample. The gHSQC experiment can also be acquired with multiplicity editing to give the same type of information as the (C)DEPT experiment.

Reference(s):

D. M. Doddrell D. T. Pegg M. R. Bendall, J. Magn. Reson. 48 (1982) 323-327. (DEPT)

M. A. Smith, H. Hu, A. J. Shaka, J. Magn. Reson. 151 (2001) 269-283. (BIP Pulses)

P. Bigler, R. Kummerle, W. Bermel, Magn. Reson. Chem. 45 (2007) 469-472. (DEPTq)
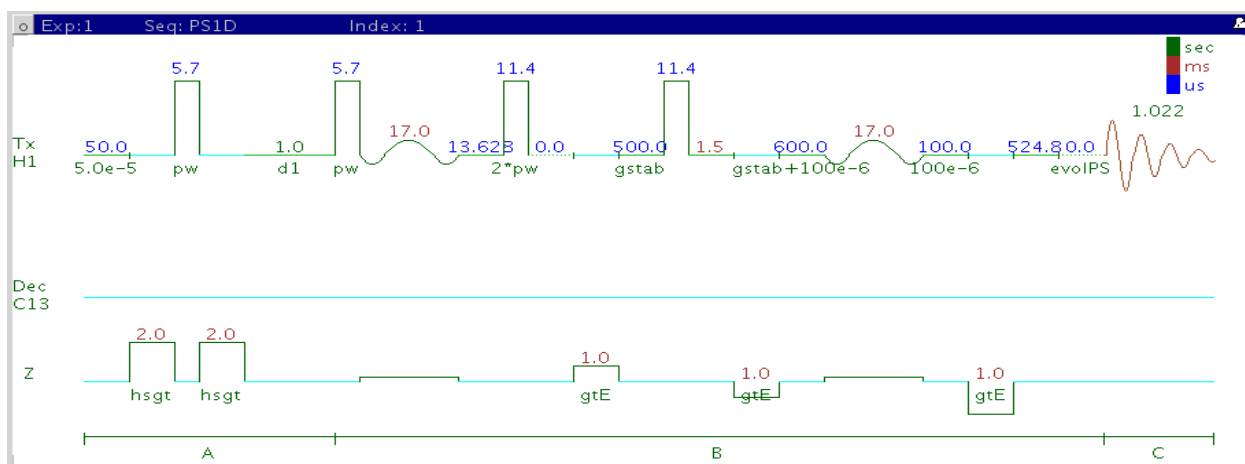
GENERAL DESCRIPTION AND USAGE

Description:

Uses a double spin-echo type experiment to acquire an effectively homonuclear-decoupled proton spectrum.

Usage:

The PureShift 1D experiment is useful to simplify complicated proton spectra dominated by overlapping multiplets. The result is a "proton-decoupled" spectrum of singlets at the true chemical shift of each proton multiplet. Note that the sensitivity will be much less than the standard proton experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent(solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Type in specific ppm values
Number of scans(nt):	Select typical choices from pull down menu*
Relaxation Delay(d1):	Select typical choices from pull down menu*- controls the delay between scans
Slice Selection Bandwidth:	Bandwidth for selective pulse- 100 Hz standard
"PureShift tau delay":	Delay for effective homonuclear decoupling

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

As the sensitivity of the PureShift 1D experiment is lacking compared to a standard PROTON, a good processing weighting choice is one which provides S/N enhancement, e.g. line broadening (lb ~0.5-1 Hz).

- Smaller bandwidths for the selective pulse will result in greater selectivity (i.e., decoupling of closely spaced coupling partners), but longer experiment times and lower sensitivity. 100 Hz is a good compromise starting point. Spectral width (sw) may also be decreased to include only the region of interest.

- The "PureShift tau delay" is inversely related to the maximum coupling constant that can be effectively decoupled. Decrease this delay for larger couplings- refer to the display in the panel for the effective maximum coupling constant.
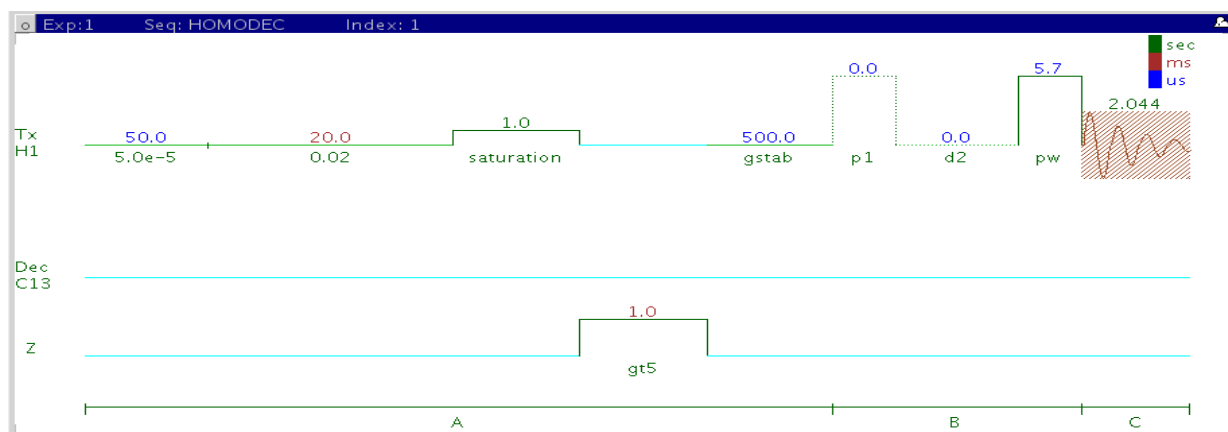
Reference(s):

P. Xu, X. Wu, R. Freeman, J. Magn. Reson. 95 (1991) 132-148. (Homonuclear Decoupling)

K. Zangger, H. Sterk, J. Magn. Reson. 124 (1997) 486-489. (Homonuclear Broadband Decoupling)

J. A. Aguilar, S. Faulkner, M. Nilsson, G. A. Morris, Angew. Chem. Int. Ed., 49 (2010) 3901-3903. (PureShift 1D)

(H)HomoDec

GENERAL DESCRIPTION AND USAGE

Description:

Uses basic irradiation to decouple a 1D-^{1}H dataset at a single frequency (hard pulse) or multiple frequencies (shaped pulse).

Usage:

Prior to widespread use of the 2D COSY and TOCSY experiments, this experiment was widely used to define homonuclear coupling partners. Irradiation at a given frequency for a multiplet will result in a collapse of coupled multiplets. Where the TOCSY and/or COSY may be difficult to interpret due to overlap, this experiment may still be a good choice for simplifying highly coupled systems. In addition to the homonuclear decoupling ("homo"), which can be used in any pulse sequence, this sequence simultaneously saturates the signal which is decoupled to remove artifacts. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and widths for the decoupling are known, and may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
Presaturate:	Check box to presaturate the selected resonances to suppress decoupling artifacts- highly recommended
Decoupling Duty Cycle:	Use to control % of time the decoupling is on- longer time improves decoupling at the expense of S/N (10% recommended)
Multifrequency Decoupling:	Check box for multiple frequencies
No., ppm, width of selective frequencies:	Use top box to set the number of selective frequencies. Boxes below used to set the desired ppm and width in Hz of each frequency

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

The processing is typically the same as that used for standard PROTON spectrum, except manual phasing may be required due to antiphase/negative peaks at the decoupling frequency. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- If the HOMODEC experiment is selected in an experiment already containing PROTON data, the decoupling band selection may be chosen interactively. Place the cursors around the multiplet to be decoupled and click "Select". Repeat for multiple decoupling bands; click "CLEAR" to erase previous choices.
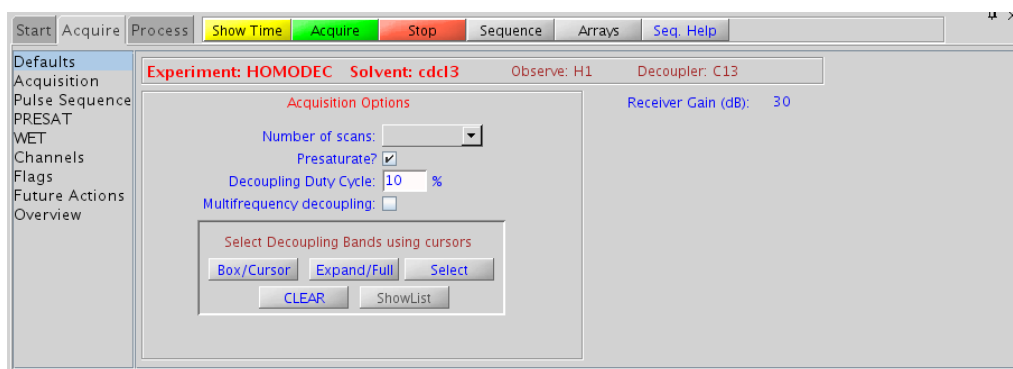
- Acquiring several spectra with single frequency decoupling usually give cleaner spectra and more easily interpretable results than one spectrum with multiple simultaneous decouplings.

- A 2D gCOSY/gDQCOSY spectrum may be a better choice for a standard structure elucidation/assignment problem as it can provide the same information for the entire molecule with a single experiment that may be obtained in <5 min. with a reasonable sample amount.

Reference(s):

J. K. M. Sanders, J. D. Mersh, Prog. NMR Spectsoc. 15 (1982) 353-400. (Difference Spectroscopy)

CARBONecho
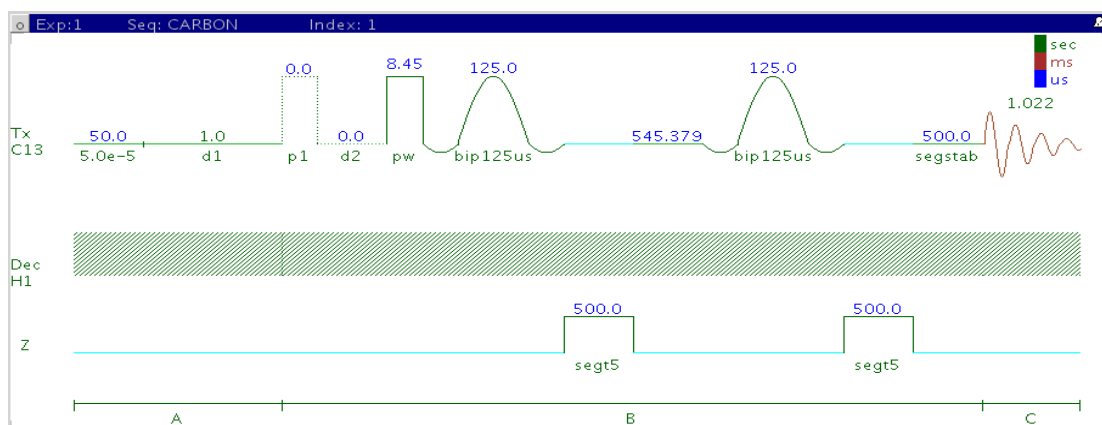
Description:

Acquires a 1D-^{13}C dataset with a double echo from bip pulses to allow delayed data acquisition without the need for linear back prediction.

Usage:

This is a highly specialized experiment to acquire 1D carbon data on a high Q probe, such as a ^{13}C cold probe, which may have long pulse ringdown times. All parameters are set from the "Acquire" tab/"Default C13" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Select typical choices from pull down menu or type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 decoupling (dm):	Select typical choices from pull down menu- turns decoupling and/or NOE enhancement on/off
Check S/N?:	Select yes or no- acquires data until S/N value between ppm indicated is > specified value
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D carbon spectrum is line broadening, with an lb setting from ~0.5-1 Hz.

- The acquisition time, which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" panel of the "Acquire" tab. Because carbon spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small coupling; one should consider lengthening the acquisition time and/or narrowing the spectral width when acquiring coupled carbon spectra.

- Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The standard proton-decoupled CARBONecho spectrum is produced by turning on the decoupling during the pulse and acquisition. It is also standard practice to leave the decoupler on during the preacquisition delay (d1), which provides an NOE enhancement to the carbon signals from attached protons. When more protons are attached to a carbon, the enhancement is larger. For example, methyl signals will appear larger than quaternary carbon signals. Turn off the NOE enhancement if integration is desired.

Reference(s):

M. A. Smith, H. Hu, A. J. Shaka, J. Magn. Reson. 151 (2001) 269-283. (BIP Pulses)

BilevelDec
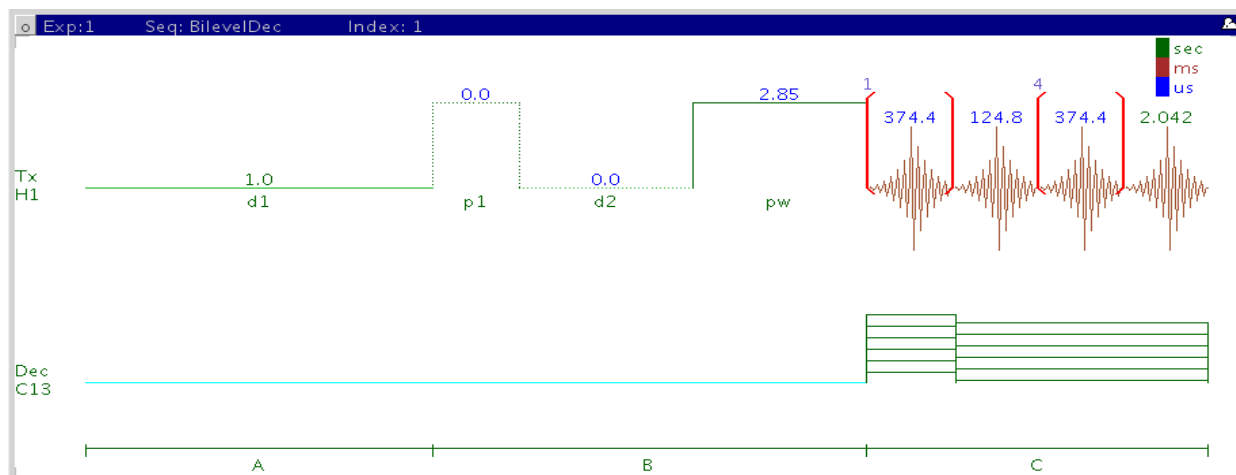
GENERAL DESCRIPTION AND USAGE

Description:
Used to acquire a carbon-decoupled 1D-^{1}H dataset.

Usage:
This is an experiment to acquire a 1D proton spectrum with heteronuclear decoupling. The bi-level style decoupling helps remove cyclic decoupling sidebands associated with large ^{1}H-X couplings by varying the length of the higher power decoupling at the beginning of the acquisition. These sidebands are often a problem when decoupling a nucleus of high abundance like ^{31}P. This experiment is also used for uniformly ^{13}C-labelled samples, which may have very complex, difficult to interpret 1D PROTON spectra from ^{13}C-^{1}H coupling.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Type in specific ppm values
Number of scans(nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
Bilevel Decoupling ON/OFF:	Check box to turn decoupling on/off
Decoupling bandwidth:	Use to the desired bandwidth for decoupling- larger bandwidth means more power
Decoupler position:	Use to set carbon decoupling frequency in ppm (50 ppm typically the center)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
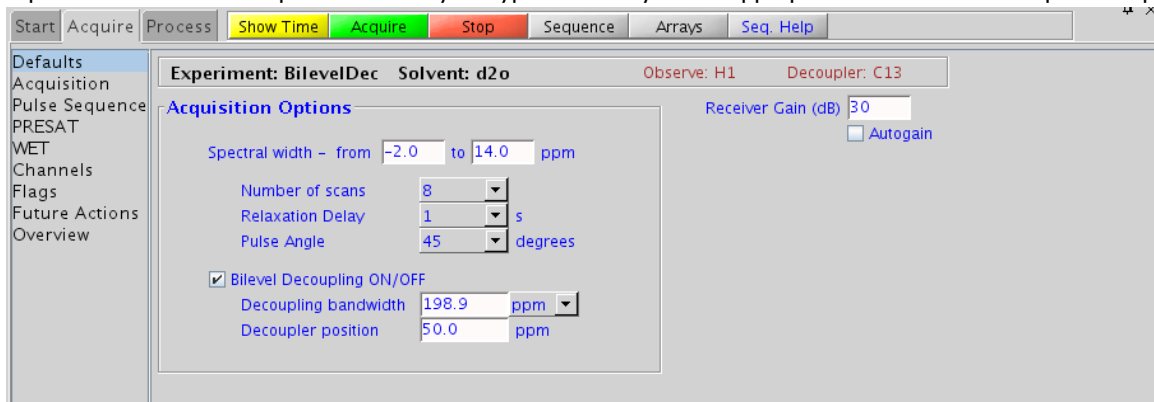
PROCESSING

The processing is typically the same as that used for standard PROTON spectrum, except manual phasing may be required due to antiphase/negative peaks at the decoupling frequency. A typical processing weighting (apodization) choice that provides S/N enhancement (at the expense of resolution) for a 1D proton spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the unfiltered peak linewidth at half-height. Other types of weighting functions are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- The appropriate receiver gain (gain) is most easily set by making sure the Autogain option is checked on right-hand side of the "Acquire" tab. The software will optimize the gain setting prior to data collection.

- If the ^{13}C-labelling is localized to a portion of the molecule, e.g. an aromatic ring, setting the decoupling position to the center of the labeled carbon region and narrowing the decoupling bandwidth can minimize the decoupling power.

- With probes of good ^{13}C efficiency (shorter 90° pulse lengths) BiLevelDEC can be used to acquire ^{13}C decoupled PROTON spectra with ^{1}H acquisition times of normal length and full band ^{13}C decoupling, provided a sufficiently long d1 relaxation delay is set, such at 10-20s. This can be useful for quantitative NMR or trace analysis where it is desirable to move the ^{13}C satellites under the main ^{1}H peak resonance.

Reference(s):

E. Kupce, R. Freeman, G. Wider and K. Wütrich, J. Magn. Reson, Ser A. 122 (1996) 81-84. (Bilevel Decoupling)

Chapter 3
Homonuclear 2D – (HH)Homo2D

Introduction

This tab of the experiment selector includes the set of 2D experiments available for proton homonuclear correlations, including both J-coupling and through-space correlation experiments. The J-coupling experiments fall into two families, the COSY family that primarily displays connectivities from two- and three-bond couplings and the TOCSY family, which can show correlations among an entire spin system. The z-filtered versions of the experiments are also available as well as gradient enhanced coherence selection for the COSY experiments. The gradient enhanced version of the experiment experiences better artifact suppression at the expense of some loss of sensitivity (factor of ~1.4). In experiments involving z-magnetization (zTOCSY, zCOSY, NOESY) zero-quantum suppression is implemented and switched on by default. The through-space correlation experiments consist of both NOESY and ROESY with an additional option of an adiabatic spinlock for the ROESY. The adiabatic spinlock is preferable as the power is lower, permitting longer mixing times for the ROESYAD.

These experiments should be considered essential data for small molecule structure elucidation following the 1D PROTON experiment. With reasonable amounts of material, the J-coupling experiments can often be acquired in 5 minutes or less. Because these experiments are proton-proton correlations, they are the most sensitive and have the best chance of providing additional structural data beyond the PROTON spectrum if the sample is limited.

gCOSY

GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional J-correlation spectroscopy with gradient coherence selection.

Usage:

This experiment will produce a 2-dimensional, absolute-value, spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between protons with with non-zero couplings, typically 2-4 chemical bonds apart. This experiment is very useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The gCOSY is a highly robust experiment and, along with TOCSY/zTOCSY, is among the most sensitive of the 2D's, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu* no required minimum for the gCOSY
t1 Increments (ni):	Select typical choices from pull down menu*-controls the resolution in F1 (minimum ~64)
COSY-beta:	Set from "Pulse Sequence" panel pull-down, choose COSY-45, 60, 90, or 135
Symmetrize:	Check box for processing option to symmetrize along the diagonal
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down menu to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are squared sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the gCOSY is set up from this experiment.

- The gCOSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. One should consider increasing the number of increments (ni) and turning off linear prediction in f1 to increase the quality of the data for such samples.

- The COSY experiment has a sensitivity advantage (factor of ~1.4) over the gCOSY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than gCOSY. The COSY also requires a multiple of 4 scans per increment, as opposed to the gCOSY, which can be run with 1 scan per increment.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default value for the tip angle of the second pulse is set to 90°, producing a gCOSY-90. To acquire a gCOSY-45, go to the "Pulse Sequence" dialog box under the "Acquire" tab and select 45 degrees from the pull-down box for COSY-beta. The gCOSY-45 will have less intense diagonal peaks than the gCOSY-90, making close chemical shift correlations easier to see at a loss of sensitivity. A 60° pulse can provide a compromise between the two.

- The d2 delay (default value 20msec) may be increased via the Pulse Sequence panel to emphasize cross-peaks originating from small couplings.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Spectral symmetrization may appear to provide an easy way of cleaning up artifacts such as t1 noise (noise along the F1 axis often observed at the chemical shift of sharp, intense peaks). However, it can produce spurious crosspeaks that appear real. It is highly recommended that when in doubt about the validity of a cross peak one should examine the unsymmetrized spectrum as well.

- If the concentration is not very low, a gDQCOSY may be considered instead of the gCOSY. It typically leads to cleaner spectra (due to suppression of singlet diagonal peaks, such as t-butyl resonances) and yields higher resolution spectra.

- Complex molecules with many overlapping spin systems may be difficult to assign from a gCOSY. One should consider using a zTOCSY in lieu of, or in conjunction with the gCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)
R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

COSY

GENERAL DESCRIPTION AND USAGE

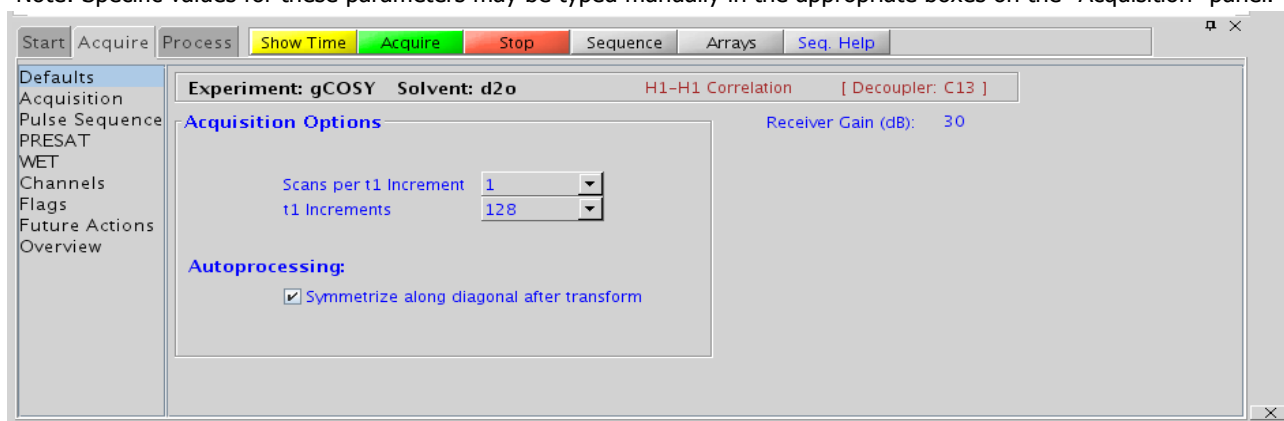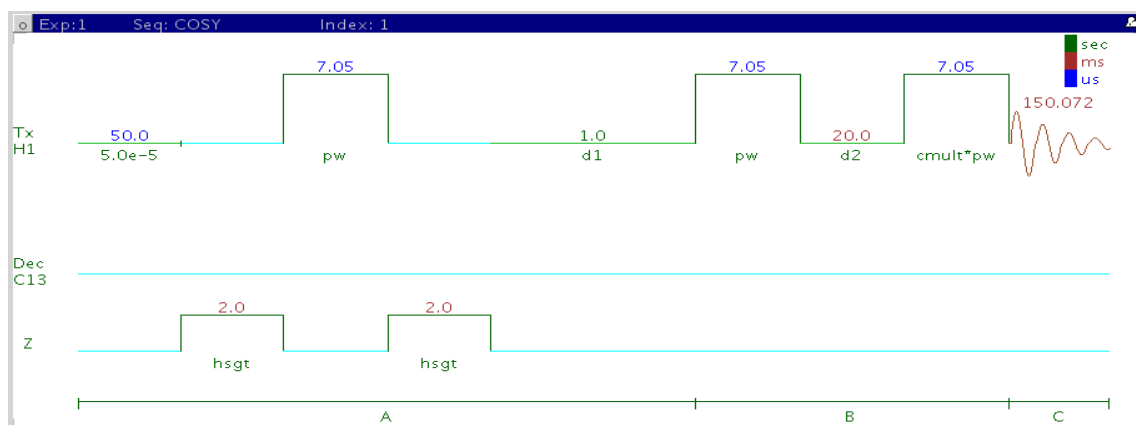**Description**:

Two Dimensional J-correlation spectroscopy.

Usage:

This experiment will produce a 2-dimensional, absolute-value, spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with with non-zero couplings, typically 2-4 chemical bonds apart. This experiment is very useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The COSY is a highly robust experiment and, along with TOCSY, is among the most sensitive of the 2D's, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
COSY-beta:	Set from "Pulse Sequence" panel pull-down, choose COSY-45, 60, 90, or 135
Symmetrize:	Processing option to symmetrize along the diagonal
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are squared sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the COSY is set up from this experiment.

- The COSY experiment has a sensitivity advantage (factor of ~1.4) over the gCOSY but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than gCOSY. The COSY also requires a multiple of 4 scans per increment, as opposed to the gCOSY, which can be run with 1 scan per increment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default value for the tip angle of the second pulse is set to 90°, producing a COSY-90. To acquire a COSY-45, go to the "Pulse Sequence" dialog box under the "Acquire" tab and select 45 degrees from the pull-down box for COSY-beta. The COSY-45 will have less intense diagonal peaks than the COSY-90, making close chemical shift correlations easier to see at a loss of sensitivity. A 60° pulse can provide a compromise between the two.

- The d2 delay (default value 20msec) may be increased via the Pulse Sequence panel to emphasize cross-peaks originating from small couplings.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- Spectral symmetrization may appear to provide an easy way of cleaning up artifacts such as t1 noise (noise along the F1 axis often observed at the chemical shift of sharp, intense peaks). However it can produce spurious crosspeaks that appear real. It is highly recommended that when in doubt about the validity of a cross peak one should examine the unsymmetrized spectrum as well.

- Complex molecules with many overlapping spin systems may be difficult to assign from a COSY- one should consider using a zTOCSY instead of, or in conjunction with the COSY data as the zTOCSY experiment is better at identifying the entire spin system of protons together.

Reference(s):

J. Jeener, Ampere International Summer School, Basko Polje, Yugoslavia 1971. (Introduction to 2D)

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)

gDQCOSY

GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional double-quantum filtered J-correlation spectroscopy with gradient coherence selection.
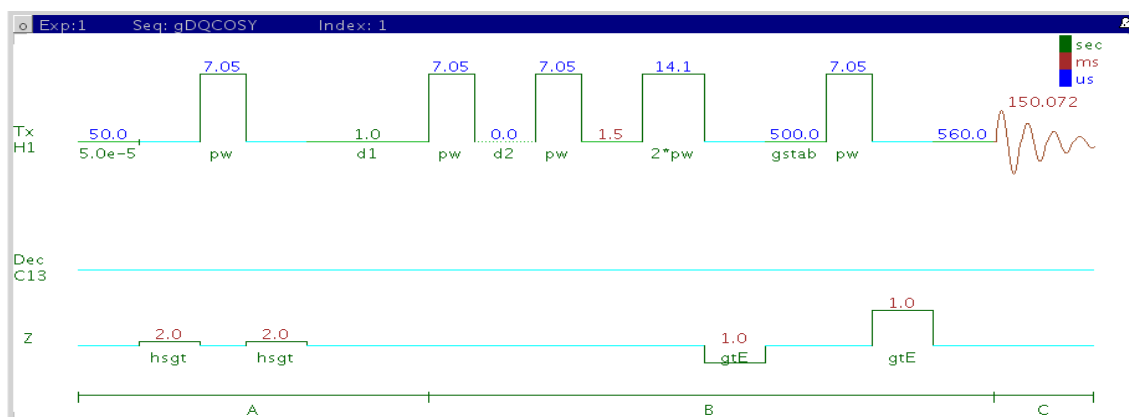
Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with with non-zero couplings, typically 2-4 chemical bonds apart. This experiment is very useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The advantage of the gDQCOSY over the gCOSY is that peaks with no double-quantum transitions (e.g. singlets) will be suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in better peak shapes. The 90° pulse width should be calibrated accurately for this experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*- no required minimum for the gDQCOSY
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are shifted sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the gDQCOSY is set up from this experiment.

- The main advantage of the gDQCOSY versus the gCOSY is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a gDQCOSY.

- The DQCOSY experiment has a sensitivity advantage (factor of ~1.4) over the gDQCOSY but may produce potentially less clean spectra (due to t1 noise from strong signals) than gDQCOSY. The DQCOSY also requires a multiple of 4 scans per increment, as opposed to the gDQCOSY, which can be run with 1 scan per increment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The 90° pulse width should to be calibrated reasonably well for best results from the gDQCOSY experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

- Because the J-couplings evolve as an echo in gDQCOSY, the crosspeaks arising from small couplings may not appear until the number of completed increments is sufficient. In general it is useful to set ni to a larger value than might be the case in an experiment such as gCOSY.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The gDQCOSY experiment can be used to measure coupling constants of overlapped multiplets as the crosspeaks contain the full multiplet structure. If used for coupling constant measurement, this experiment should be run with large number of increments (ni typically to 400 or higher). The zCOSY is a better alternative for coupling constant measurements than the gDQCOSY.

- Complex molecules with many overlapping spin systems may be difficult to assign from a gDQCOSY-one should consider using a zTOCSY instead of, or in conjunction with the gDQCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982) 6800-6801. (Double Quantum Filter)

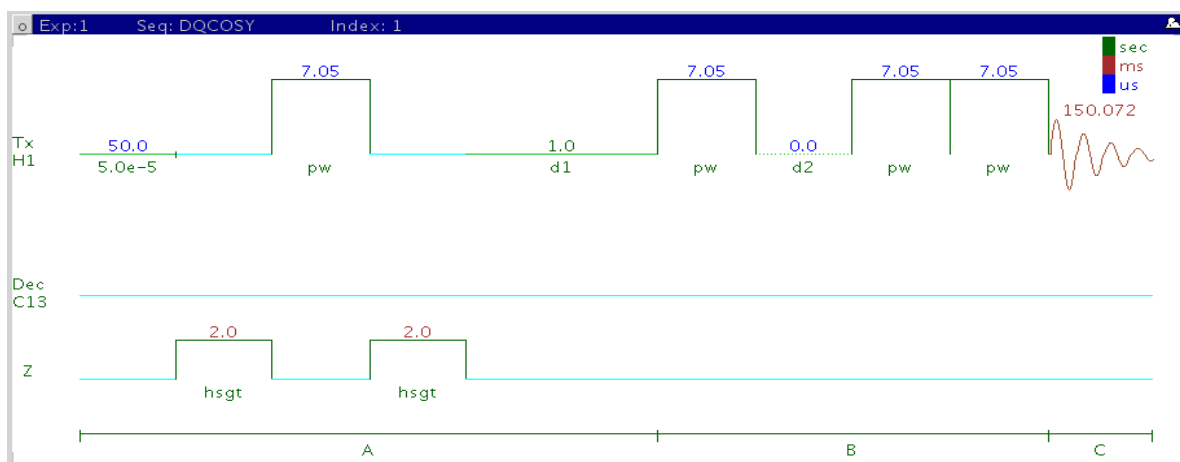R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

DQCOSY

Description:

Two Dimensional double-quantum filtered J-correlation spectroscopy.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with 2- (geminal) or 3- (vicinal)-bond couplings. This experiment is very useful for assignment, particularly when some of the peak identities are known- the others can then be deduced from the crosspeaks observed. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. The advantage of the DQCOSY over the COSY is that peaks with no double-quantum transitions (e.g. singlets) will be suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in better peak shapes. The 90° pulse width should be calibrated accurately for this experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are shifted sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain)-settings will transfer from a PROTON if the DQCOSY is set up from this experiment.

- The main advantage of the DQCOSY versus the COSY is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a DQCOSY.

- The DQCOSY experiment has a sensitivity advantage (factor of ~1.4) over the gDQCOSY but may produce potentially less clean spectra (due to t1 noise from strong signals) than gDQCOSY. The DQCOSY also requires a multiple of 4 scans per increment, as opposed to the gDQCOSY, which can be run with 1 scan per increment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The 90° pulse width needs to be calibrated accurately for best results from the DQCOSY experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

- Because the J-couplings evolve as an echo in DQCOSY, the crosspeaks arising from small couplings may not appear until the number of completed increments is sufficient. In general it is useful to set ni to a larger value than might be the case in an experiment such as gCOSY.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The DQCOSY experiment can be used to measure coupling constants of overlapped multiplets as the crosspeaks contain the full multiplet structure. If used for coupling constant measurement, this experiment should be run with large number of increments (ni typically to 400 or higher).

- Complex molecules with many overlapping spin systems may be difficult to assign from a DQCOSY- one should consider using a zTOCSY instead of, or in conjunction with the DQCOSY data as the zTOCSY experiment is better at identifying the entire spin system of protons.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)
U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982) 6800-6801. (Double Quantum Filter)

TOCSY
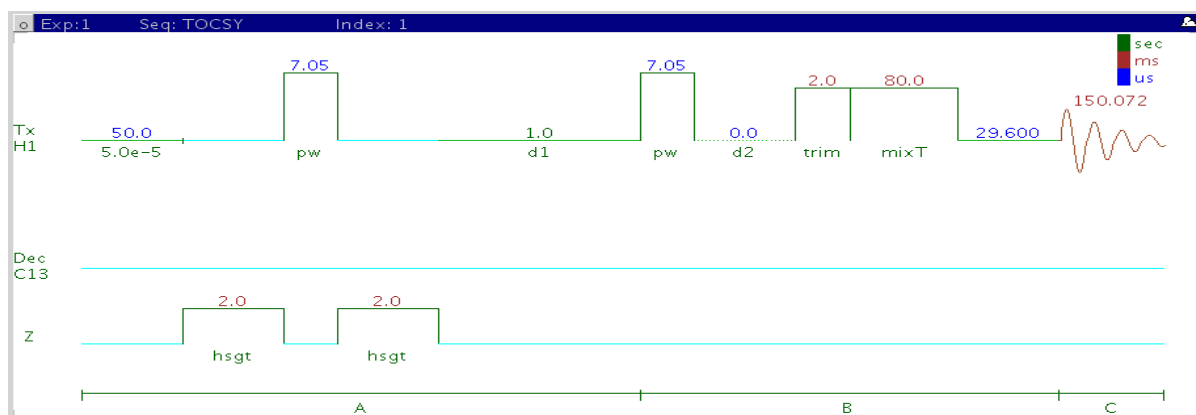
GENERAL DESCRIPTION AND USAGE

Description:

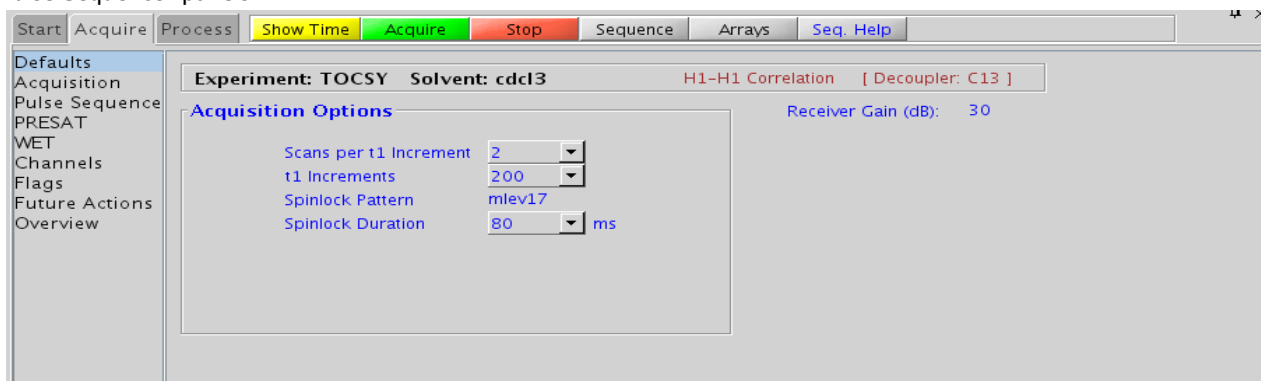Two dimensional total J-correlation spectroscopy for scalar coupled spin systems.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks potentially between all protons in a scalar coupled spin system. This experiment is very useful for assignment, particularly for complex molecules with multiple overlapping spin systems, e.g. peptides. For structure elucidation, the molecular fragments containing individual spin systems can be mapped out from the correlations. The TOCSY is a highly robust experiment and, along with gCOSY, is among the most sensitive of the 2D's, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Spinlock Pattern:	Pull-down menu on the "Pulse Sequence" panel- MLEV17 or DIPSI2 work well for small molecules, clean MLEV17 or DIPISI2 are good choices for large molecules
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the TOCSY is set up from this experiment.

- The TOCSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. One should consider increasing the number of increments (ni) to increase the quality of the data for such samples.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, e.g. 30ms or less. Such short spinlock TOCSY is a good alternative to gCOSY as TOCSY gives pure absorptive lineshape and potentially improved resolution.

- The MLEV17 and DIPSI2 spinlock patterns are good choices for small molecules, and the DIPSI3 and clean MLEV are typically used for larger molecules.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The zTOCSY experiment contains a zero-quantum filter and usually results in cleaner spectra than the TOCSY experiment. Only zTOCSY experiment can be combined with zero-quantum filters.
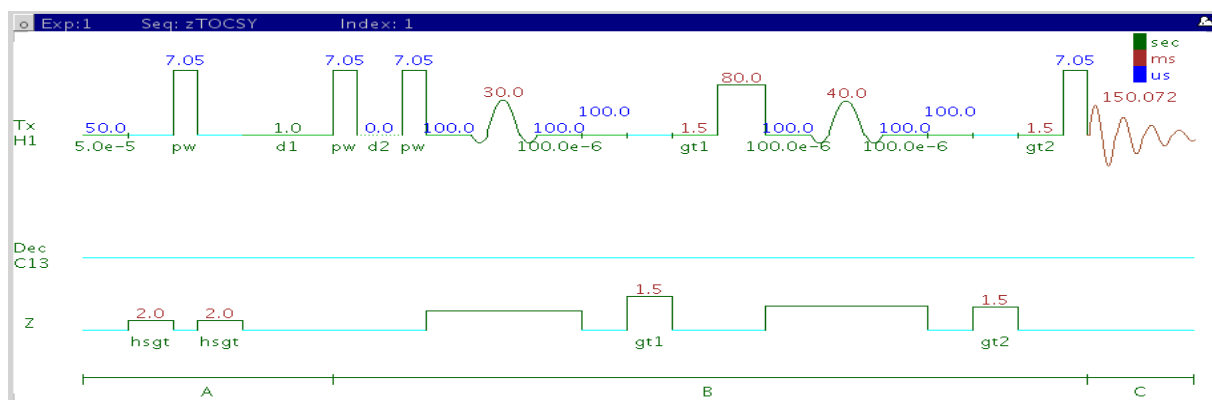
Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

C. Griesinger, G. Otting, K. Wuethrich, R. R. Ernst, J. Am. Chem. Soc. 110 (1988) 7870-7872. (TOCSY with clean MLEV17)

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

zTOCSY

Description:

Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with a zero-quantum filter for artifact suppression.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks potentially between all protons in a scalar coupled spin system. This experiment is very useful for assignment, particularly for complex molecules with multiple overlapping spin systems, e.g. peptides. For structure elucidation, the molecular fragments containing individual spin systems can be mapped out from the correlations. The zTOCSY is a highly robust experiment and, along with gCOSY, is among the most sensitive of the 2D's, making it a popular choice for the organic chemistry NMR lab. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Spinlock Pattern:	Pull-down menu on the "Pulse Sequence" panel- DIPSI2 works well for small molecules, DIPSI3 is a good choice for large molecules
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the zTOCSY is set up from this experiment.

- The TOCSY is a high sensitivity experiment and data can often be collected using one scan per increment with reasonably concentrated samples. One should consider increasing the number of increments (ni) to increase the quality of the data for such samples

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, e.g. 30ms or less Such short spinlock zTOCSY is a good alternative to gCOSY as zTOCSY gives pure absorptive lineshape and potentially improved resolution.

- The DIPSI2 spinlock pattern is a good choice for small molecules, and the DIPSI3 is typically used for larger molecules

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The zTOCSY experiment contains a zero-quantum filter and usually results in cleaner spectra than the TOCSY experiment.

Reference(s):

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

ROESY

Description:

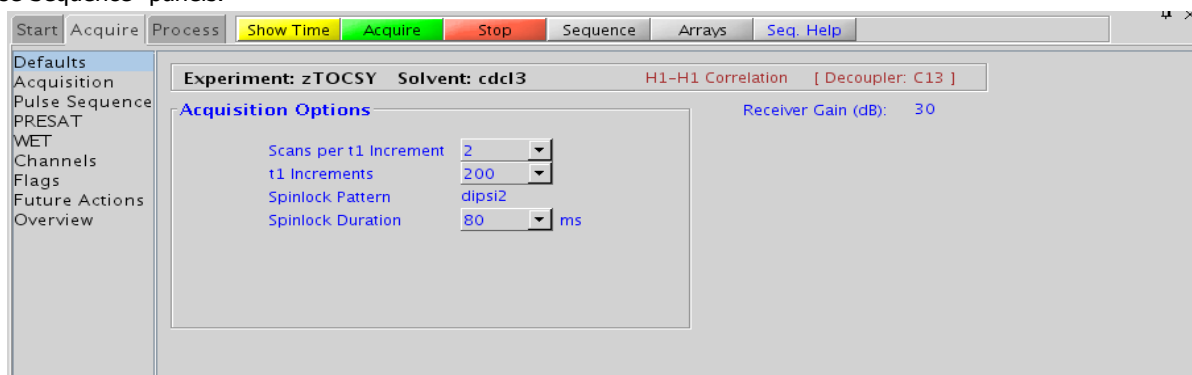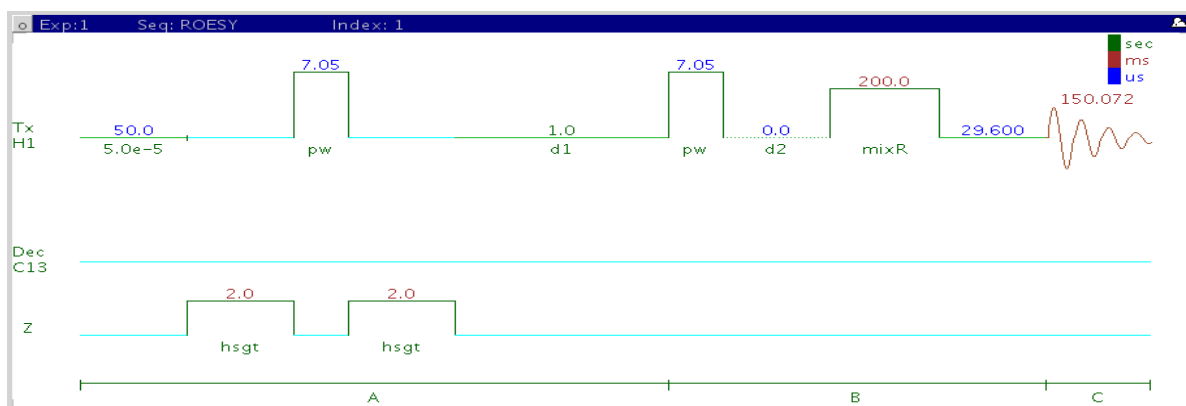Two Dimensional through-space correlation spectroscopy in the rotating frame.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <4 Å). As such the ROESY experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but non-coupled portions of a molecule. The ROESY is the experiment of choice for intermediate-sized molecules (typically between 800-1000 MW) where the NOE may be zero. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1):	Select typical choices from pull down menu*-controls the delay between scans
Spinlock Pattern:	Pull-down menu on "Pulse Sequence" panel- choose troesy (recommended) or cw
Spinlock Mixing time:	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the ROE peaks will be negative. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the ROESY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the ROESY is limited, weaker correlations may not be observable. ROESY is the experiment of choice over NOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running NOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Crosspeaks in the ROESY spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time or TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

- The ROESYAD experiment uses adiabatic pulses for the spinlock, which may be preferred over the ROESY.

Reference(s):

A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, J. W. Jeanloz, J. Am. Chem. Soc. 106 (1984) 811-813. (CAMELSPIN)

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)

ROESYAD

Description:

Two Dimensional through-space correlation spectroscopy in the rotating frame with adiabatic pulses for the spinlock.
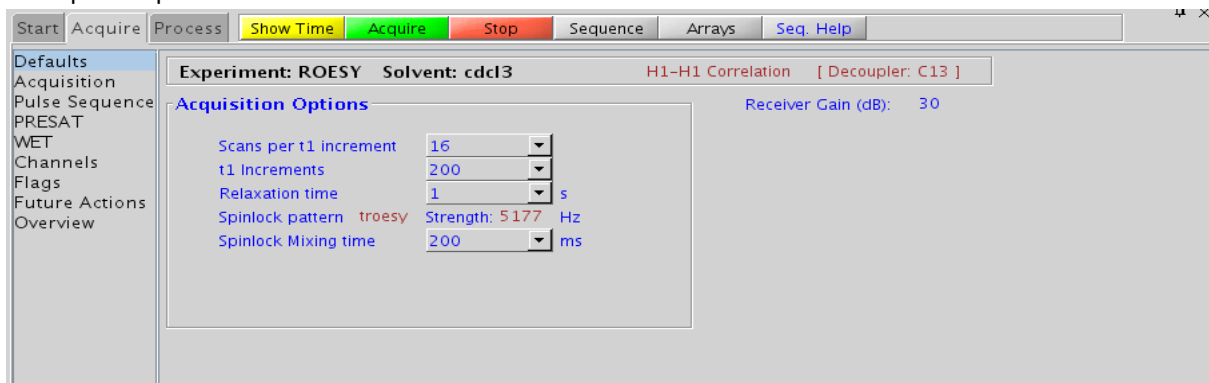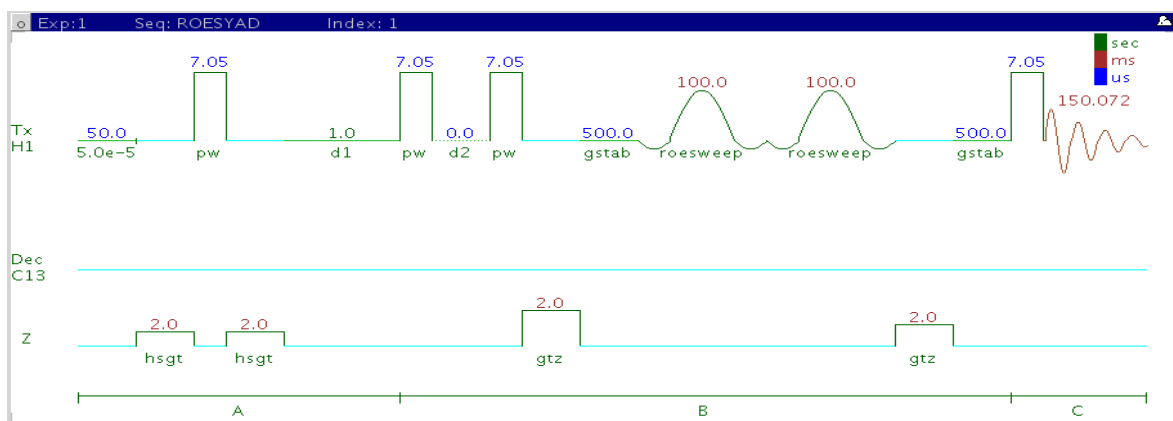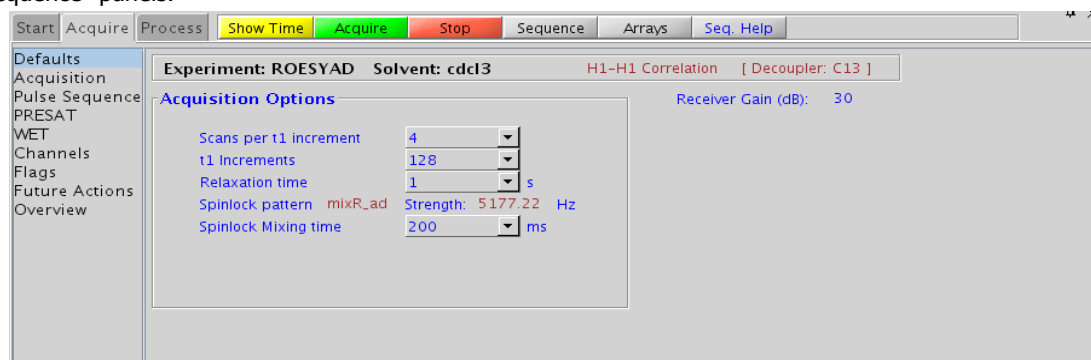
Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <4 Å). As such the ROESYAD experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but non-coupled portions of a molecule. The ROESYAD is the experiment of choice for intermediate-sized molecules (typically between 800-1000 MW) where the NOE may be zero. Because of its lower-power adiabatic spinlock the ROESYAD is usually superior to the traditional ROESY experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1):	Select typical choices from pull down menu*-controls the delay between scans
Spinlock Mixing time:	Select from pull down menu- 200ms works well for most small molecules*
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the ROE peaks will be negative. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the ROESYAD is set up from this experiment.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the ROESYAD is limited, weaker correlations may not be observable. ROESYAD is the experiment of choice over NOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running NOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Crosspeaks in the ROESYAD spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time or TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

- The ROESYAD experiment uses adiabatic pulses for the spinlock, which may be preferred over the ROESY.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)
T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)
D. F. Hansen, L. E. Kay, J. Biomol. NMR, 37 (2007) 245-255. (Adiabatic Spinlock)

NOESY
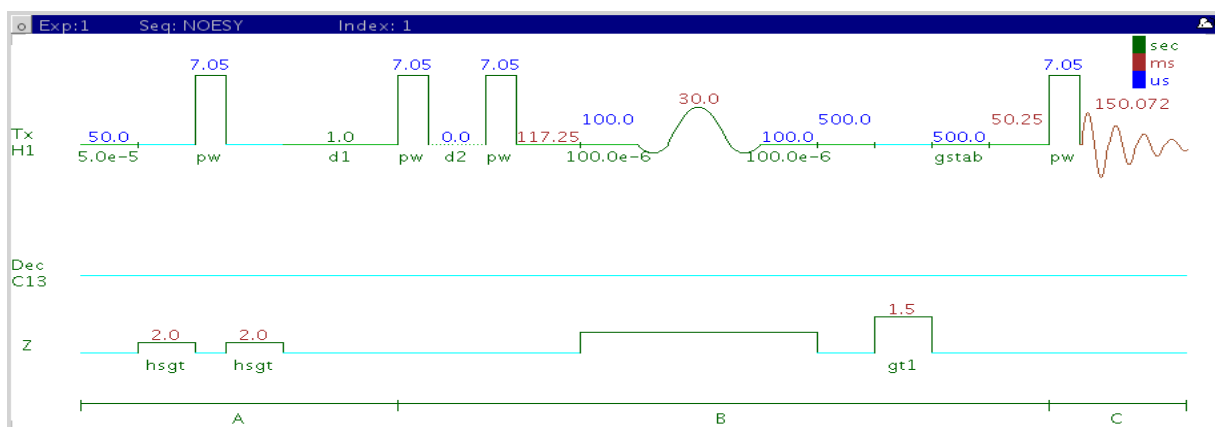
Description:
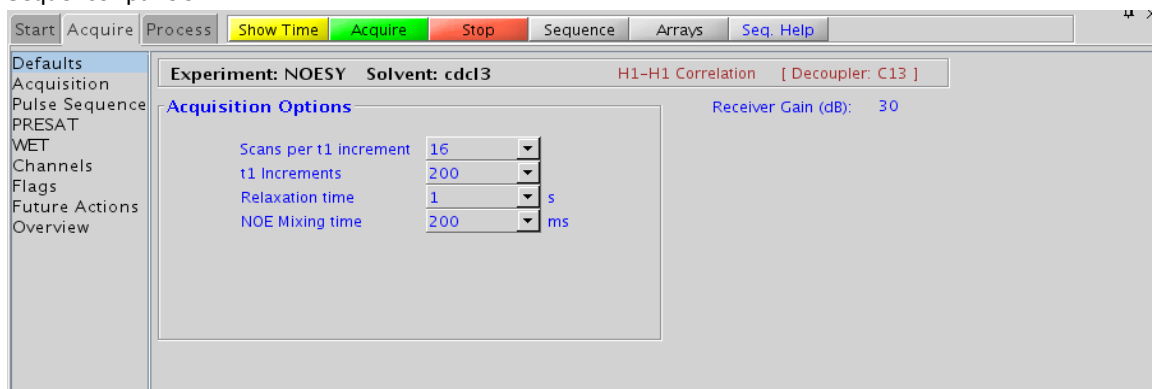
Two dimensional through-space correlation spectroscopy.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <5 Å). As such the NOESY experiment is often used to help determine stereochemistry for rigid ring systems or to confirm attachments between proximal, but non-coupled portions of a molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1):	Select typical choices from pull down menu*-controls the delay between scans
NOE Mixing time (mixT):	Select from pull down menu- 500ms works well for most small molecules*
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
ZQ filter:	Check box on the "Pulse Sequence" panel- zero-quantum filter for artifact suppression. Strongly recommended- default is on.

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the NOE peaks will be negative for smaller molecules. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the NOESY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.

- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOE's. One should consider acquiring a ROESYAD for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and will have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

Reference(s):
S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)
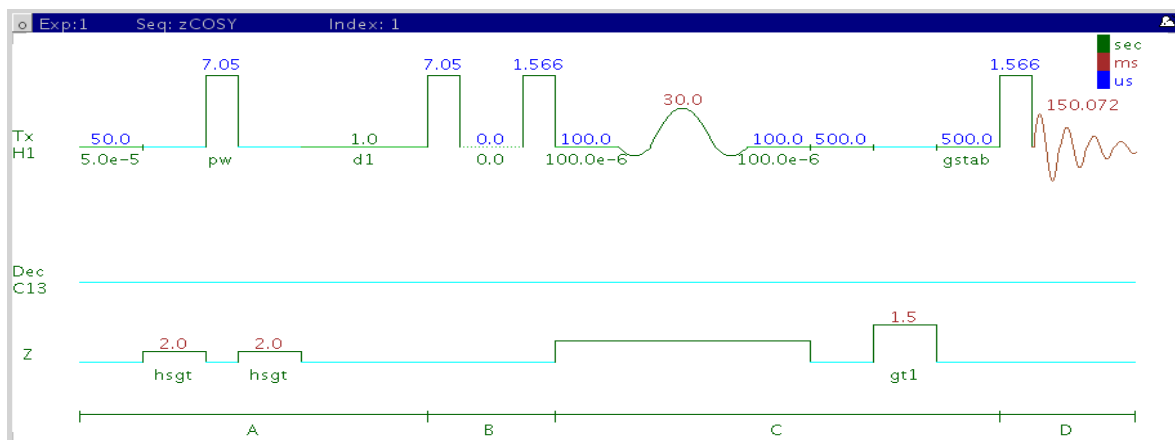
GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional J-correlation spectroscopy.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons with with non-zero couplings, typically 2-4 chemical bonds apart. This experiment is mostly used to determine homonuclear coupling constants, especially smaller long-range couplings. These are typically obstructed by the complex coupling pattern in a traditional COSY but are accessible in zCOSY because of its E.COSY (Exclusive COSY) pattern. For structure elucidation, the protonated molecular fragments can be mapped out from the couplings. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Relaxation time (d1):	Select typical choices from pull down menu*-controls the delay between scans
Anti-z COSY:	Check box to acquire an anti-z COSY (broadband proton decoupled)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the zCOSY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The zCOSY is an attractive alternative to the traditional E.COSY (Exclusive COSY) experiment. zCOSY gives the same crosspeak pattern as E.COSY but can be run with single scan per increment.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)
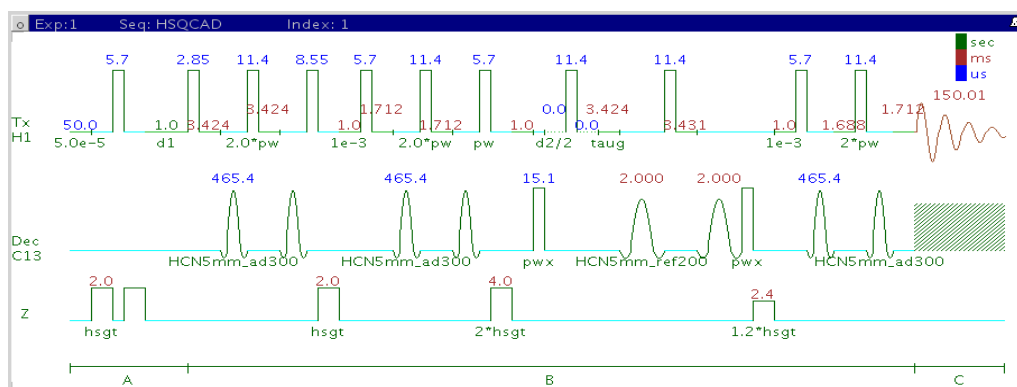
Chapter 4
Heteronuclear 2D 1-bond - J1(CH)corr

Introduction

This tab of the experiment selector contains 2D experiments for correlating directly attached protons to carbons. HSQC and its variants are the most commonly used family, as they typically provide the best sensitivity and peak shape. These experiments are available both with and without gradient coherence selection and adiabatic 180° carbon pulses. The gradient coherence selected versions of the experiments experience better artifact suppression at the expense of losses of sensitivity (factor of ~1.4). The adiabatic versions, HSQCAD and gHSQCAD are highly recommended, as they provide more uniform inversion profiles for a wide ^{13}C spectral width, and, at the same time, compensate for any ^{13}C pwx imperfections in the 180° pulses. Gradient and non-gradient HMQC experiments are also available, as well as a "fast" experiment called ASAPHMQC, which can provide a complete HX correlation spectrum with reasonable resolution in about a minute or less for samples of ~40mM concentration. Finally, there are both gradient and non-gradient versions of the ^{13}C-detected HETCOR experiment. The HSQC and HETCOR experiment (and their variants) also support multiplicity-editing, where signals from the methylene group appear with opposite sign from the signals arising from methyl and methine groups. Note that although the default parameters for these experiments are setup for ^{1}H-^{13}C correlations, other X-nuclei may be used instead of carbon provided the appropriate calibrations for these nuclei are in the probe file.

The 1-bond proton-carbon correlation is extremely useful for structure confirmation and elucidation. Peak assignments are easily made from this data; if a particular carbon assignment is known, its attached proton assignment can be derived, and vice versa. Knowledge gained from carbon chemical shift prediction can also be applied to the proton assignments with this type of data. The proton-detected experiments, HSQC and HMQC, are also considerably more sensitive than a 1D CARBON and thus permit access to carbon data for much less concentrated samples.

HSQCAD

GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses.
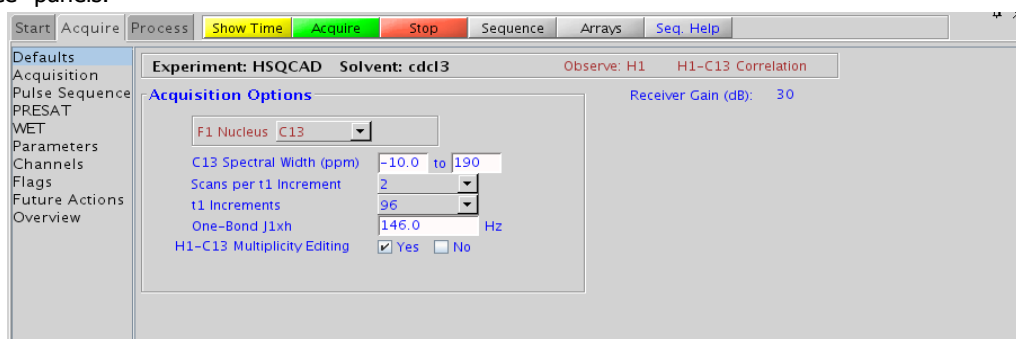
Usage:

The HSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HSQCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This results in cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constant and carbon chemical shift. Carbons with coupling constants that deviate significantly from this relationship (for example cyclopropanes or ethylene oxides) will result in imperfect editing.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.

- The HSQCAD experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCAD, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- The HSQCAD uses adiabatic 180° carbon pulses and will often give better results than the HSQC, due to much improved uniform inversion over a wide ^{13}C spectral width.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (HSQCAD / CRISIS-HSQC)
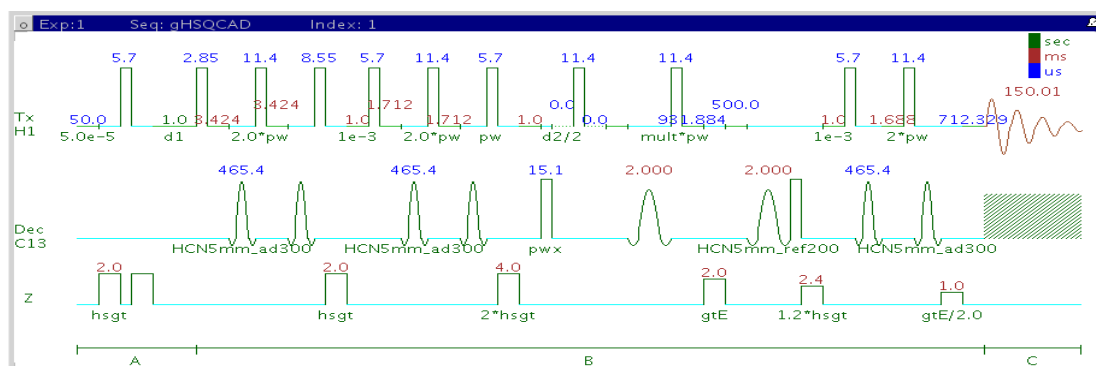
gHSQCAD

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and gradient coherence selection.
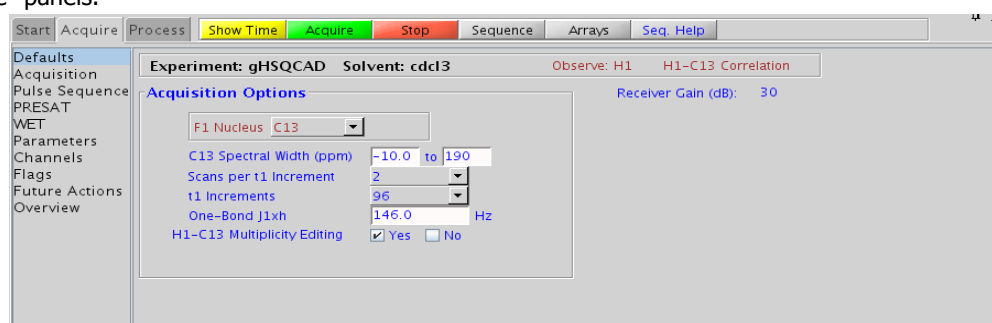
Usage:

The gHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHSQCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This results in cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.

- The HSQCAD experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCAD, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- The gHSQCAD uses adiabatic 180° carbon pulses and will often give better results than the gHSQC, due to much improved uniform inversion over a wide ^{13}C spectral width.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.
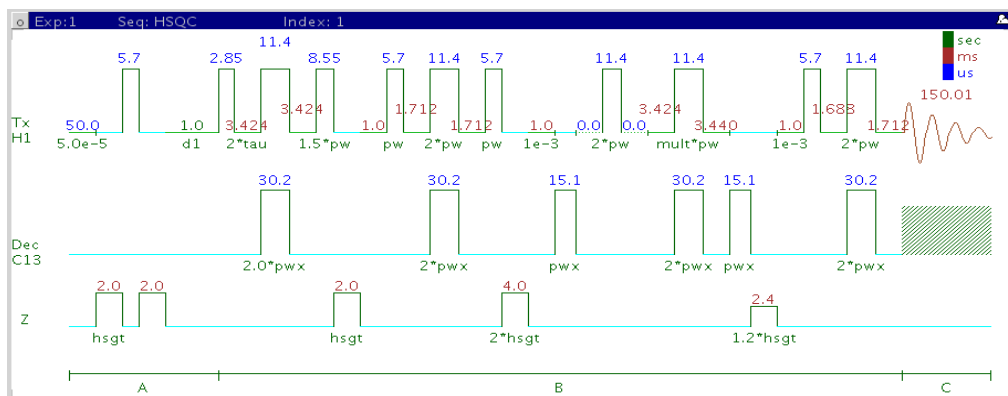
Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (gHSQCAD / CRISIS-gHSQC)

GENERAL DESCRIPTION AND USAGE

Description:

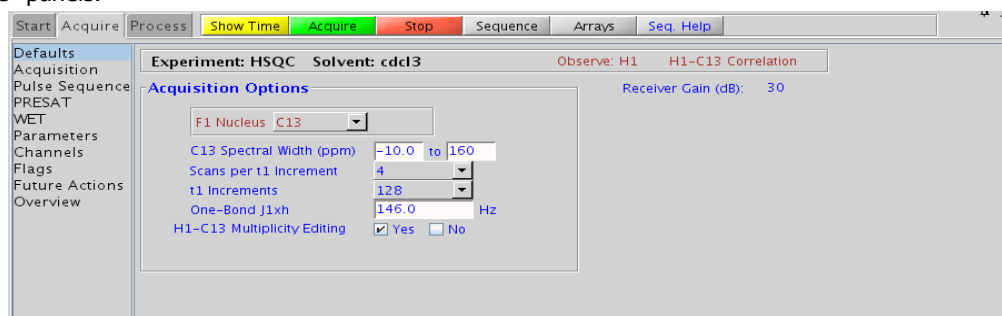Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy.

Usage:

The HSQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HSQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. There will be a sensitivity loss with this feature due to unmatched coupling constants and echo time, but the extra information about multiplicity is usually worth the loss in sensitivity. Hence this feature is on by default. Alternately, one should consider using HSQCAD to minimize this sensitivity loss.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.

- The HSQC experiment has a sensitivity advantage (factor of ~1.4) over the gHSQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- The HSQCAD and gHSQCAD use adiabatic 180° carbon pulses and will invariably give better results than the HSQC.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)
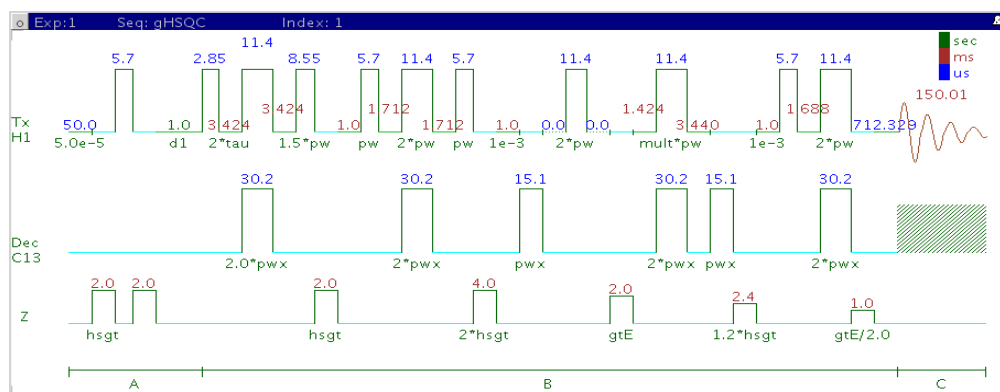
gHSQC

GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with gradient coherence selection.
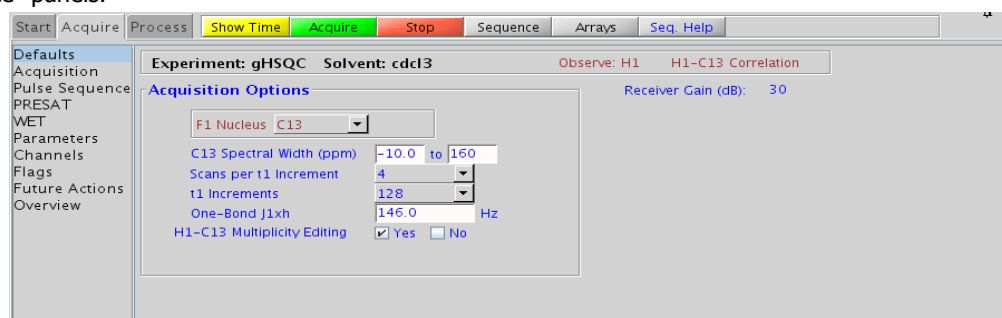
Usage:

The gHSQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHSQC is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. There will be a sensitivity loss with this feature due to unmatched coupling constants and echo time, but the extra information about multiplicity is usually worth the loss in sensitivity. Hence this feature is on by default. Alternately, one should consider using gHSQCAD to minimize this sensitivity loss.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution.

- The HSQC experiment has a sensitivity advantage (factor of ~1.4) over the gHSQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- The HSQCAD and gHSQCAD use adiabatic 180° carbon pulses and will invariably give better results than the gHSQC

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
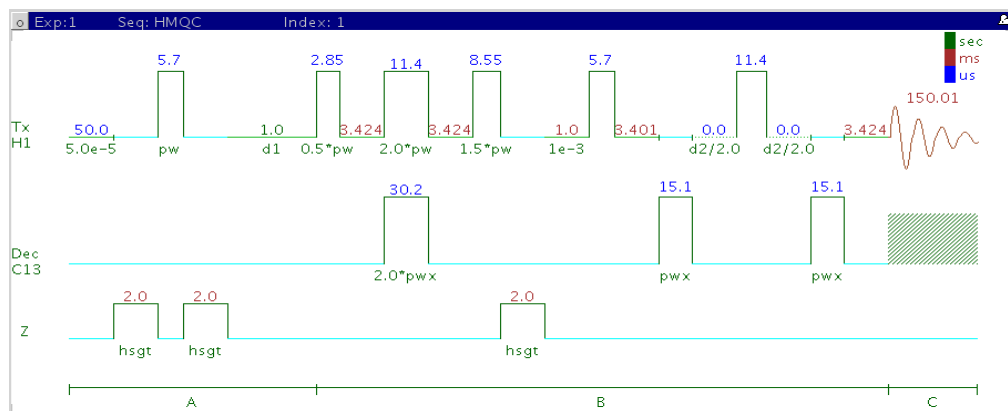
GENERAL DESCRIPTION AND USAGE

Description:

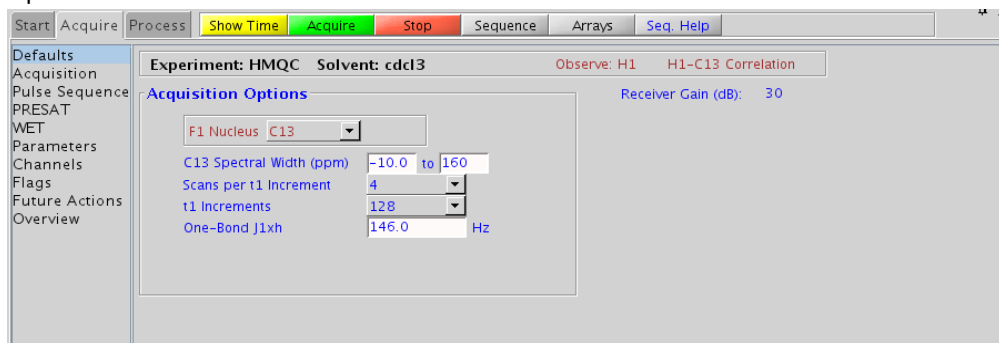Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy.

Usage:

The HMQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.
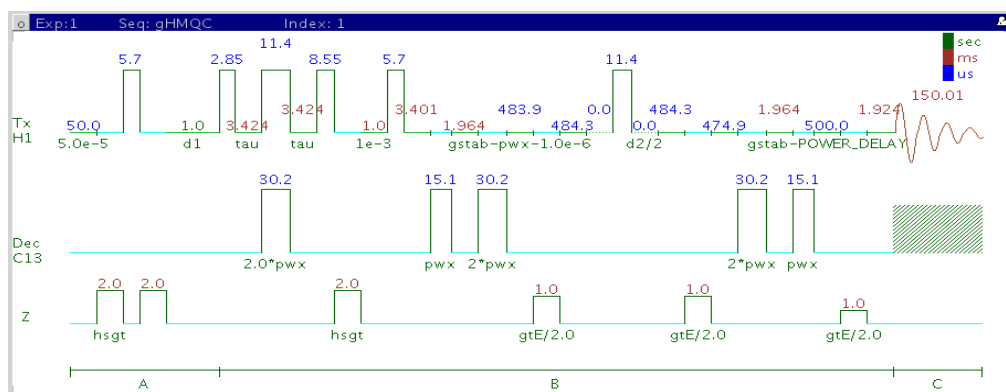
TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The HMQC experiment has a sensitivity advantage (factor of ~1.4) over the gHMQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution. In addition, the HSQC experiments have the added ability to do multiplicity- editing.

Reference(s):

L. Muller, J. Am. Chem. Soc. 101 (1979) 4481-4484. (HMQC)

A. Bax, R. H. Griffey, B. L. Hawkins, J. Magn. Reson. 55 (1983) 301-315. (HMQC)

gHMQC

GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with gradient coherence selection.
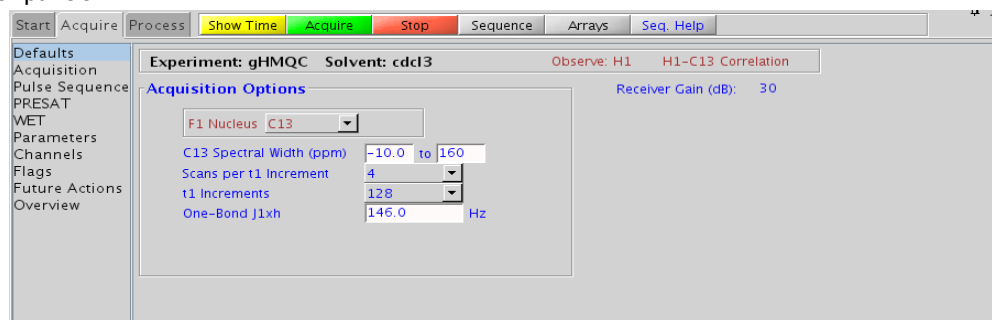
Usage:

The gHMQC experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The HMQC experiment has a sensitivity advantage (factor of ~1.4) over the gHMQC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, leading to typically better S/N and resolution. In addition, the HSQC experiments have the added ability to do multiplicity editing.

Reference(s):

L. Muller, J. Am. Chem. Soc. 101 (1979) 4481-4484. (HMQC)

A. Bax, R. H. Griffey, B. L. Hawkins, J. Magn. Reson. 55 (1983) 301-315. (HMQC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
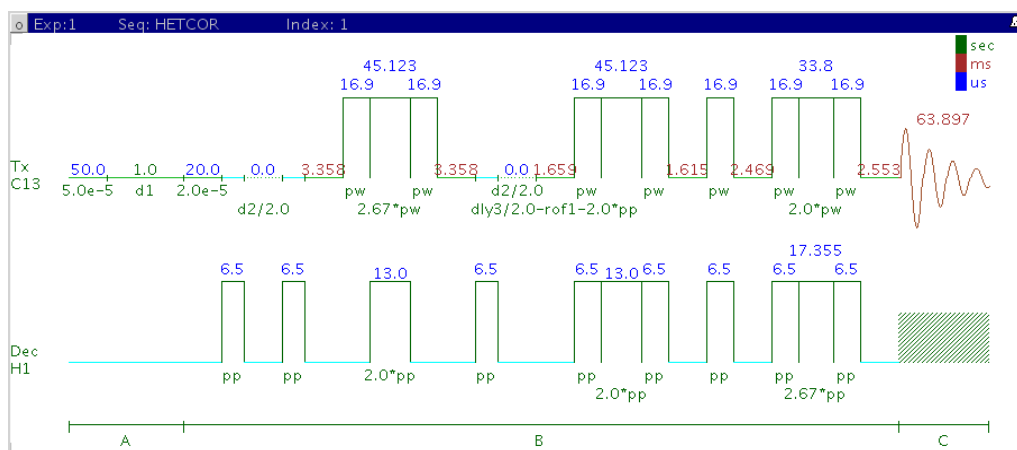
HETCOR

Description:

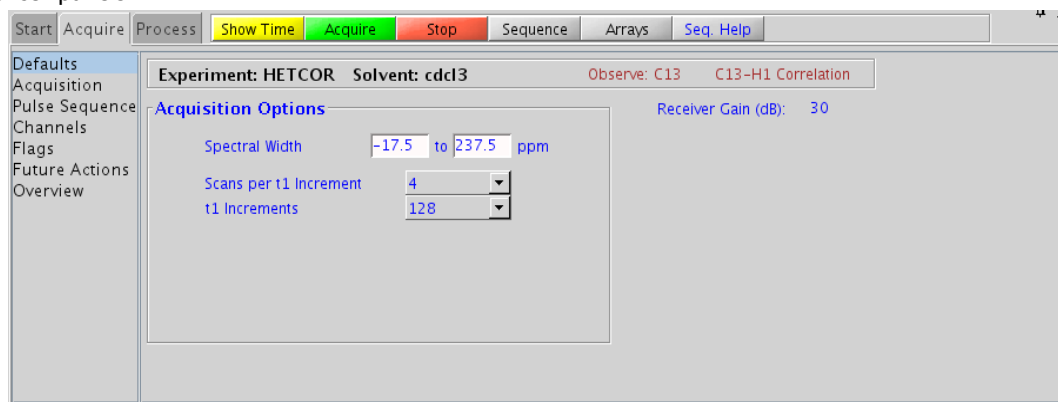Two dimensional heteronuclear 1-bond shift correlation with carbon observe.

Usage:

The HETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. However unlike the HSQC/HMQC experiments HETCOR is carbon-detected, meaning the F1 dimension is ^{1}H and the F2 dimension is ^{13}C. The HETCOR, thus, is significantly less sensitive than the proton-detected HSQC/HMQC, and should only be used under special circumstances where higher resolution is desired in the carbon dimension. The proton decoupling power (during acquisition) used is also usually less. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired CARBON or type in desired values
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond Coupling:	Set value for the average $^1J_{X,H}$ on "Pulse Sequence" panel- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a CARBON if the HETCOR is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

- The embedded multiplicity- editing results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons.

- The HETCOR also generates an effective ^{1}H broadband decoupling along the F1 (H1) dimension.

- The HETCOR experiment has a sensitivity advantage (factor of ~1.4) over the gHETCOR, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- In general, the HSQC-type experiments are far more sensitive than the HETCOR. The advantages to the HETCOR are lower decoupling power and potentially higher resolution for carbon. The band-selective HSQC experiment, (HC)bsHSQCAD (see Chapter 7), however, is a much more sensitive experiment and may provide a more convenient way to achieve higher ^{13}C resolution.

Reference(s):

A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981), 501-505. (HETCOR)
D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)
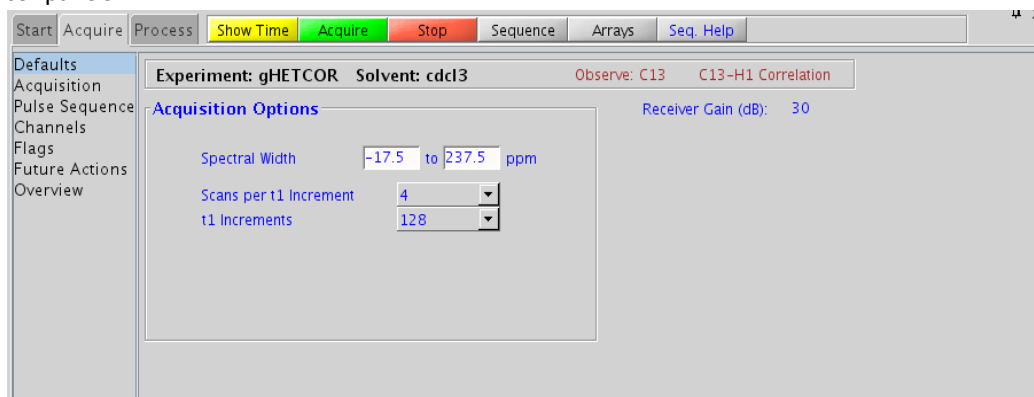
gHETCOR
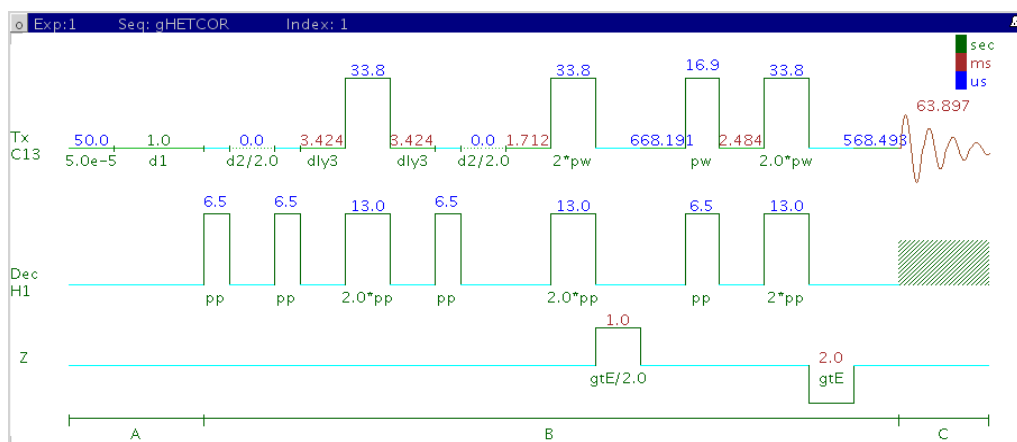
Description:

Two dimensional heteronuclear 1-bond shift correlation with carbon observe and gradient coherence selection.

Usage:

The gHETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. However unlike the gHSQC/gHMQC experiments gHETCOR is carbon-detected, meaning the F1 dimension is ^{1}H and the F2 dimension is ^{13}C. The gHETCOR , thus, is significantly less sensitive than the proton-detected gHSQC/gHMQC, and should only be used under special circumstances where higher resolution is desired in the carbon dimension. The proton decoupling power (during acquisition) used is also usually less. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired CARBON or type in desired values
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond Coupling:	Set value for the average ^{1}J$_{X,H}$ on "Pulse Sequence" panel- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a CARBON if the gHETCOR is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

- The embedded multiplicity editing results in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons.

- The gHETCOR also generates an effective ^{1}H broadband decoupling along the F1 (H1) dimension.

- The HETCOR experiment has a sensitivity advantage (factor of ~1.4) over the gHETCOR, but due to t1 noise from strong signals from t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- In general, the gHSQC-type experiments are far more sensitive than the gHETCOR. The advantages to the gHETCOR are lower decoupling power and potentially higher resolution for carbon. The band-selective HSQC experiment, (HC)bsgHSQCAD (see Chapter 7), however, is a much more sensitive experiment and may provide a more convenient way to achieve higher ^{13}C resolution.

Reference(s):

A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981), 501-505. (HETCOR)

D. L. Turner, Prog. NMR. Spectrosc. 17 (1985) 281-358. (2D NMR)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
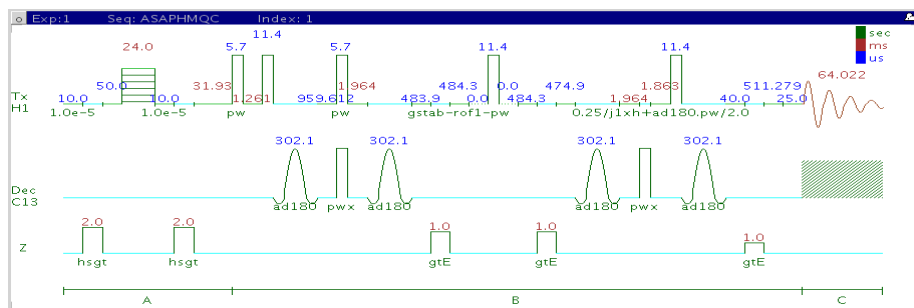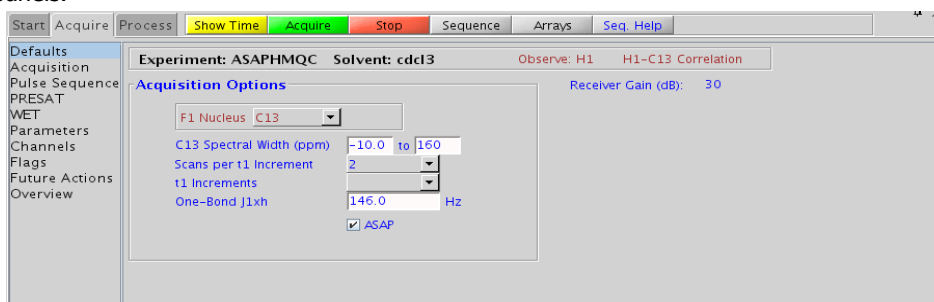
ASAPHMQC

GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with adiabatic 180° X-nuclei pulses and an "ASAP" feature for rapid recycle times.

Usage:

This experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The experiment is the most useful of the HMQC series as it contains a feature that allows for very fast recycle time. With a reasonably concentrated sample, it is possible to obtain an ASAPHMQC spectrum with adequate resolution in about 1 minute. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s for ASAP
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
ASAP:	Check box to turn on/off ASAP feature (off will give an HMQC with adiabatic 180° X-nuclei pulses
Relaxation time (d1):	Set from "Acquisition" panel- controls the delay between scans (default is 0.064s)
Mixing time (mix):	Set from" Pulse Sequence" panel- mixing time for adiabatic pulses (default is 25ms)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the ASAPHMQC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C-labelled.

- ASAPHMQC as a fast method gives significantly better sensitivity than other members of the heteronuclear one-bond correlation experiments for a given spectrometer time. This alone overweighs other advantages of traditional experiments.

- During the short relaxation delay (d1), the ASAPHMQC employs a TOCSY-type magnetization transfer from ^{12}C-attached protons (which are kept along z during the pulse sequence) to the observed ^{13}C-attached protons. It should be noted that because of this transfer the ASAPHMQC works best if one or several protons are in the neighborhood (i.e., spin system) of the observed ^{13}C-^1H pair. Lone ^{13}C-^1H pairs without neighboring protons may therefore show up at a much lower intensity. In such cases it may help to increase d1 from the default 60ms.

- The adiabatic pulses and rapid recycle time make the ASAPHMQC experiment a good choice for obtaining carbon information in automation. This experiment is also a good way to rapidly obtain "survey" carbon information for a subsequent band-selective experiment (see Chapter 7).

Reference(s):

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
E. Kupce, R. Freeman, Magn. Reson. Chem. 45 (2007) 2-4. (ASAPHMQC)

# Chapter 5
Heteronuclear 2D multiple-bond - Jn(CH)corr

Introduction

This tab of the experiment selector contains 2D experiments showing long-range couplings from protons to carbon nuclei. The most commonly used experiment type, the HMBC, is available both with and without adiabatic 180° carbon pulses, and also with and without gradient coherence selection. The gradient enhanced version of the experiment has better artifact suppression at the expense of a loss of sensitivity (factor of ~1.4). The adiabatic version, gHMBCAD is very highly recommended, as it provides more uniform inversion profiles for a wide ^{13}C spectral width, and at the same time compensates for any ^{13}C pwx imperfections in the 180° pulses. There is a multiplicity-edited version of this experiment, gHMBCmeAD, and an experiment that provides similar information to differentiate between 2-bond and >2-bond couplings, the gHMBCRELAY. The H2BC experiment (adiabatic and non-adiabatic) is used to show specifically 2-bond proton-carbon correlations involving non-quaternary carbons. This experiment is, in principle, an HMQC-COSY equivalent. Finally, there are the adiabatic and non-adiabatic CIGAR experiments. While the HMBC experiments are typically optimized for a single Jn(XH) coupling constant, the CIGAR experiments attempt to optimize a range for this coupling constant. This experiment is particularly effective for ^{15}N, where the Jn(NH) coupling constants can vary widely.

Long-range proton-carbon correlations are often used in structure elucidation to provide key connectivity data between separated spin systems and to quaternary carbons. The HMBC type experiments are generally more sensitive than 1D CARBON spectra for detecting quaternary carbons (with the exception of carbons which are 3 or 4 bonds away from any proton). The sensitivity of these experiments does vary widely from correlation to correlation. When the HMBC type experiments are used with less concentrated samples, while it might not show all correlations, even a partial long range correlation data set is highly valuable for structure elucidation.

Most of these experiments (except gHMBCRELAY, gH2BC and gH2BCAD) rely on ^1H-^{13}C correlations via long-range coupling constants. In general 3-bond coupling constants (~8 Hz) are larger than 2-bond coupling constants (~2-3 Hz) and typically tend to give stronger crosspeaks when the experiment parameters are optimized for 8 Hz. However, care must be exercised in interpreting these crosspeaks from their intensity as arising from 2 or 3 bond correlations, as they can only be definitively identified as crosspeaks due to smaller coupling constants (and may be 2, 3, or 4 bonds

away). The 2-bond correlation experiments gH2BC and gH2BCAD, although less sensitive than the HMBC, can be used to distinguish between 2- and 3-bond correlations.
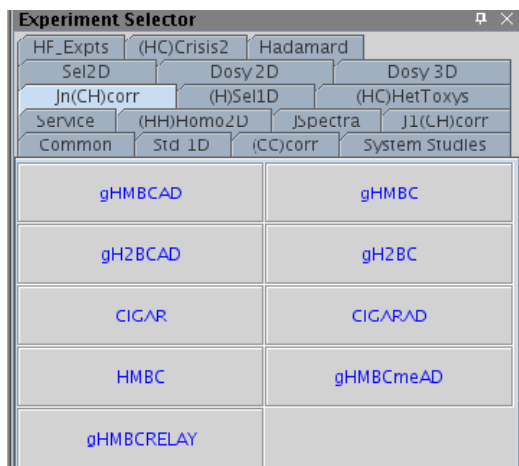
gHMBCAD
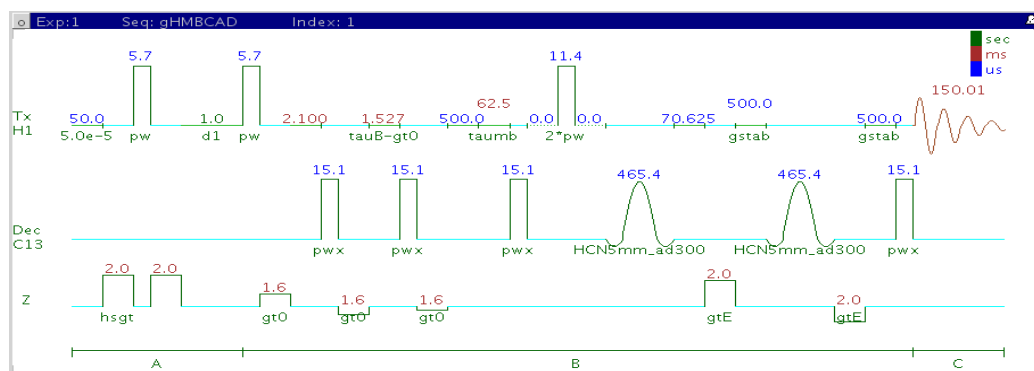
GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses and gradient coherence selection.
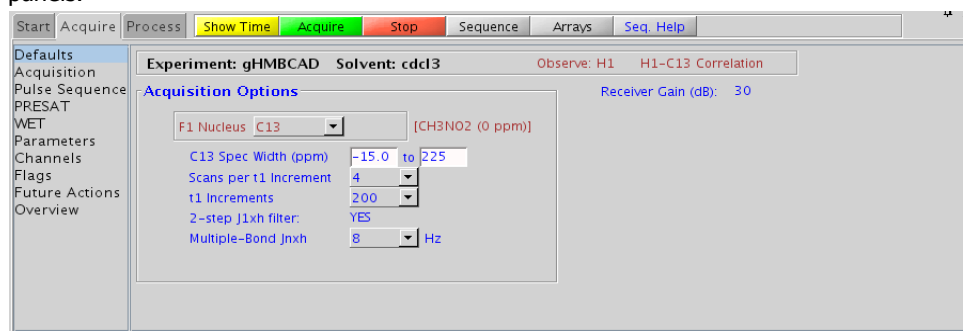
Usage:

The gHMBCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The gHMBCAD experiment is also one of the most sensitive ways to obtain information about quaternary carbon chemical shifts and connectivities. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$- 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMBCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGARAD experiment is better suited on a reasonably concentrated sample for detecting longer range correlations from a range of coupling constants, but at the expense of sensitivity.

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The gHMBCAD uses adiabatic 180° carbon pulses and will invariably give better results than the gHMBC.

Reference(s):

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

C. E. Hadden, Magn. Reson. Chem. 43 (2005) 330-333. (Adiabatic Pulses)
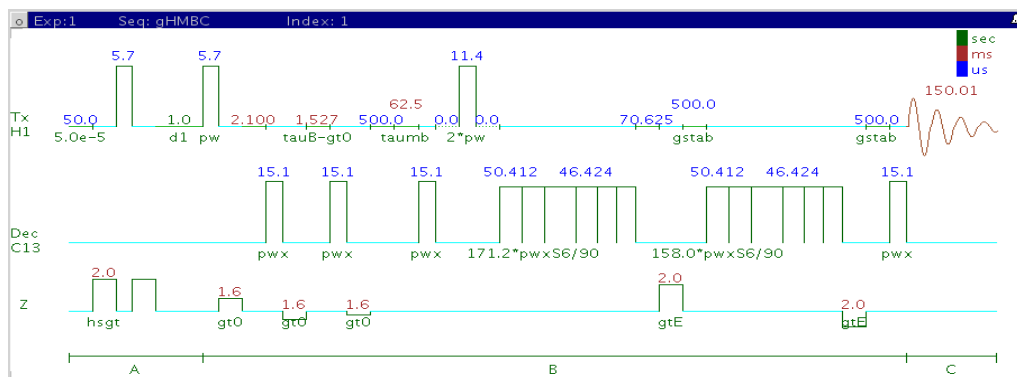
gHMBC

GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection.

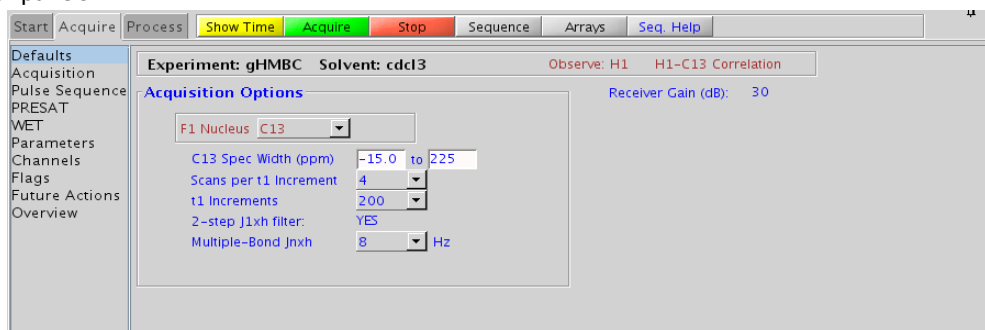**Usage:**

The gHMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The gHMBC experiment is also one of the most sensitive ways to obtain information about quaternary carbon chemical shifts and connectivities. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$- 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMBC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The HMBC experiment has a sensitivity advantage (factor of ~1.4) over the gHMBC, but due to t1 noise from strong signals such as t-butyl may produce potentially less clean spectra than its gradient counterpart.

- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGAR experiment is better suited on a reasonably concentrated sample for detecting longer-range correlations from a range of coupling constants, but at the expense of sensitivity.

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The gHMBC uses the Shaka6 (S6) pulse to achieve broadband inversion. Parameters relating to this pulse can be found on the "Pulse Sequence" tab.

- The gHMBCAD uses adiabatic 180° carbon pulses and will invariably give better results than the gHMBC.

Reference(s):

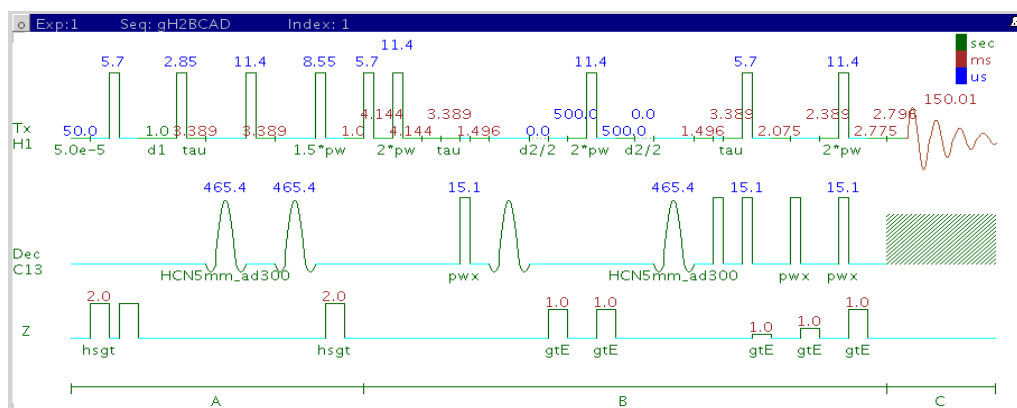A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

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gH2BCAD

Description:

Two dimensional heteronuclear 2-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses and gradient coherence selection.
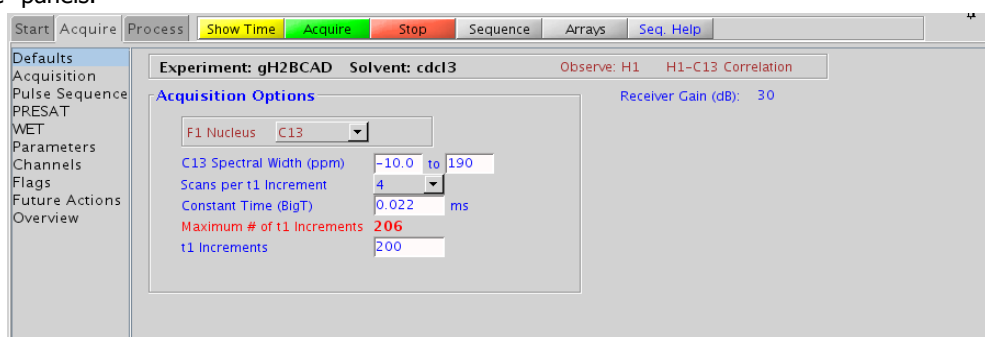
Usage:

The gH2BCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons through ^{1}H-^{1}H vicinal and ^{1}H-^{13}C one bond coupling connectivity. Although less sensitive than the gHMBCAD, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
Constant Time (BigT):	Type in value for constant time delay- 22ms (default) works for most samples
t1 Increments (ni):	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (256k x 256k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also available (defaults are gaussian in both F1 and F2 dimensions). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gH2BCAD is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gH2BCAD is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.

- The gH2BCAD experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gHMBCAD experiment. As such, the gH2BCAD is best used as supplemental information to the gHMBCAD data to distinguish between 2- and 3-bond correlations.

- The gH2BCAD uses adiabatic 180° carbon pulses and will invariably give better results than the gH2BC.

Reference(s):

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
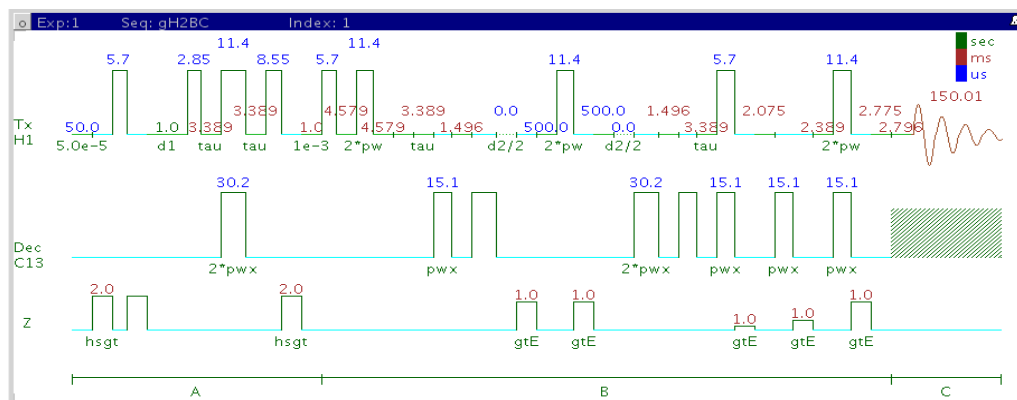
gH2BC

Description:

Two dimensional heteronuclear 2-bond J-correlation spectroscopy with gradient coherence selection.

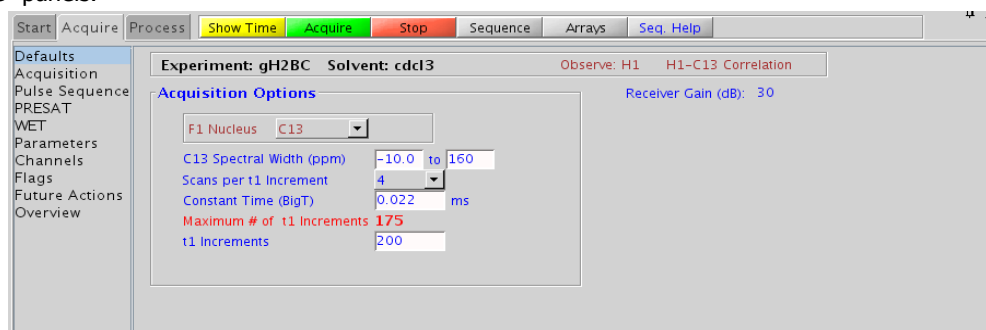**Usage:**

The gH2BC experiment is used to acquire a 2dimensional spectrum to correlate protons with carbons through^{1}H-^{1}H vicinal and ^{1}H-^{13}C one-bond coupling connectivity. Although less sensitive than the gHMBC, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
Constant Time (BigT):	Type in value for constant time delay- 22ms (default) works for most samples
t1 Increments (ni):	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (256k x 256k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also available (defaults are gaussian in both F1 and F2 dimensions). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gH2BC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gH2BC is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.

- The gH2BC experiment is specialized to show only 2-bond correlations, however it is less sensitive than the gHMBC experiment. As such, the H2BC is best used as supplemental information to the gHMBC data to distinguish between 2- and 3-bond correlations.

Reference(s):

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)
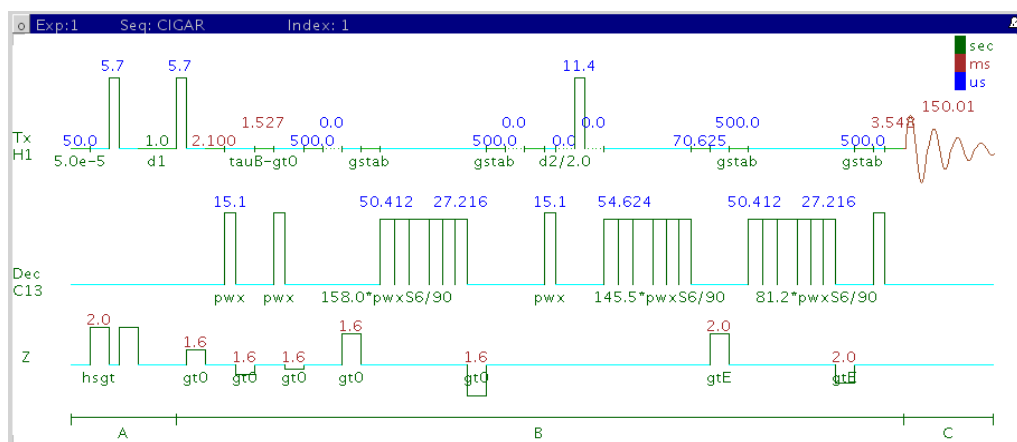
CIGAR

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy.

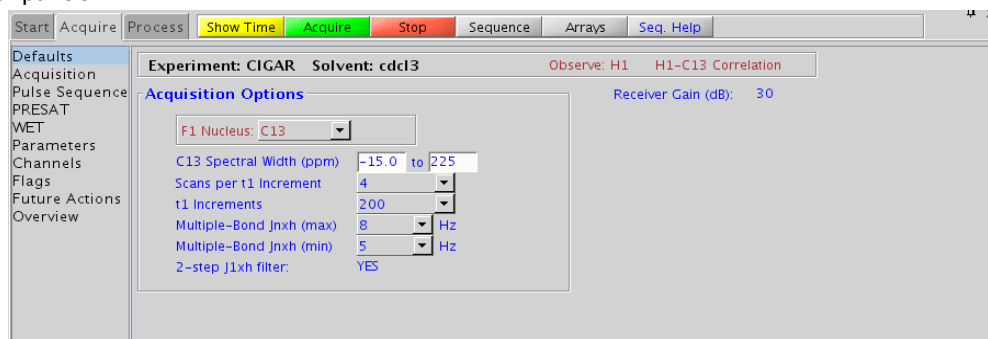**Usage:**

The CIGAR experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than its gHMBC counterpart, this experiment is useful when there is a need to see longer range correlations (>3 bonds and/or with small coupling constants), or when there is a large range of $^nJ_{X,H}$ coupling constants, such as with proton-nitrogen. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond jnxh (max/min):	Set values for the maximum/minimum $^nJ_{X,H}$
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the CIGAR is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The parameter jscaleU and jscaleD (accessed in the "Pulse Sequence" panel) are typically set to 0 for best sensitivity, which are the default values. However, one could set these to non-zero values to achieve homonuclear J dependent peak "skewing" for either improved peak identification in a crowded region (jscaleU) or to differentiate J^2 vs. J^3 crosspeaks (jscaleD).

- The CIGAR experiment is specialized to allow input of a range for $^nJ_{X,H}$. However, it is less sensitive than the gHMBC experiment (at least by a factor of 2). As such, the CIGAR is best used under circumstances where there is a large range for the $^nJ_{X,H}$ coupling constants, such as for proton-nitrogen. The CIGAR experiment may also be used when the sample is reasonably concentrated and there is a need to see longer range correlations (e.g. >3-bond).

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The CIGARAD uses adiabatic 180° carbon pulses and will invariably give better results than the CIGAR.

Reference(s):
C. E. Hadden, G. E. Martin, V. V. Krishnamurthy, Magn. Reson. Chem. 38 (2000) 143-147. (CIGAR)

M. Kline, S. Cheatham, Magn. Reson. Chem. 41 (2003) 307-314. (^{15}N-optimized CIGAR)

CIGARAD
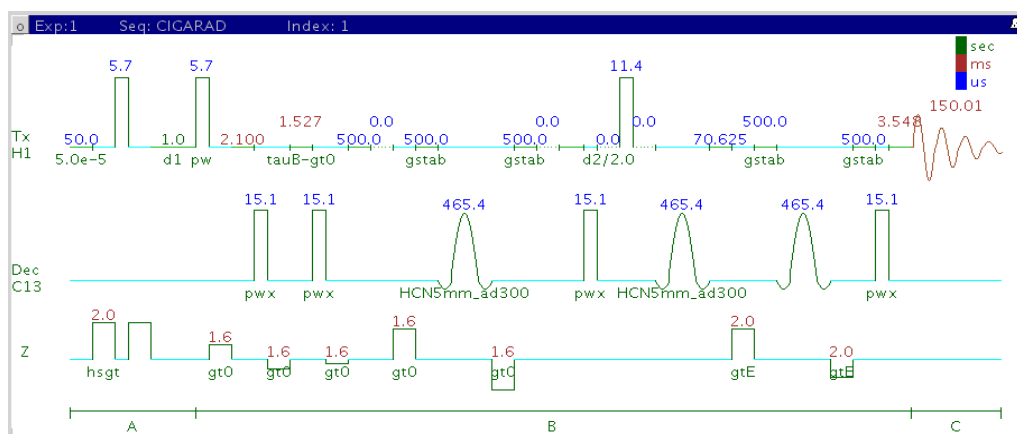
GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses.

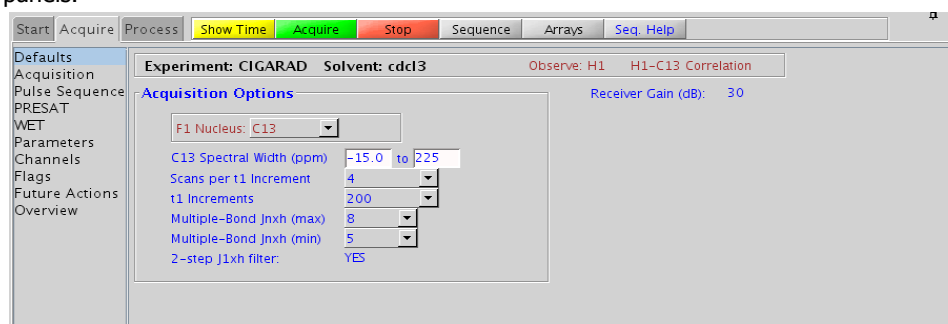**Usage:**

The CIGARAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than its gHMBC counterpart, this experiment is useful when there is a need to see longer range correlations (>3 bonds and/or with small coupling constants), or when there is a large range of $^nJ_{X,H}$ coupling constants, such as with proton-nitrogen. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond jnxh (max/min):	Set values for the maximum/minimum $^nJ_{X,H}$
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the CIGARAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The parameter jscaleU and jscaleD (accessed in the "Pulse Sequence" panel) are typically set to 0 for best sensitivity, which are the default values. However, one could set these to non-zero values to achieve homonuclear J dependent peak "skewing" for either improved peak identification in a crowded region (jscaleU) or to differentiate J^2 vs. J^3 crosspeaks (jscaleD).

- The CIGARAD experiment is specialized to allow input of a range for $^nJ_{X,H}$, however it is less sensitive than the gHMBC experiment (at least by a factor of 2). As such, the CIGARAD is best used under circumstances where there is a large range for the $^nJ_{X,H}$ coupling constants, such as for proton-nitrogen. The CIGARAD experiment may also be used when the sample is reasonably concentrated and there is a desire to see longer range correlations (e.g. >3-bond).

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The CIGARAD uses adiabatic 180° carbon pulses and will invariably give better results than the CIGAR.

Reference(s):

C. E. Hadden, G. E. Martin, V. V. Krishnamurthy, Magn. Reson. Chem. 38 (2000) 143-147. (CIGAR)

C. E. Hadden, Magn. Reson. Chem. 43 (2005) 330-333. (Adiabatic Pulses)
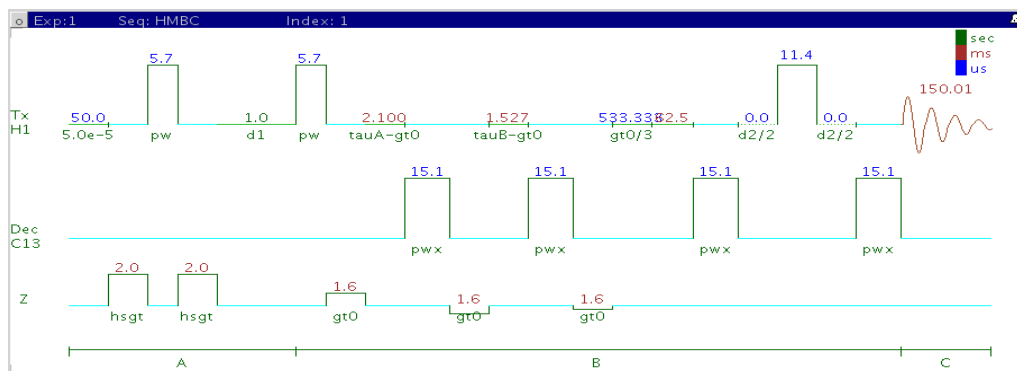
Description:

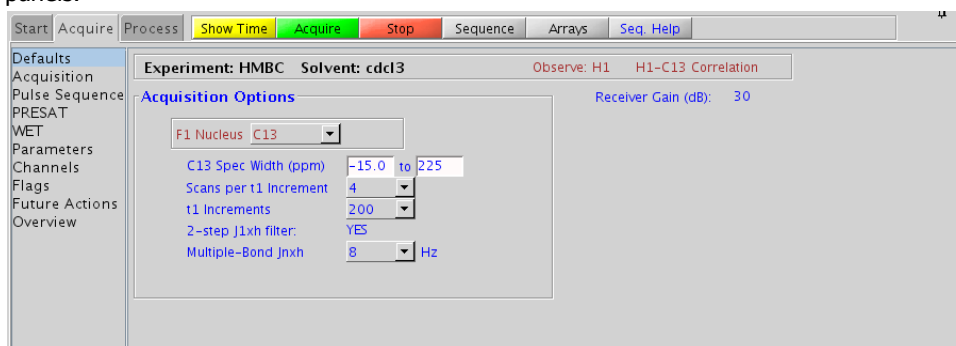Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection.

Usage:

The HMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons with carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. The HMBC experiment is also one of the most sensitive ways to obtain information about quaternary carbons chemical shifts and connectivities. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull-down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$ - 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations on/off- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HMBC is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The HMBC experiment has a sensitivity advantage (factor of ~1.4) over the gHMBC, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart. Invariably the gradient coherence selected variants (such as gHMBC or gHMBCAD) are preferred over HMBC.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss..

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

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Reference(s):
A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)
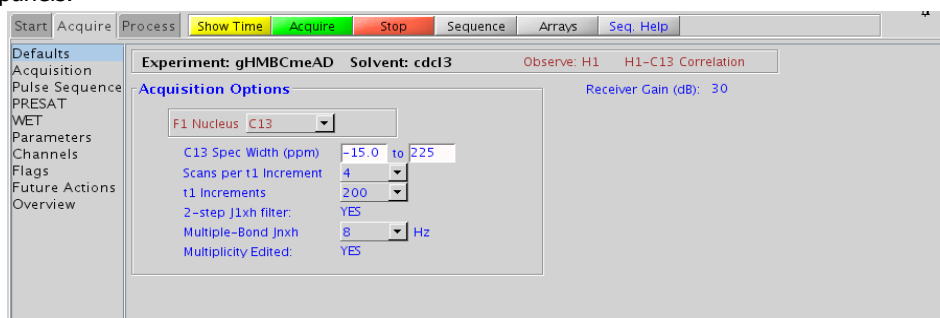
gHMBCmeAD
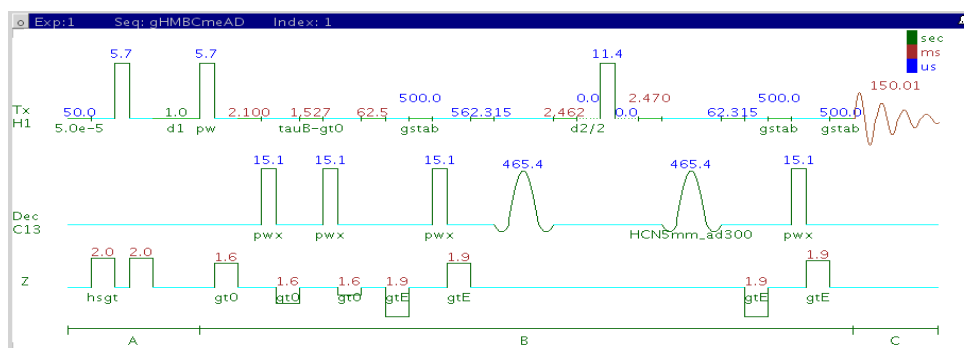
GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear multiple-bond J-correlation spectroscopy with multiplicity-editing (adiabatic 180° X-nuclei pulses and gradient coherence selection).

Usage:

The gHMBCmeAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The experiment is highly useful for structure elucidation as the longer range allows connectivities between isolated proton spin systems to be confirmed. The gHMBCmeAD experiment is a multiplicity edited version of gHMBCAD. This experiment is acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be reliably predicted with software. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$ - 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

The multiplicity- editing feature requires running an array of two spectra (default setup). The "Process" tab/"Default" panel has three buttons under the "Transform" heading to select the desired subspectrum. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase sensitive mode. This arrangement allows for the best balance of resolution and sensitivity.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMBCmeAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constant and carbon chemical shift. Carbons with coupling constants that deviate significantly from this relationship will result in imperfect editing.

- As the gHMBCmeAD is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

Reference(s):

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743. (gHMBCme)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (CRISIS)

gHMBCRELAY
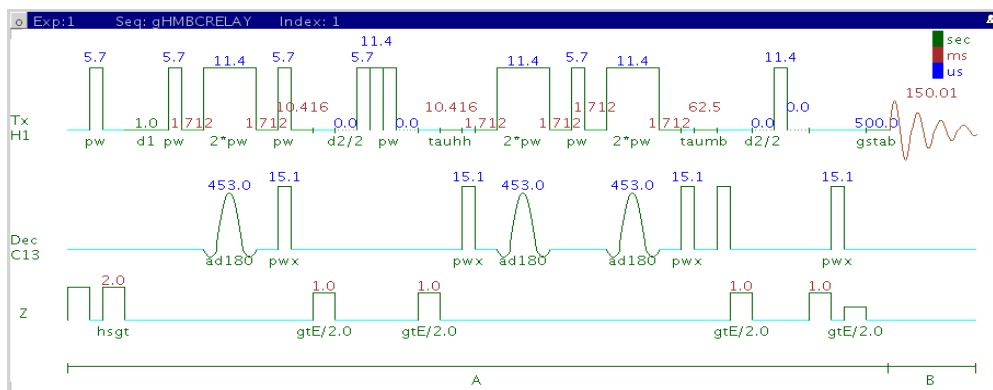
GENERAL DESCRIPTION AND USAGE

Description:

Two dimensional heteronuclear spectroscopy for the simultaneous detection of $^2J_{C,H}$ and $^nJ_{C,H}$ with adiabatic 180° X-nuclei pulses and gradient coherence selection.
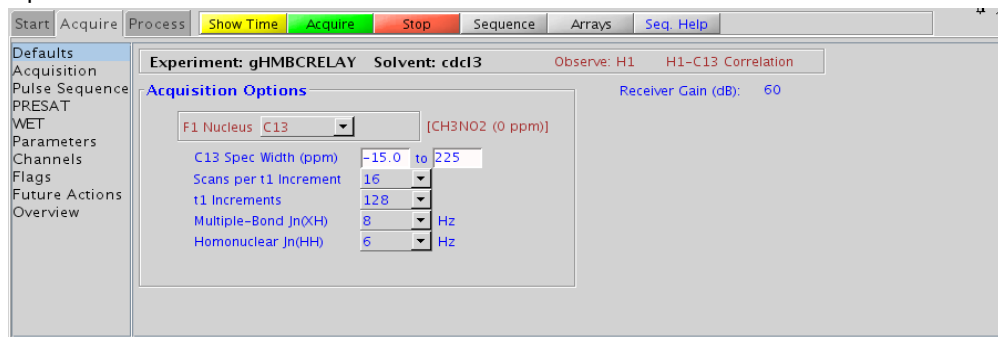
Usage:

The gHMBCRELAY experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over multiple bonds. Although less sensitive than the gHMBCAD, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations, as the 2-bond correlations are created through a separate pathway and may be separated into a subspectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^nJ_{X,H}$ - 8 Hz works well for most molecules
Homonuclear Jn(HH):	Pull-down menu to set value for the optimized $^nJ_{H,H}$
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition usually requires no further adjustment. The "Process" tab/"Default" panel has three buttons under the "Transform" heading to select the desired subspectrum. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are squared sinebells). More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMBCRELAY is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gHMBCRELAY experiment is specialized to be able to show subspectra containing only 2-bond H-C correlations, or >2-bond correlations, however it is less sensitive than the gHMBCAD experiment. As such, the gHMBCRELAY is best used only when it is important to be able to distinguish between 2- and 3-bond correlations.

Reference(s):

T. Sprang, P. Bigler, Magn. Reson. Chem. 42 (2004) 55-60. (HMBCRELAY)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

M. R. Bendall, M. Garwood, K. Ugurbill, D. T. Pegg, Magn. Reson. Med. 4 (1987) 493-499. (Adiabatic Pulses)

Chapter 6
Selective 1D – (H)Sel1D

Introduction

This tab of the experiment selector includes the set of 1D experiments performed on small, user-defined bands or resonances of the proton spectrum using selective pulses. They are useful to obtain targeted information about single resonances or small areas of a complicated proton spectrum. Included are J-coupled experiments (TOCSY1D and zTOCSY1D), through-space experiments (NOESY1D, ROESY1D), as well as a simple selective excitation experiment (selexcit). The zTOCSY1D contains a z-filter to suppress zero-quantum artifacts and will, in general, give better results than TOCSY1D. The stepNOESY1D is a specialized experiment that first performs a TOCSY 1D and then a second selective NOESY1D excitation off one of the TOCSY relayed peaks.

The most direct way to set up these experiments is to first acquire a proton spectrum and then choose the appropriate selective 1D experiment. The proton spectrum can then be used as a reference to select the desired peaks/regions for the experiment. Alternatively, if the regions are known *a priori*, the selective 1D experiment can be chosen initially and the regions entered manually.
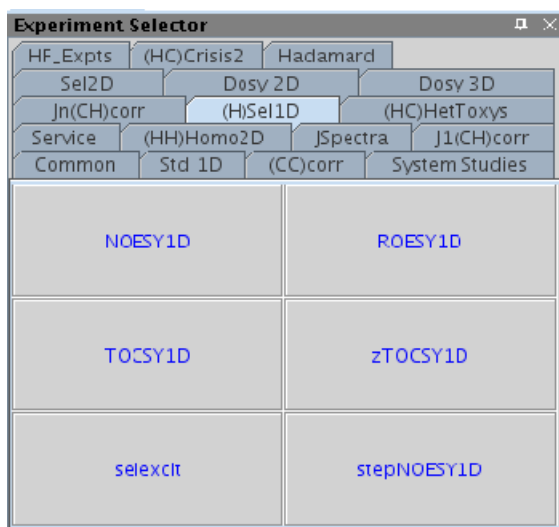
PAGE LEFT BLANK INTENTIONALLY

NOESY1D
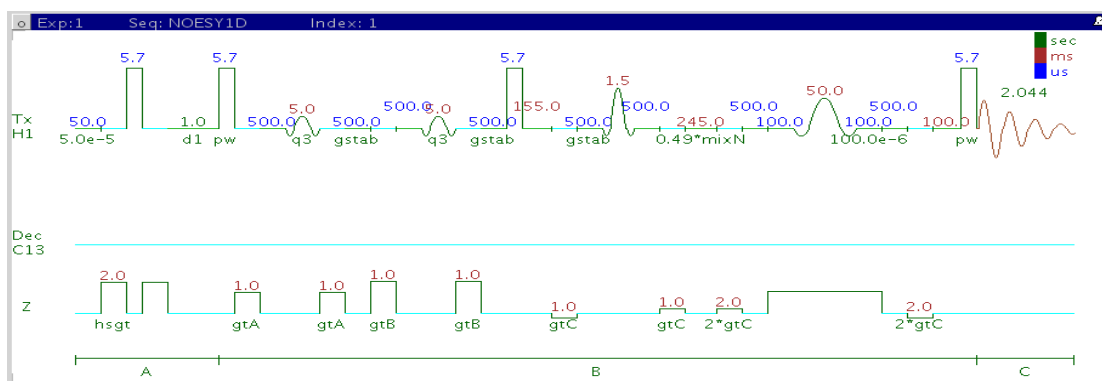
Description:

A pulse sequence to acquire a 1D NOESY dataset with a selective pulse to obtain NOE information from a specific frequency.

Usage:

The NOESY1D experiment is an important tool for structure elucidation as it does not require through-bond coupling connectivity for correlations, rather the NOE (Nuclear Overhauser Effect) interaction is seen between protons that are close in space (typically <5 Å). As such the NOESY1D experiment is often used to help determine stereochemistry for rigid ring systems, or to confirm attachments between proximal (but non-coupled) portions of a molecule. The selective NOESY1D experiment has an advantage over the 2D NOESY version for simplifying complicated spectra with a lot of overlapping peaks. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for NOE analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
NOE Mixing time (mixT):	Select from pull down menu- 500ms works well for most small molecules*
Run a mix=0 spectrum:	Check box to acquire a control spectrum with a mixing time of 0ms- will create an array for mixT
No., ppm, width of selective frequencies:	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes.
ZQ filter:	Check box on the "Pulse Sequence" panel- zero quantum filter for artifact suppression. Strongly recommended- default is on.

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panel

Same processing as for standard PROTON spectrum, except that manual phasing may be required as the NOE peaks may be negative for smaller molecules. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the NOESY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) for NOE analysis, and the "Default" panel will appear differently (see below). Place the cursor(s) on or around the peak and click ▭Select▭. Repeat for additional peaks. Results will be best if isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this will create shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will avoid interference from nearby resonances.

- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time. In addition, the slope of such a build up curve can be used to determine bond distances between selected spin pairs (using methods such as PANIC)

- Molecules with multiple ring systems or a ring system combined with a long carbon chain may require different optimal mixing times for different parts of the molecule. In general, more flexible parts of the molecule will require longer mixing times and more rigid, shorter.

- The NOESY1D contains zero quantum suppression element to suppress artifacts (typically antiphase in nature and interfering with spectral analysis), which is switched on by default.

- The sensitivity of the NOESY1D experiment is not equivalent to a PROTON, nor is an 8-scan NOESY1D equivalent to an 8-scan, 256-increment NOESY. Do not hesitate to acquire this experiment with a larger number of scans, particularly for weak/long-distance NOE's.

- When acquiring the NOESY1D with a larger number of scans, it is advisable to acquire a mixN=0 experiment with the same number of scans (using ▭Run a mix=0 spectrum 8 scans▭), to see peaks that appear from any potential pulse imperfections, so as not to mistake them for NOE correlations. The mixN=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixN≠0 spectrum with no observed NOE.

- Medium-sized molecules (MW ~700-1000 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOE's. One should consider acquiring a ROESY1D for these molecules. Alternatively one may consider (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (this results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii)selecting a lower temperature (if the solvent allows it), or (iii)running the experiment on a spectrometer with higher field strength (if available).

Reference(s):

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

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ROESY1D
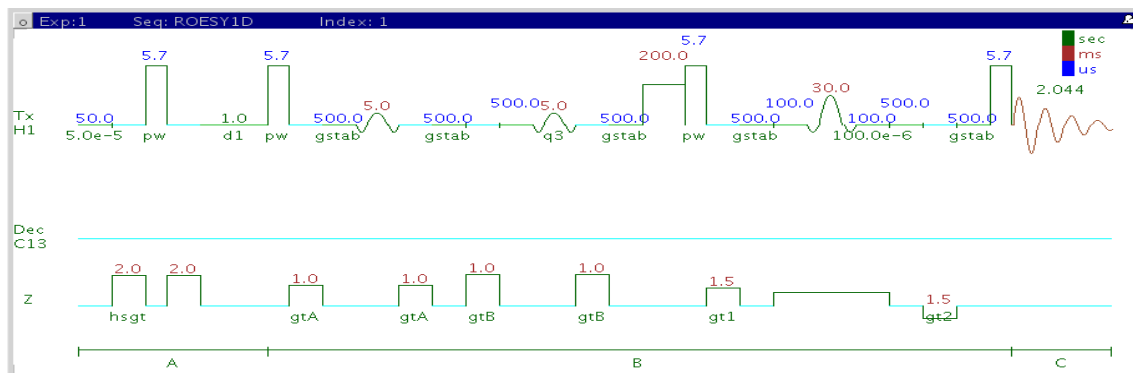
GENERAL DESCRIPTION AND USAGE

Description:

A pulse sequence to acquire a 1D ROESY dataset with a selective pulse to obtain ROE information from a specific frequency.

Usage:

The ROESY1D experiment is an important tool for structure elucidation as it does not require through-bond coupling connectivity for correlations, rather the ROE (rotating frame Overhauser effect) interaction is seen between protons that are close in space (typically <4 Å). As such, the ROESY1D experiment if often used to help determine stereochemistry for rigid ring systems, or to confirm attachments between proximal but non-coupled portions of a molecule. The selective ROESY1D experiment has an advantage over the 2D ROESY version for simplifying complicated spectra with a lot of overlapping peaks. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for ROE analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
SpinLock time:	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Run a mix=0 spectrum:	Check box to acquire a control spectrum with a mixing time of 0ms- will create an array
No., ppm, width of selective frequencies:	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes
ZQ filter:	Check box on the "Pulse Sequence" panel- zero quantum filter for artifact suppression. Strongly recommended- default is on.

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panel.

Same processing as for standard PROTON spectrum, except that manual phasing may be required as the ROE peaks will be negative. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

- Choosing the ROESY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) for ROE analysis, and the "Default" panel will appear differently (see below). Place the cursor(s) on or around the peak and click ⬚ Select ⬚. Repeat for additional peaks. Results will be best if the isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this will create shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will avoid interference from nearby resonances.

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the ROESY1D is limited, weaker correlations may not be observable. The ROESY1D is the experiment of choice over NOESY1D for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running NOESY1D experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (this results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- The ROESY1D contains zero quantum suppression element to suppress artifacts (typically antiphase in nature and interfering with spectral analysis), which is switched on by default.

- The sensitivity of the ROESY1D experiment is <u>not</u> equivalent to a PROTON, nor is an 8-scan ROESY1D equivalent to an 8-scan, 256-increment ROESY. Do not hesitate to acquire this experiment with a larger number of scans, particular for weak/long-distance ROE's.

- When acquiring the ROESY1D with a larger number of scans, it is advisable to acquire a mixR=0 experiment with the same number of scans (using ⬚ Run a mix=0 spectrum 8 scans ⬚), to see peaks that appear from any potential pulse imperfections so as not to mistake them for ROE correlations. The mixR=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixR≠0 spectrum with no observed ROE.

- Sensitivity permitting, the slope of a ROESY1D buildup curve (by an array of mixing time) can be equally used as a NOESY1D buildup curve to determine bond distances between selected spin pairs. (using methods such as PANIC)

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)
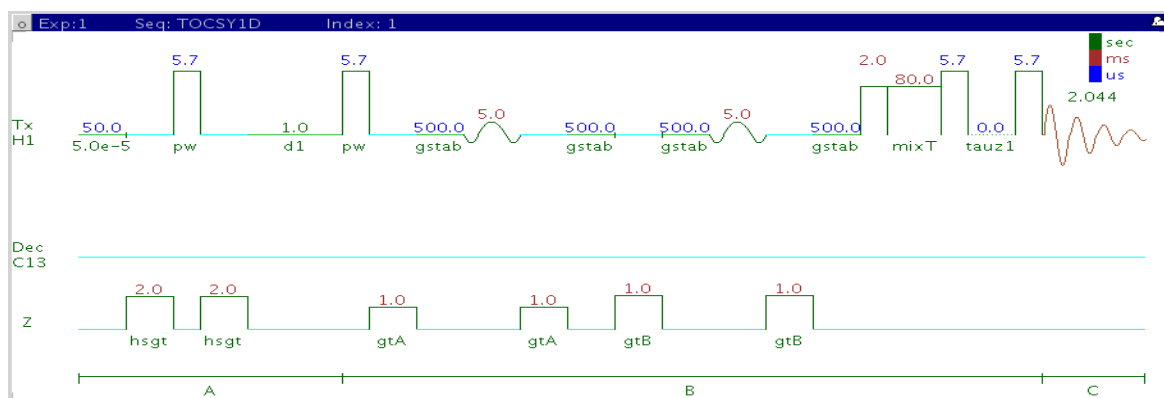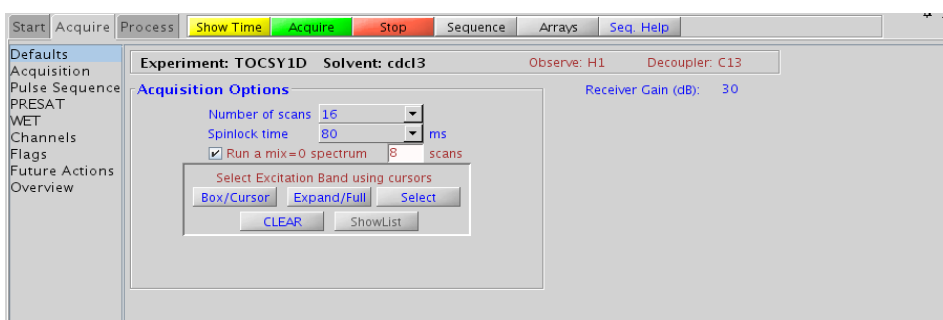
M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

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TOCSY1D

GENERAL DESCRIPTION AND USAGE

Description:

A pulse sequence to acquire a 1D TOCSY dataset with a selective pulse to obtain coupling information from a specific frequency.

Usage:

The TOCSY1D experiment is an important tool for structure elucidation as it can show all protons in a chosen spin system. The selective TOCSY1D experiment has an advantage over the 2D TOCSY version for simplifying complicated spectra with invariably shorter total experiment time. If one proton in a given spin system is resolved, selecting this proton for TOCSY1D analysis will produce an edited 1D spectrum containing only the resonances in this spin system. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for TOCSY1D analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
SpinLock time:	Select from pull down menu- 80ms will work best for large spin systems*
Run a mix=0 spectrum:	Check box to acquire a control spectrum with a mixing time of 0ms- will create an array
No., ppm, width of selective frequencies:	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes
SpinLock Pattern:	Pull-down menu on the "Pulse Sequence" panel- MLEV17 or DIPSI2 works well for small molecules, clean MLEV17 is a good choice for large molecules

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panel.

Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the TOCSY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) for analysis, and the "Default" panel will appear differently (see below). Place the cursor(s) on or around the peak and click [Select]. Repeat for additional peaks. Results will be best if the more isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this will create shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will prevent interference from nearby resonances.

- The zTOCSY1D experiment contains a zero-quantum filter to suppress artifacts and will often give better results than the TOCSY1D.

- The spinlock time ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, e.g. 30ms or less.

- The MLEV17 and DIPSI2 spinlock patterns are good choices for small molecules and the DIPSI3 and clean MLEV for larger molecules.

- The sensitivity of the TOCSY1D experiment is <u>not</u> equivalent to a PROTON, nor is an 8-scan TOCSY1D equivalent to an 8-scan, 256-increment TOCSY. Do not hesitate to acquire this experiment with a larger number of scans, particular for weak couplings or extended spin systems.

- When acquiring the TOCSY1D with a larger number of scans, it is advisable to acquire a mixT=0 experiment, to see peaks that appear from any potential pulse imperfections, so as not to mistake them for TOCSY correlations. The mixT=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixT≠0 spectrum with no observed TOCSY correlation.

- It is also advisable to run a mixT=0 "reference" spectrum (using [✓ Run a mix=0 spectrum 8 scans]), when TOCSY1D is acquired with longer spinlock mixing time. Unlike typical NOESY1D or ROESY1D, the selected resonance (i.e., the originally selected peak) may have intensity similar to the TOCSY peak and hence may not be obviously discernable.
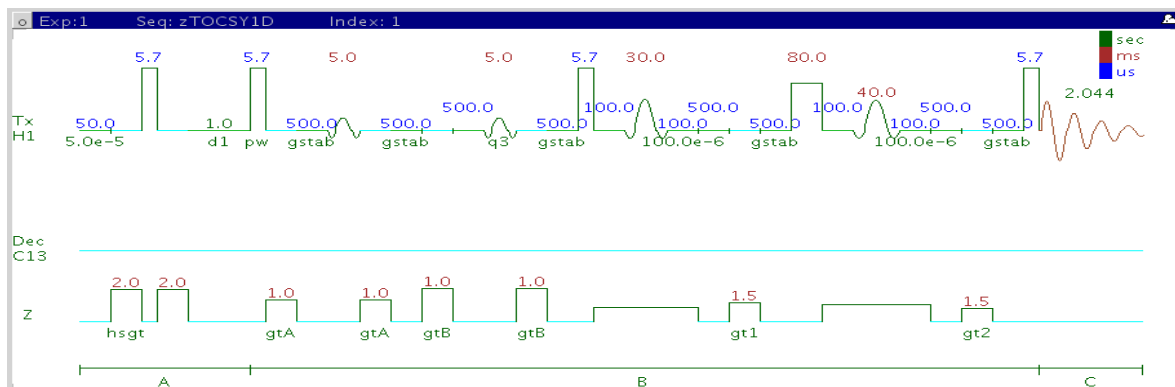
Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)
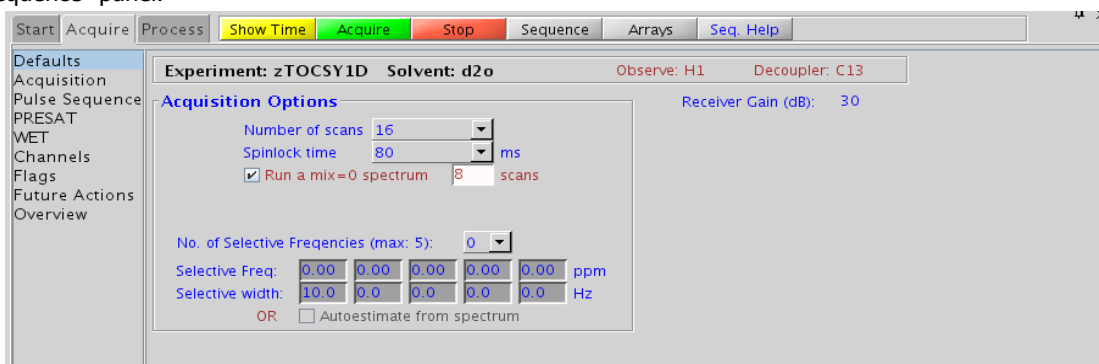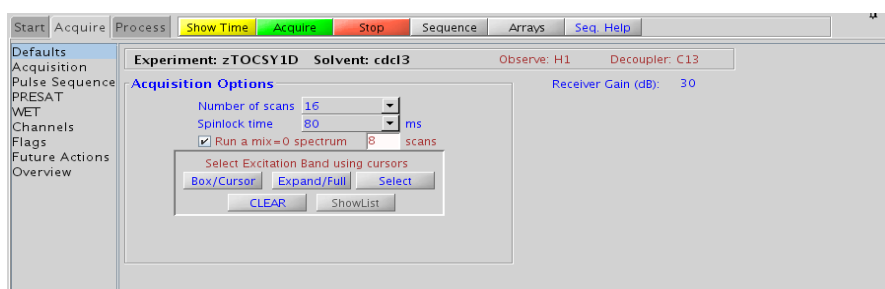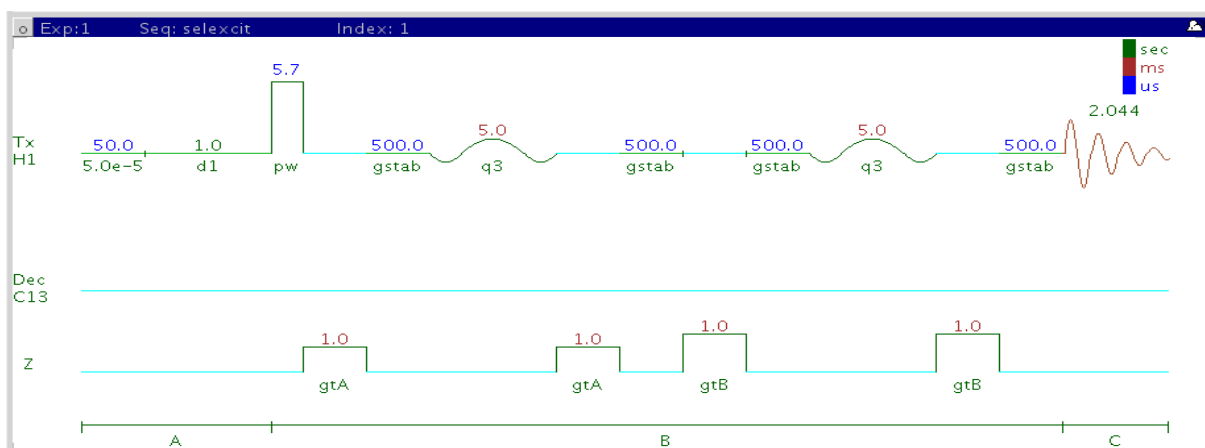
zTOCSY1D

Description:

A pulse sequence to acquire a 1D zTOCSY dataset with a selective pulse to obtain coupling information from a specific frequency.

Usage:

The zTOCSY1D experiment is an important tool for structure elucidation as it can show all protons in a chosen spin system. The selective zTOCSY1D experiment has an advantage over the 2D zTOCSY version for simplifying complicated spectra with a lot of overlapping peaks with invariably shorter total experiment time. If one proton in a given spin system is resolved, selecting this proton for zTOCSY1D analysis will produce an edited 1D spectrum containing only the resonances in this spin system. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for zTOCSY1D analysis may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
SpinLock time:	Select from pull down menu- 80ms will work best for large spin systems*
Run a mix=0 spectrum:	Check box to acquire a control spectrum with a mixing time of 0ms- will create an array
No., ppm, width of selective frequencies:	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes
SpinLock Pattern:	Pull-down menu on the "Pulse Sequence" panel- DIPSI2 works well for small molecules, DIPSI3 is a good choice for large molecules

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panel.

Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the zTOCSY1D experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) for analysis, and the "Default" panel will appear differently (see below). Place the cursor(s) on or around the peak and click ___Select___. Repeat for additional peaks. Results will be best if the more isolated peaks are chosen for correlation analysis. It is advisable not to keep the widths of the selected peak too narrow as it will create shaped pulses with long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will prevent interference from nearby resonances.

- The zTOCSY1D experiment contains a zero-quantum filter to suppress artifacts and will often give better results the than TOCSY1D.

- The spinlock time ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings (such as for a COSY), select a shorter spinlock time, e.g. 30ms or less.

- The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 for larger molecules.

- The sensitivity of the zTOCSY1D experiment is <u>not</u> equivalent to a PROTON, nor is an 8-scan zTOCSY1D equivalent to an 8-scan, 256-increment zTOCSY. Do not hesitate to acquire this experiment with a larger number of scans, particularly for weak couplings or extended spin systems.

- When acquiring the zTOCSY1D with a larger number of scans, it is advisable to acquire a mixT=0 experiment, to see peaks that appear from any potential pulse imperfections, so as not to mistake them for TOCSY correlations. The mixT=0 spectrum can also be used to confirm the selective excitation occurred as expected, verifying a result from the mixT≠0 spectrum with no observed TOCSY correlation.

- It is also advisable to run a mixT=0 "reference" spectrum (using [✓ Run a mix=0 spectrum 8 scans]), when TOCSY1D is acquired with longer spinlock mixing time. Unlike typical NOESY1D or ROESY1D, the selected resonance (i.e., the originally selected peak) may have intensity similar to the TOCSY peak and hence may not be obviously discernable.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

selexcit

Description:

A pulse sequence to acquire a 1D ^{1}H dataset with a selective pulse to excite only a portion of the spectrum.

Usage:

The selexcit experiment is most commonly used to set up band-selective 2D experiments (see Chapter 7). It can be used, however, to edit the proton spectrum and improve the dynamic range, when there may be large amounts of impurities and/or solvents signals. An example might be a sample from a biological matrix such as bile, which may have large signals in the aliphatic region and it may be desirable to selectively excite the aromatic region of the spectrum only. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies and bandwidths for selective excitation are known, and may be chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent:	Set from "Start" tab/"Standard" panel
Spectral Width (sw):	Set from previously acquired proton*
Number of scans:	Select typical choices from pull down menu*
No., ppm, width of selective frequencies:	Select number of frequencies from the drop down menu. Enter frequency position(s) and bandwidth(s) in the entry boxes

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Same processing as for standard PROTON spectrum. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- Choosing the selexcit experiment from a previously acquired PROTON data set allows for interactive selection of the peak(s) for analysis, and the "Default" panel will appear differently (see below). Place the cursor(s) on or around the peak and click [Select]. Repeat for additional peaks. Results will be best if the more isolated peaks are chosen for correlation analysis. It is advisable not to make the widths of the selected peak too narrow as this will create shaped pulses of long durations, which, in turn, can result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will prevent interference from nearby resonances from being selected.
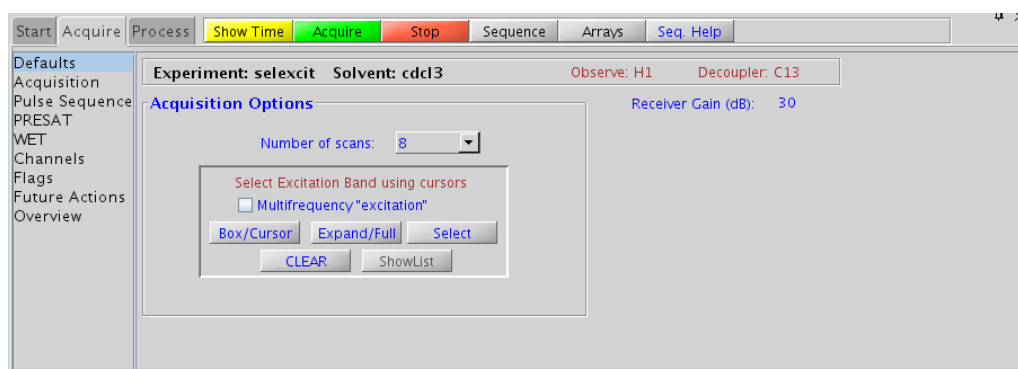
- The selexcit experiment has a check option to do multifrequency excitation. Choosing multiple bands (interactively from the PROTON spectrum) with this check box "ON" results in a single spectrum wherein all of the selected bands are included. However, selection of multiple bands with this check box "OFF" results in multiple spectra each with a single selected band.

- The sensitivity of the selexcit experiment is usually not equivalent to a PROTON, due to some potential loss from the selective pulse.

- Setting up the desired regions from a selexcit experiment is the easiest way to configure a band-selective 2D experiment (see Chapter 7).

Reference(s):

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

stepNOESY1D
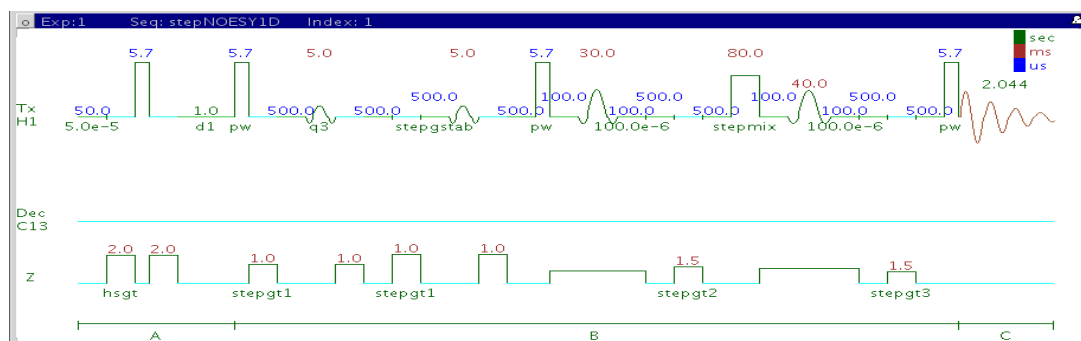
GENERAL DESCRIPTION AND USAGE

Description:

A pulse sequence to acquire a 1D TOCSYNOESY dataset with a selective pulse to obtain TOCSYNOESY information from a specific frequency. It is effectively a 1D equivalent of a 3D experiment.

Usage:

The stepNOESY1D experiment first acquires a TOCSY1D on a selected peak from a PROTON spectrum, and then performs a NOESY1D on one of the TOCSY correlations. This experiment, while somewhat insensitive, can be helpful to simplify and obtain subspectra of impure samples and/or complex spectra when there is one resolved peak from the desired molecule of interest. This is a unique method to obtain NOE information from completely obscured protons in a complex molecule. This experiment is most easily set up from a previously acquired PROTON spectrum so the frequencies for the TOCSY and subsequent NOE analysis are chosen interactively with the cursor. All parameters are set from the "Acquire" tab/"Default" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from "Start" tab/"Standard" panel
Spectral Width(sw):	Set from previously acquired PROTON*
Number of scans(nt):	Select typical choices from pull down menu*
TOCSY STEP ON/OFF:	Check box to turn off TOCSY to set up STEP band (turn off NOE also); turn on for analysis
STEP mixing time:	Select from pull down menu- 80ms will work best for large spin systems*
Set STEP band, STEP peak (ppm), width (Hz):	Select desired TOCSY correlation with cursor type in ppm/width, click "Select and makeshape" button
NOE ON/OFF:	Check box to turn off NOE to set up NOE band; turn on for analysis
NOE mixing time:	Select from pull down menu- 500ms will work best well for most small molecules*
Set NOE band:	Select desired TOCSY correlation with cursors, click "Select and makeshape button"

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panel.

Same processing as for standard PROTON spectrum, except that manual phasing may be required as the NOE peaks may be negative for smaller molecules. A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is line broadening equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel.

TIPS, TRICKS AND NEXT STEPS

- The STEP band for the TOCSY may be selected from a previously acquired PROTON dataset, or a quick stepNOESY1D experiment with the TOCSY STEP and NOE off can be used to acquire a quick sample spectrum. Place the cursor(s) on or around the peak to be selected and click `Select and makeshape`. Alternatively, the frequency (ppm) and width (Hz) for the STEP peak may be entered in the boxes provided and `makeshape` clicked. The NOE band may also be selected from a previously acquired TOCSY1D or zTOCSY1D spectrum. Alternately, a quick stepNOESY1D experiment with the TOCSY STEP on and NOE off can be acquired and used for NOE band selection. Place the cursor(s) on or around the peak to be selected and click `Select and makeshape`. It is advisable to not keep the widths of the selected peaks too narrow, as it will create shaped pulses with long durations, which can, in turn, result in some sensitivity loss. The peak of interest does not necessarily have to be centered in the selection window if placing it off-center will prevent interference from nearby resonances.

- Spinlock (STEP mixing time) durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. A STEP mixing time of 30-80ms is typical for most small molecules, depending on the length of the coupling network and the size of the intervening coupling constants. An 80ms STEP mixing time is a good choice for molecules with extended spin systems.

- The TOCSY spinlock pattern can be selected from the "STEP" tab. The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 is typically used for larger molecules.

- A mixing time for the NOE of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. As in NOESY1D, the NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time. In addition, the slope of such a build up curve can be used to determine bond distances between selected spin pairs (using methods such as PANIC).

- The sensitivity of the stepNOESY1D experiment is quite low, as it is essentially a 1D equivalent of a 3D experiment, which typically takes a long time to acquire. Do not hesitate to acquire this experiment with a larger number of scans. It may also be helpful to optimize the STEP and NOE mixing times with TOCSY1D and NOESY1D experiments prior to acquiring the stepNOESY1D.

Reference(s):

H. Hu, S. Bradley, K. Krishnamurthy, J. Magn. Reson., 171 (2004), 201 (STEPNOESY1D)

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

Chapter 7
Selective 2D - Sel2D

Introduction

The experiments in the "Sel2D tab" are F1 band-selected 2D experiments. Band-selection in the F1 dimension saves time and improves resolution in any 2D experiment by reducing the number of increments needed in that dimension, while allowing the operator to focus on the correlation region of interest. In the heteronuclear experiments this can be especially useful for resolving nearly-degenerate ^{1}H-^{13}C resonances into unambiguous correlations. With homonuclear spectra it can shed light, for example, into a crowded aromatic region of a NOESY. Such high resolution in the traditional (broadband version) experiment could only be obtained at the cost a very high number of increments (and hence spectrometer time) .

The homonuclear experiments include (HH)bsROESY, (HH)bsTOCSY, and (HH)bsNOESY. All of these experiments can be setup either from a PROTON spectrum, a selexcit experiment (selective 1D), or by simple entry of the desired F1 region in the setup panels. All necessary selective pulses are created automatically and the parameters set appropriately. The homonuclear experiments support the option for broadband homonuclear decoupling in the F1 dimension, thereby further improving resolution.

The heteronuclear experiments are proton-carbon by default, with ^{13}C as the band-selected dimension. If the selective 2D experiment is setup from a traditional 2D data set of the same type, e.g. HSQC or HMBC, the operator can choose the band-selected frequencies with cursors from the existing spectra. Alternatively, the desired F1 window can be chosen by entering the desired region into the parameter entries. All required shaped pulses are created automatically.

There are two experiments that only appear in the Sel2D tab, (HC)bsHSQCNOESY and (HC)bsHSQCROESY. Because the F1 window can be customized, the HSQCNOESY/ROESY is available for a wide range of structural problems. The information conveyed is similar to the HetToxy family of experiments, e.g. "proton A has NOE to a proton B, whose carbon is at that chemical shift", but with the potential of added resolution for the carbon shifts.

The (HC)EXSIDE is an experiment that provides a method to measure long-range H/C coupling constants. Typically the (HC)EXSIDE is setup from a selexcit experiment, where the multi-band option is used to select all desired protons, taking care to exclude pairs from the same spin system. The coupling constants are seen as in-phase pairs in the F1 dimension, scaled up by a J-scaling parameter.

(HC)bsHSQCAD

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses and band-selection in F1.
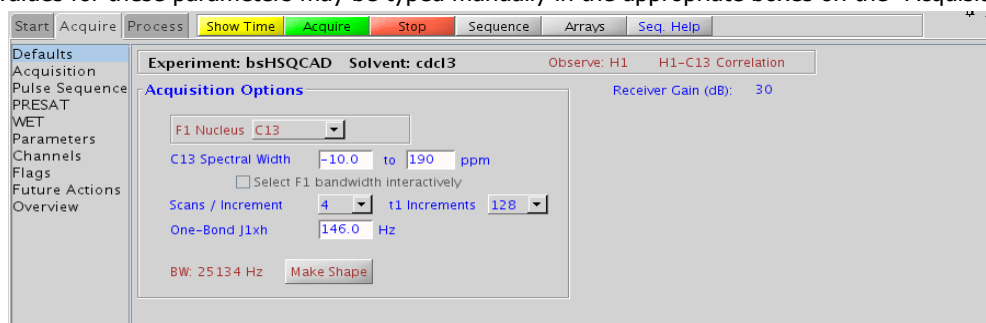
Usage:

The (HC)bsHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons in a band-selected region along F1. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired HSQC-type experiment
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- check box to turn on/off TANGO-Gradient ^{12}C suppression (default is on- highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HC)bsHSQCAD is set up from this experiment. All HSQC-related parameters will transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The (HC)bsHSQCAD experiment has a sensitivity advantage (factor of ~1.4) over the (HC)bsgHSQCAD, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liquart, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

(HC)bsgHSQCAD

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with adiabatic 180º X-nuclei pulses, gradient coherence selection, and band-selection in F1.
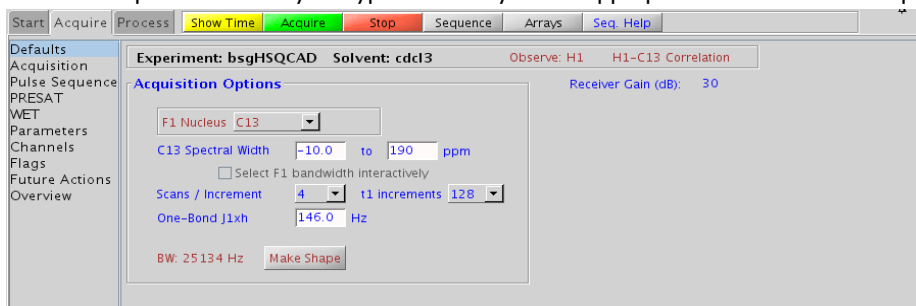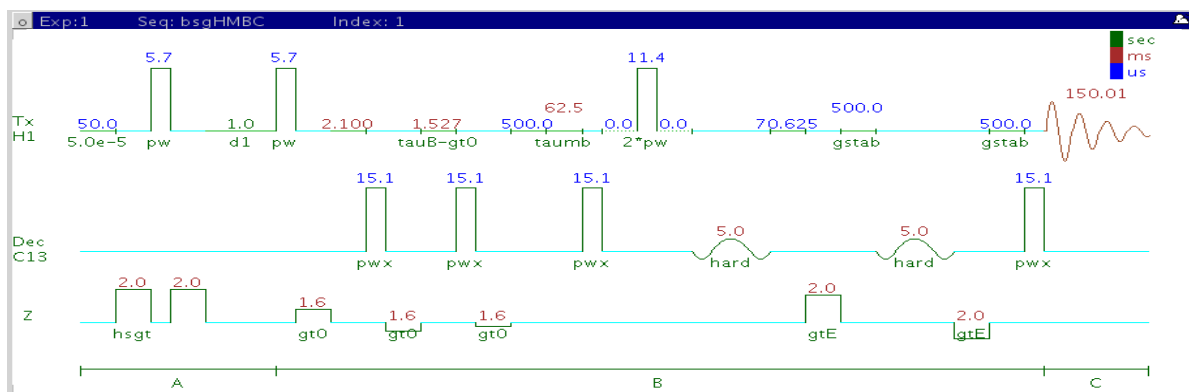
Usage:

The (HC)bsgHSQCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons in a band-selected region along F1. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- check box to turn on/off TANGO-Gradient ^{12}C suppression (default is on- highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HC)bsgHSQCAD is set up from this experiment. All HSQC-related parameters will transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The (HC)bsHSQCAD experiment has a sensitivity advantage (factor of ~1.4) over the (HC)bsgHSQCAD, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liquart, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

(HC)bsgHMBC

GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with gradient coherence selection and band-selection in F1.
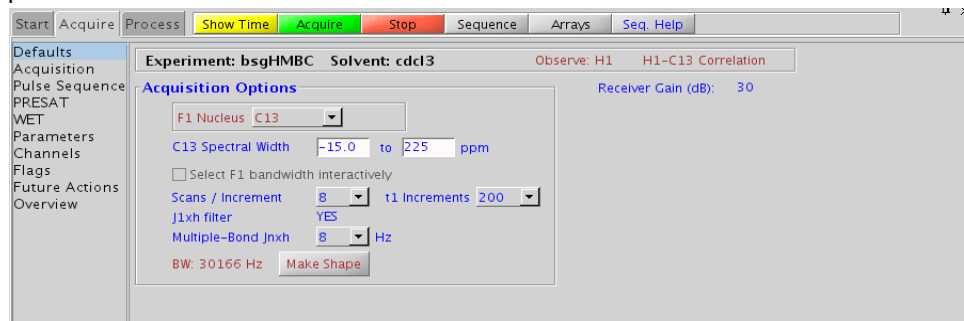
Usage:

The (HC)bsgHMBC experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons in a band-selected region along F1, usually over 2-3 bonds with the 3-bond correlations typically being stronger. Using band-selection is an efficient way to increase the resolution in the F1 carbon dimension without adding large numbers of increments and, hence, experiment time. The selected band can either be typed in or chosen interactively from a previously acquired HSQC- or HMBC-type data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired (CH) correlation experiment
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$ - 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HC)bsgHMBC is set up from this experiment. All HMBC-related parameters will transfer if the experiment is set up from a previously acquired HMBC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity.

Reference(s):

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liquart, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

(HC)EXSIDE
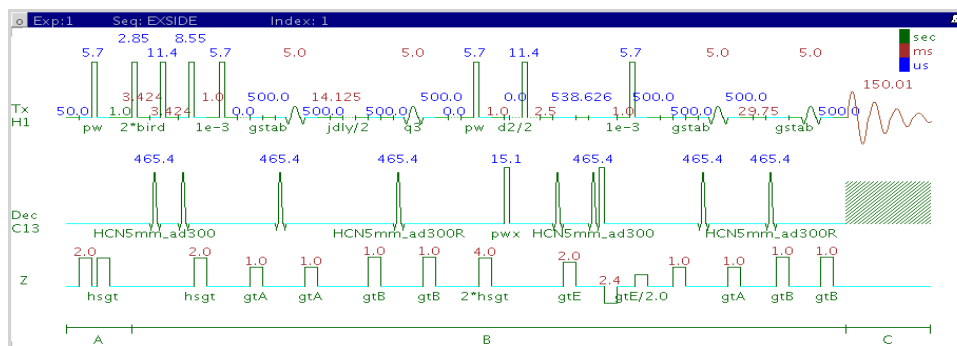
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional band-selective HSQC-type experiment for measuring long-range ^{1}H-^{13}C coupling constants.

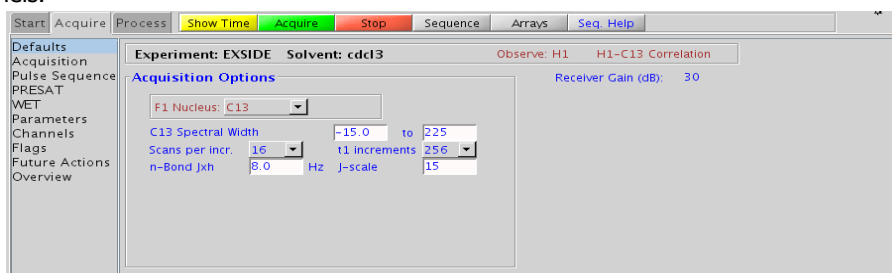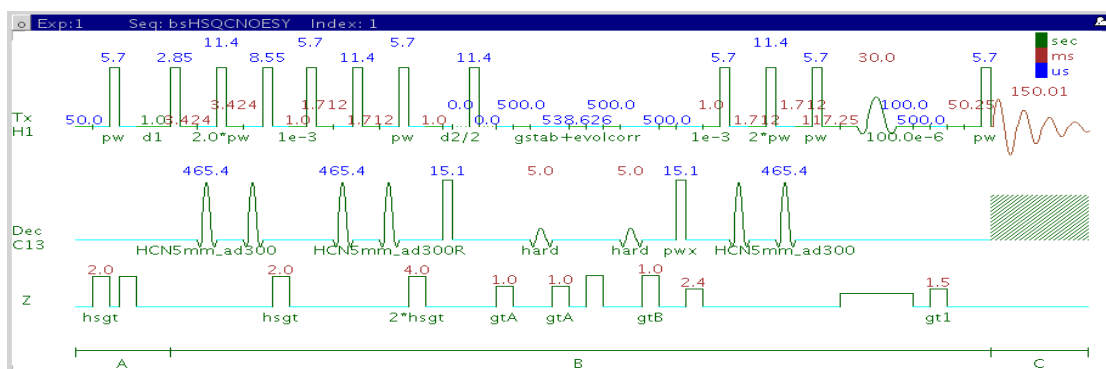**Usage:**

The (HC)EXSIDE experiment is used primarily to obtain information about long-range ^{1}H-^{13}C coupling constants. Setup is from a previously acquired selexcit experiment. The data appears as a 2D 1-bond ^{1}H-^{13}C correlation with proton chemical shifts in F2 and carbon in F1. The long-range ^{1}H-^{13}C coupling constants are measured from in-phase pairs in the F1 dimension scaled up by the j-scaling factor. The specific advantage of (HC)EXSIDE in measuring long-range coupling constants is that (i) they are measured along F1 as doublets with the peak separation scaled by a user definable scaling factor and (ii) only the heteronuclear active coupling is observed without any interference from ^{1}H-^{1}H homonuclear couplings. Proton-carbon coupling constants are often used to help define *e/z* stereochemistry of double bonds or molecular conformations, e.g. the torsion angles of peptides. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired selexcit
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
n-Bond jxh (jnxh):	Pull-down menu to set value for the average ^{n}J$_{X,H}$ - 8 Hz works well for most molecules
J-scale (jscale):	Scaling factor for the coupling constants in F1
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- This experiment is setup from a previously acquired selexcit experiment. The multi-band option should be selected and all desired protons should be chosen, taking care to select only protons which do not share a mutual homonuclear coupling. Ideally a COSY-type experiment should be acquired and should be used to guide selection of resonances (not coupled to each other) for the selexcit. If heteronuclear coupling constants need to be determined from two mutually coupled protons , two EXSIDE experiments must be acquired. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The jnxh parameter sets the optimal value of the proton-carbon coupling constant for detection.

- Proton-carbon long range coupling constants are often used to help define *e/z* stereochemistry of double bonds or molecular conformations, e.g. the torsion angles of peptides.

Reference(s):

V. V. Krishnamurthy, J. Magn. Reson. Ser. A121 (1996) 33-41. (EXSIDE)

(HC)bsHSQCNOESY

Description:

Two dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with the addition of a noesy mixing time Contains adiabatic 180° X-nuclei pulses and band-selection in F1.

Usage:

The (HC)bsHSQCNOESY experiment is used to acquire a 2-dimensional spectrum to collect information correlating NOE and carbon chemical shift information, e.g. Proton A has an NOE to proton B at carbon chemical shift X. The band-selection along the F1 dimension gives the (HC)bsHSQCNOESY an advantage of better resolution than a non-selective HSQCNOESY. The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type spectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
NOE Mixing time (mixN):	Select from pull down menu- 500ms works well as a starting point*
Steady State (ss):	Set from second "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HC)bsHSQCNOESY is set up from this experiment. All HSQC-related parameters will transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.

- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOE's. One should consider acquiring a (HC)bsHSQCROESY for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and will have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

- (HC)bsHSQCNOESY spectra may sometimes contain signals with partial antiphase character. Zero quantum suppression is embedded (and switched on by default) into the (HC)bsHSQCNOESY pulse sequence to suppress such artifacts.

Reference(s):

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

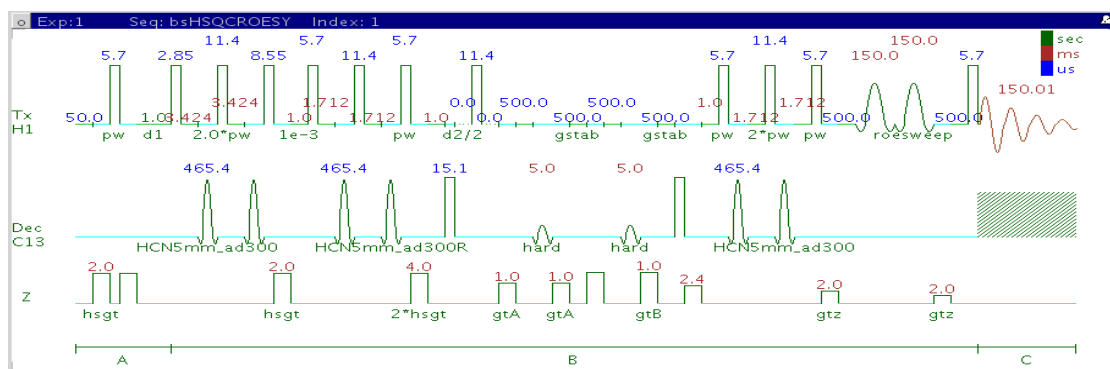E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liquart, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

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(HC)bsHSQCROESY

GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with the addition of a roesy mixing time Contains adiabatic 180º X-nuclei pulses and band-selection in F1.

Usage:

The (HC)bsHSQCROESY experiment is used to acquire a 2-dimensional spectrum to collect information correlating NOE and carbon chemical shift information, e.g. Proton A has an ROE to proton B at carbon chemical shift X. The band-selection along F1 dimension gives the (HC)bsHSQCROESY an advantage of a better resolution than a non-selective HSQCROESY. . The selected band can either be typed in or chosen interactively from a previously acquired HSQC-type spectrum. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension or check box to select interactively from previously acquired gHSQCAD
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration:	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
Steady State (ss):	Set from second "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HC)bsHSQCROESY is set up from this experiment. All HSQC-related parameters will transfer if the experiment is set up from a previously acquired HSQC-type experiment and the F1 band may be selected interactively. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the (HC)bsHSQCROESY is limited, weaker correlations may not be observable. (HC)bsHSQCROESY is the experiment of choice over (HC)bsHSQCNOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running (HC)bsHSQCNOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Crosspeaks in the (HC)bsHSQCROESY spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

Reference(s):
A. Bax, D. G. Davies, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

C. Gaillet, C. Liquart, P. Deberie, J. M. Nuzillard, J. Magn. Reson. 139 (1999) 454-459. (Band Selective HSQC/HMBC)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

(HH)bsROESY
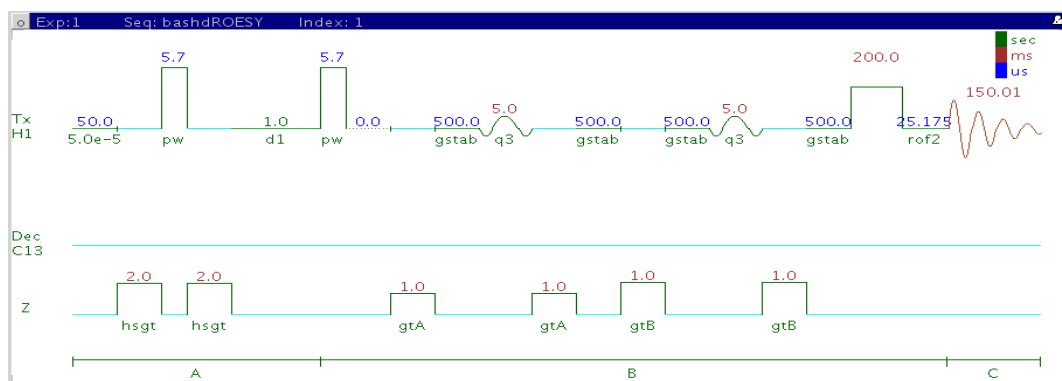
GENERAL DESCRIPTION AND USAGE

Description:

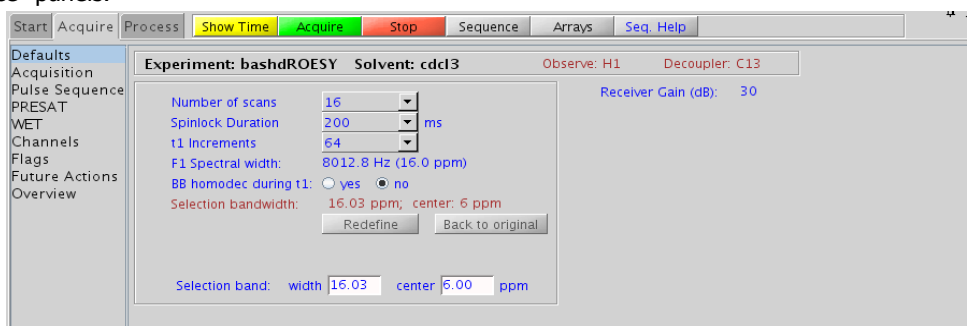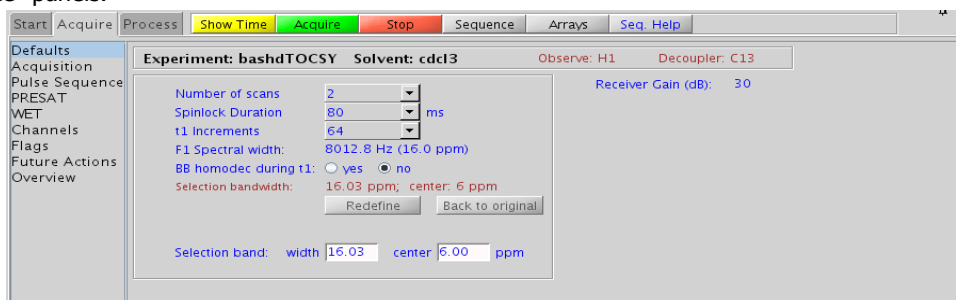Two Dimensional through-space correlation spectroscopy in the rotating frame with band-selection in F1.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <4 Å). This experiment has an added feature above the ROESY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large number of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsROESY can be very useful to obtain high-resolution through-space correlations to an overlapped area of the spectrum, for example the α-proton region of peptides. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Relaxation time (d1):	Set from "Acquisition" panel, controls the delay between scans
Number of scans (nt):	Select typical choices from pull down menu*
Spinlock Duration:	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec):	Select yes/no for homonuclear decoupling in F1
Selection bandwidth:	Set from previously acquired selexcit or type in specific values
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the ROE peaks will be negative. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HH)bsROESY is set up from this experiment. The F1 band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings will transfer from a selexcit if the (HH)bsROESY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The homonuclear decoupling option (homodec) along F1 necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the (HH)bsROESY is limited, weaker correlations may not be observable. (HH)bsROESY is the experiment of choice over (HH)bsNOESY for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running (HH)bsNOESY experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enchancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Crosspeaks in the (HH)bsROESY spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc 114 (1992) 3157-3159. (EASY-ROESY)

A. Kaerner, D. L. Rabenstein, Magn. Reson. Chem. 36 (1998) 601-607. (Band Selective ROESY)

(HH)bsTOCSY
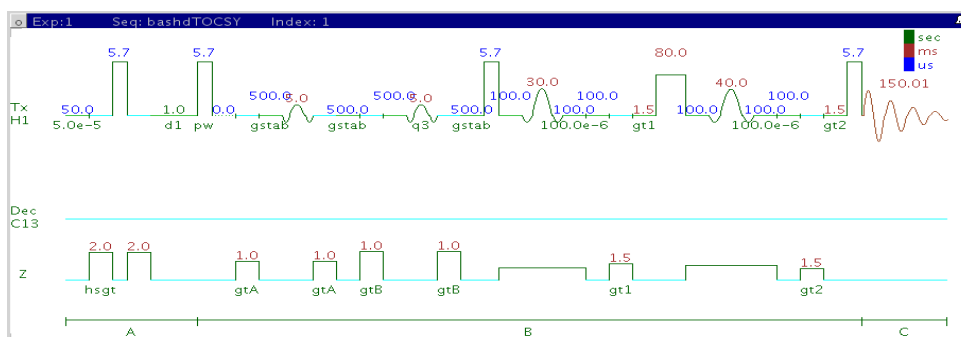
Description:

Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with a zero-quantum filter for artifact suppression and band-selection in F1.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and 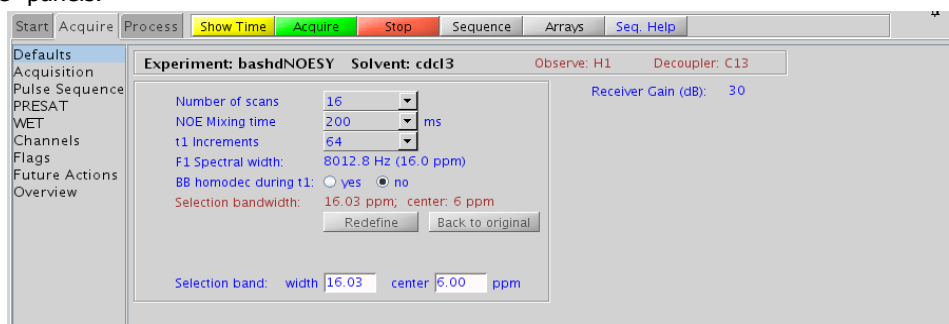crosspeaks between several protons in a scalar coupled spin system. This experiment has an added feature above the zTOCSY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large numbers of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsTOCSY can be very useful to obtain high-resolution through-bond correlations to a overlapped area of the spectrum, for example the α-proton region of peptides.. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Relaxation time (d1):	Set from "Acquisition" panel, controls the delay between scans
Number of scans (nt):	Select typical choices from pull down menu*
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec):	Select yes/no for homonuclear decoupling in F1
Selection bandwidth:	Set from previously acquired selexcit or type in specific values
Spinlock Pattern:	Pull-down menu on the "Pulse Sequence" panel- dipsi2 or dipsi3
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the (HH)bsTOCSY is set up from this experiment. The F1 band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings will transfer from a selexcit if the (HH)bsTOCSY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The homonuclear decoupling option (homodec) along F1 necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The DIPSI2 spinlock pattern is a good choice for small molecules and the DIPSI3 is typically used for larger molecules

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The (HH)bsTOCSY experiment contains a zero-quantum filter, and usually results in cleaner spectra. The zero-quantum suppression filter is switched on by default.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

J. Cavanaugh, M. Rance, J. Magn. Reson. 96 (1992) 670-678. (TOCSY with DIPSI2)

V. V. Krishnamurthy, Magn. Reson. Chem. 35 (1997) 9-12. (Band Selective TOCSY)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

(HH)bsNOESY
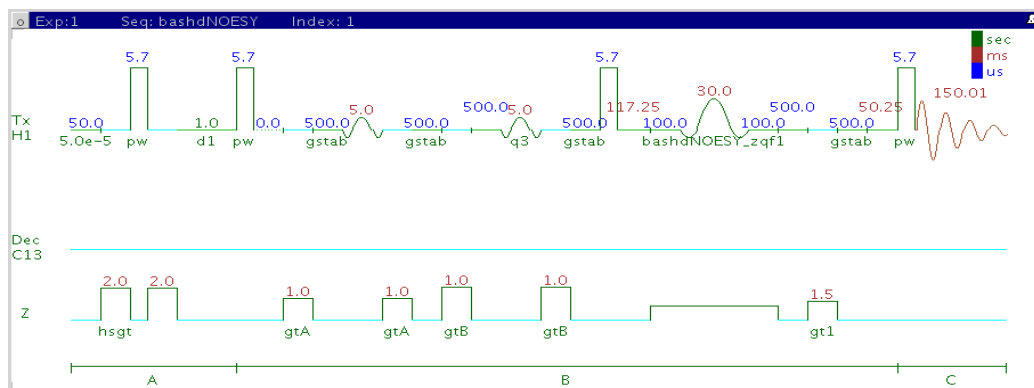
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional through-space correlation spectroscopy with band-selection in F1.

Usage:

This experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <4 Å). This experiment has an added feature above the NOESY of band-selection in F1, which is an efficient way to increase the resolution in the F1 dimension without adding a large number of increments and, hence, experiment time. There is an added experimental option for homonuclear decoupling in F1 to further improve the resolution. The (HH)bsNOESY can be very useful to obtain high-resolution through-space correlations to a overlapped area of the spectrum, for example the α-proton region of peptides. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
Relaxation time (d1):	Set from "Acquisition" panel, controls the delay between scans
Number of scans (nt):	Select typical choices from pull down menu*
NOE Mixing time (mixN):	Select from pull down menu- 500ms works well as a starting point*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
BB homodec during t1 (homodec):	Select yes/no for homonuclear decoupling in F1
Selection bandwidth:	Set from previously acquired selexcit or type in specific values
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the NOE peaks will be negative for smaller molecules. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

- Spectral width (sw) setting will transfer from a PROTON if the (HH)bsNOESY is set up from this experiment. The F1 band is then selected interactively. Optimized selected bandwidth, sw, and receiver gain settings will transfer from a selexcit if the (HH)bsNOESY is set up from this experiment.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.
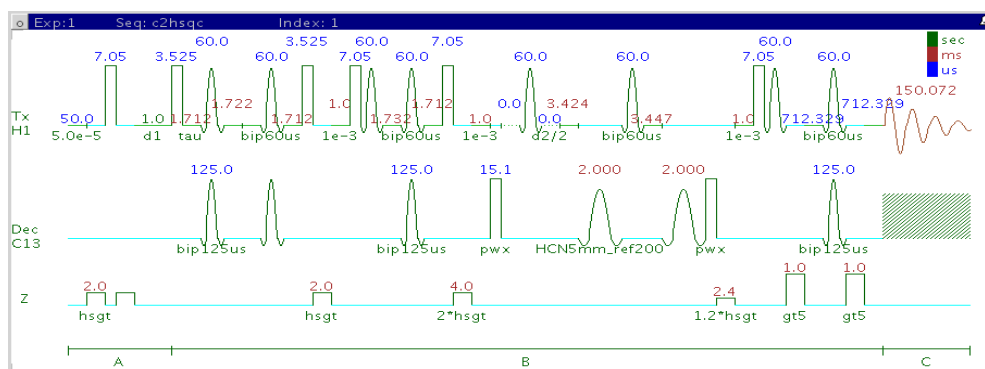
- The homonuclear decoupling option (homodec) along F1 necessitates that the selected resonances do not have mutual homonuclear coupling among themselves.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.

- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOE's. One should consider acquiring a (HH)bsROESY for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and will have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

- (HH)bsNOESY spectra may sometimes contain signals with partial antiphase character. Zero quantum suppression is embedded (and switched on by default) into the bsNOESY pulse sequence to suppress such artifacts.

Reference(s):

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

K. Stott, J. Stonehouse, J. Keeler, T. L. Hwang, A. J. Shaka, J. Am. Chem. Soc. 117 (1995) 4199-4200. (Excitation Sculpting)

M. J. Trippleton, J. Keeler, Angew. Chem. Int. Ed. 42 (2003) 3938-3941. (Zero Quantum Suppression)

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Chapter 8
Heteronuclear CRISIS2-(HC)Crisis2

Introduction

The (HC)Crisis2 experiments are a suite of commonly-used heteronuclear 2D experiments that differ by having bip (broadband inversion pulse) pulses or adiabatic pulses in both the ^{13}C and ^1H channels. For all the experiments in this tab, one can use either bip or adiabatic inversion pulses in one or both channels by the appropriate selection of the bipflg parameter. The bipflg parameter value typically has two characters. The first character represents channel 1 (1H) and the 2nd character represents channel 2 (13C). A value of "y" uses bip inversion pulse while a value of "n" uses adiabatic inversion pulses. The advantage of adiabatic pulses is that they are tunable to any specific bandwidth using Pbox utility while the bip pulse shapes are supplied for a given bandwidth and duration, Setup and use of all of these experiments is exactly the same as the corresponding experiments found in the J1(CH)corr (Chapter 4) and the Jn(CH)corr (Chapter 5) tabs. These experiments can be particularly useful when acquiring a collection of samples in automation that may adversely and variably affect the probe tuning/calibrations because of variations in salt concentrations, different solvents, etc. The bip pulses can compensate for imperfect calibrations, resulting in good data quality under these difficult conditions. Crisis2 experiments, because of their broadband inversion pulses on both channels, are also the best choice for 19F-X correlation.
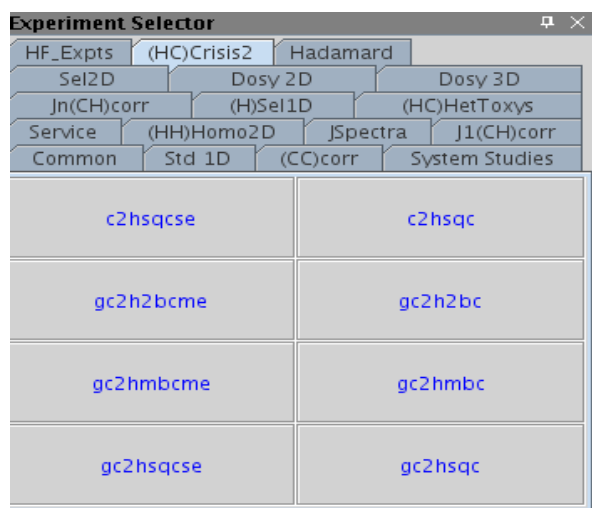
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c2hsqcse
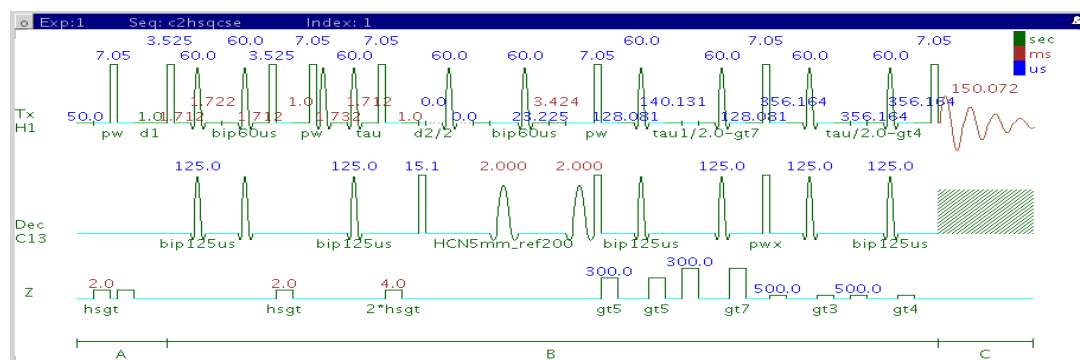
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and sensitivity enhancement.
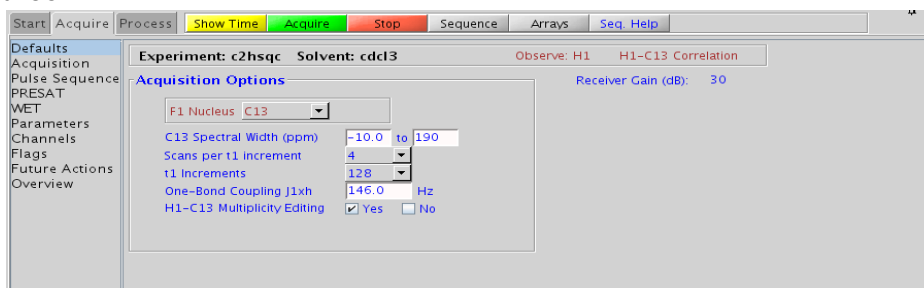
Usage:

The c2hsqcse experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the c2hsqcse is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^1H-^{13}C (or ^1H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- The c2hsqcse experiment has comparable sensitivity to the gc2hsqcse, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- The c2hsqcse uses adiabatic or bip 180º carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

- The c2hsqcse has a sensitivity enhancement feature which, in theory, can result in an increase of approximately a factor of 1-1.4 in sensitivity over c2hsqc.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (gHSQCAD / CRISIS-gHSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

c2hsqc

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses.

Usage:

The c2hsqc experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^{1}J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.
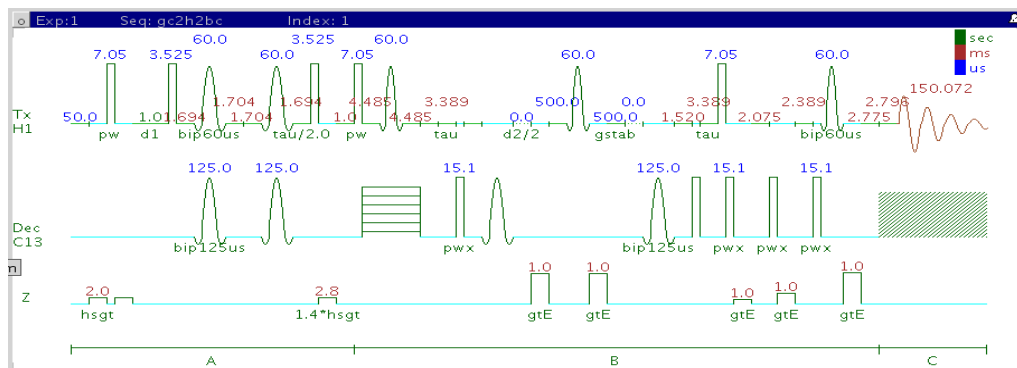
TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the c2hsqc is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- The c2hsqc experiment has a sensitivity advantage (factor of ~1.4) over the gc2hsqc, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- The c2hsqc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (gHSQCAD / CRISIS-gHSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2h2bcme
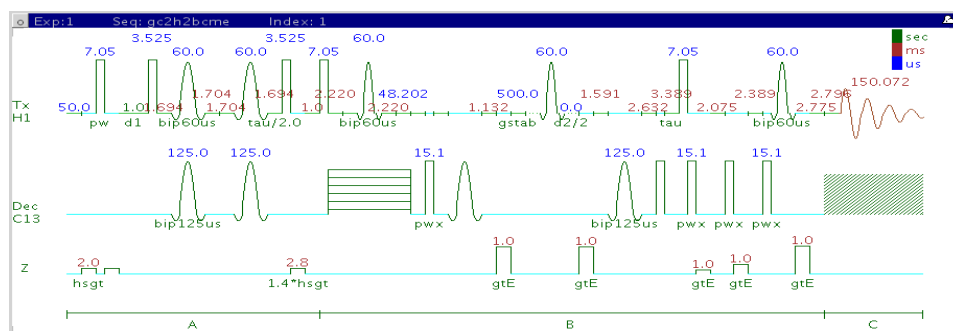
Description:

Two Dimensional heteronuclear 2-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, gradient coherence selection, and multiplicity editing.
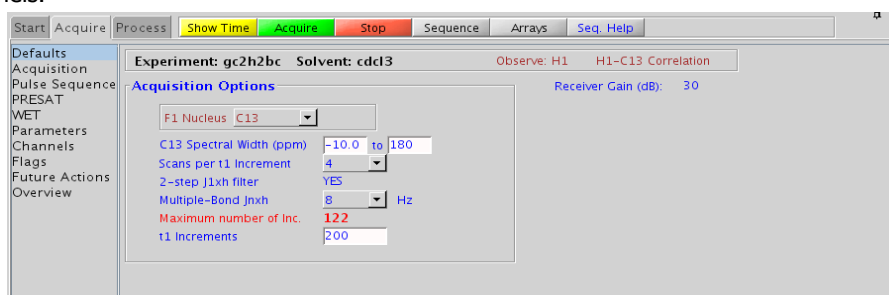
Usage:

The gc2h2bcme experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over 2 bonds. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. Although the gc2h2bcme is less sensitive than the gc2hmbcme, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^nJ_{X,H}$ - 8 Hz works well for most molecules
Constant Time (BigT):	Type in value on the "Pulse Sequence" panel for constant time delay- 0.022ms (default) works for most samples
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. The "Process" tab/"Default" panel has three buttons under the "Transform" heading to select the desired subspectrum. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2h2bcme is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gc2h2bcme is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.

- The gc2h2bcme experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gc2hmbcme experiment. As such, the gc2h2bcme is best used as supplemental information to the gc2hmbcme data to distinguish between 2- and 3-bond correlations.

- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constants and carbon chemical shifts. Carbons with coupling constants that deviate significantly from this relationship will result in imperfect editing.

- As the gc2h2bcme is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.

- The gc2h2bcme uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection

Reference(s):
A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743. (Multiplicity Edited HMBC)

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

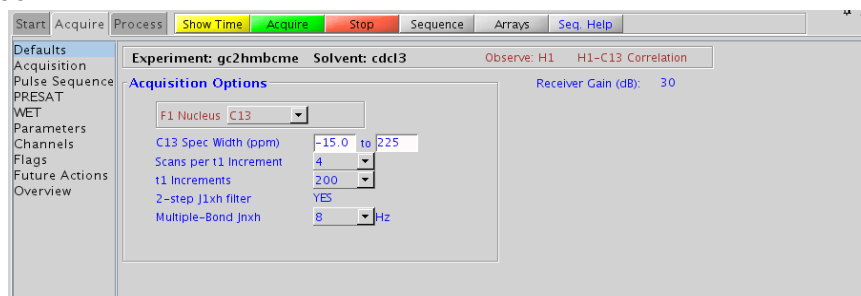# gc2h2bc

GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear 2-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.

Usage:

The gH2BCAD experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons over 2 bonds. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. Although the gc2h2bcme is less sensitive than the gc2hmbcme, this experiment is useful as supplemental information to distinguish between 2- and 3-bond correlations. Note that the experiment is constant time in F1, so the constant time delay (BigT) determines the maximum number of increments (ni). All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Type in value- controls the resolution in F1 (note maximum allowed from the constant time delay (BigT)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{X,H}$ - 8 Hz works well for most molecules
Constant Time (BigT):	Type in value on the "Pulse Sequence" panel for constant time delay- 0.022ms (default) works for most samples
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- turn on/off TANGO-gradient suppression- default is on

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2h2bc is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gc2h2bc is in principle a gHMQC-COSY type experiment. By this very nature, it can only show 2-bond correlations to protonated carbons but not for quaternary carbons. A 22ms bigT delay is an optimal value for COSY magnetization transfer.

- The gc2h2bc experiment is specialized to show only 2-bond correlations, but it is less sensitive than the gc2hmbc experiment. As such, the gc2h2bc is best used as supplemental information to the gc2hmbc data to distinguish between 2- and 3-bond correlations.

- The gc2h2bc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

Reference(s):

N. T. Nyberg, J. O. Duus, O. W. Sorensen, J. Am. Chem. Soc. 127 (2007) 6154-6155. (H2BC)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hmbcme
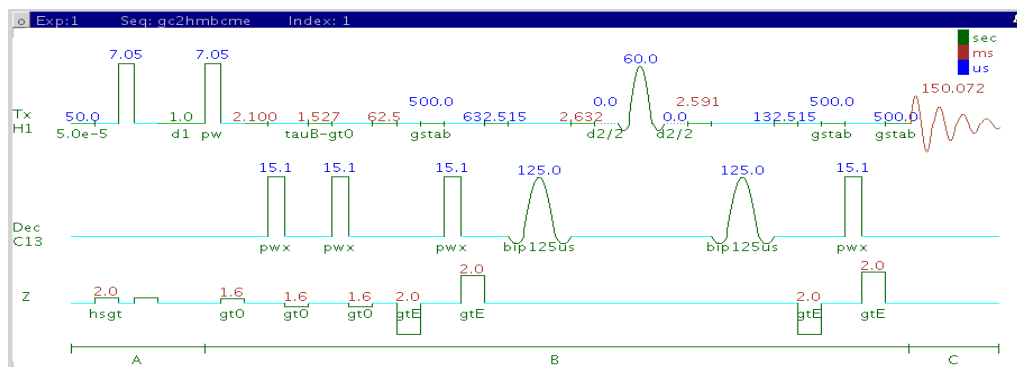
Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, gradient coherence selection, and multiplicity editing.
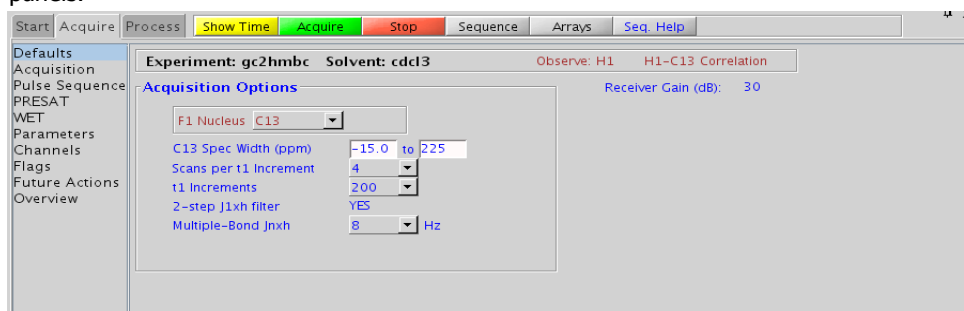
Usage:

The gc2hmbcme experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be reliably predicted with software. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^nJ_{X,H}$ - 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

This is an edited experiment acquired as an array of 2 spectra and needs to be processed in two different ways to generate the edited 2D spectra. The "Process" tab/"Default" panel has three buttons under the "Transform" heading to select the desired subspectrum. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2hmbcme is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The multiplicity-editing feature uses the CRISIS technique that relies on a hypothetical relationship between one-bond coupling constants and carbon chemical shifts. Carbons with coupling constants that deviate significantly from this relationship will result in imperfect editing.

- As the gHMBCmeAD is acquired as an array of two spectra, it takes twice as long to acquire and there is a small sensitivity loss with the multiplicity-editing.

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The gc2hmbcme uses adiabatic or bip 180º carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

Reference(s):

A. J. Benie, O. W. Sorensen, Magn. Reson. Chem. 44 (2006) 739-743. (Multiplicity Edited HMBC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)
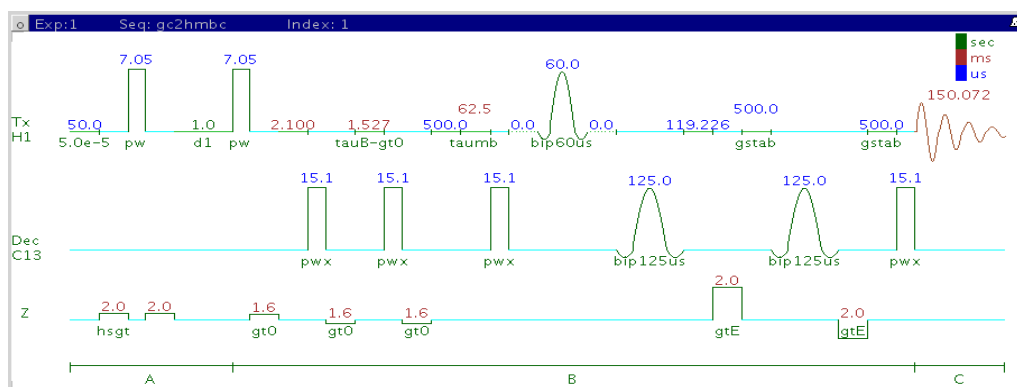
gc2hmbc

Description:

Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.

Usage:

The gc2hmbc experiment is used to acquire a 2-dimensional spectrum to correlate protons to carbons, usually over 2-3 bonds with the 3-bond correlations typically being stronger The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is highly useful for structure elucidation as the longer range correlations allow connectivities between isolated proton spin systems to be identified. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be reliably predicted with software. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
Multiple-Bond Jnxh (jnxh):	Pull-down menu to set value for the average $^{n}J_{x,H}$ - 8 Hz works well for most molecules
One-Bond suppression:	Set from "Pulse Sequence" panel- turn on/off suppression of one-bond correlations- default is on
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussian in F1 and sqsinebell in F2); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination.
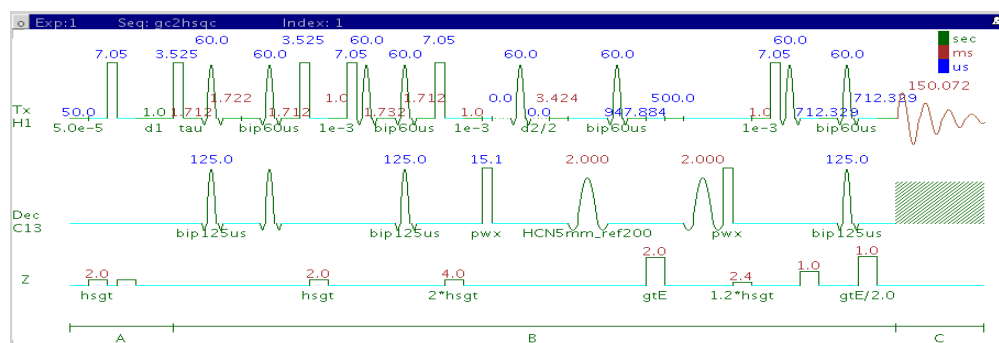
TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2hmbc is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥ 30), unless the sample is extremely concentrated or ^{13}C-labelled.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- In general, the HMBC-type experiments have the best sensitivity of the Jn(CH)corr experiments- the other experiments provide specialized data for specific information types.

- The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity loss. The CIGARAD experiment is better suited on a reasonably concentrated sample for detecting longer range correlations from a range of coupling constants, but at the expense of sensitivity.

- The experiment features a 2-step low-pass filter that can eliminate undesired crosspeaks from one-bond couplings. Select two coupling constants on the "Pulse Sequence" panel around which one-bond couplings should be eliminated (default values are 165 and 130 Hz). These filters are rather narrow-band, however, and if the spread of coupling constants is such that some couplings lie considerably outside these values (for example 115 or 145 Hz), its signals may still be visible in the spectrum. As HMBC-type spectra are recorded without ^{13}C decoupling, such signals can usually still be discerned from long-range crosspeaks by their one-bond splitting.

- The gc2hmbcme uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

Reference(s):

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1996) 2093-2094 (HMBC)

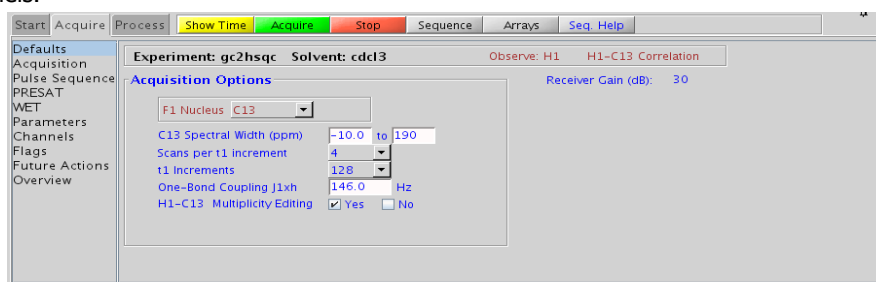H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hsqcse
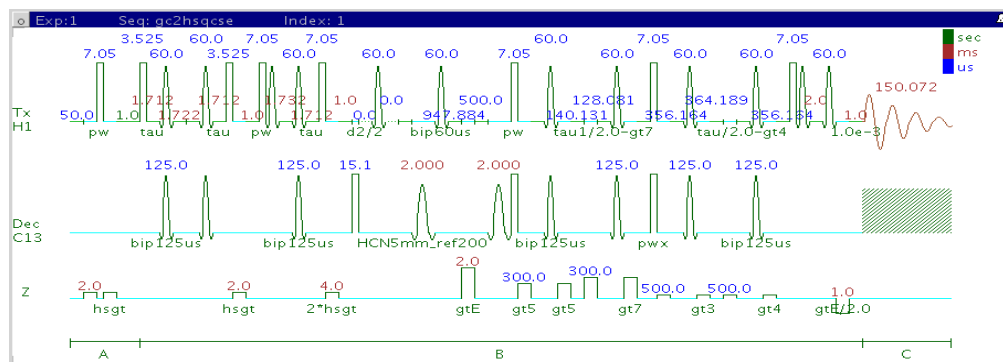
Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses, sensitivity enhancement, and gradient coherence selection.

Usage:

The gc2hsqcse experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2hsqcse is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- The c2hsqcse experiment has a comparable sensitivity to the gc2hsqcse, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- The gc2hsqcse uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

- The gc2hsqcse has a sensitivity enhancement feature which, in theory, can result in an increase of approximately a factor of 0.7-1.4 over c2hsqc and a factor of 1 to 2 over gc2hsqc.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

gc2hsqc

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with bip and/or adiabatic 180° pulses and gradient coherence selection.
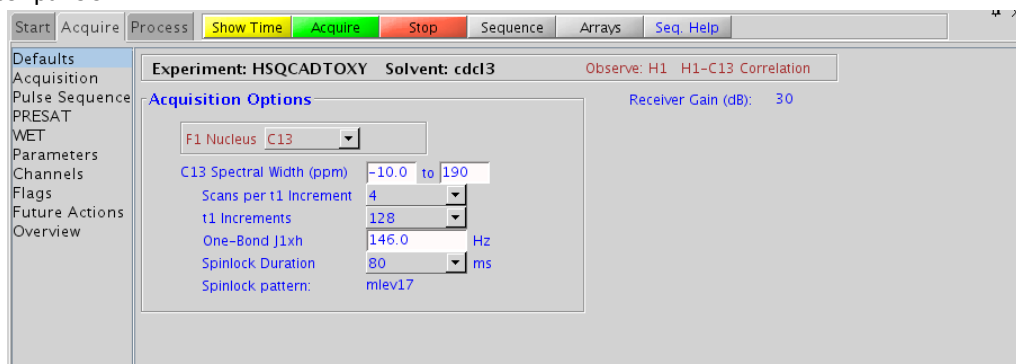
Usage:

The gc2hsqc experiment is used to acquire a 2-dimensional spectrum to correlate protons with directly attached carbons. The CRISIS2 (c2) version of this experiment uses either bip or adiabatic 180° pulses in both channels. The experiment is very useful for proton and carbon assignments, because if the proton assignment is known, the carbon assignment can be deduced directly from this data and *vice versa*. For structure elucidation, carbon chemical shifts are particularly useful as they are highly sensitive to local functional groups and can be more reliably predicted. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
H1-C13 Multiplicity Editing:	Check box to turn on/off multiplicity editing
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
C12-H1 suppression:	Set from "Pulse Sequence" panel- controls type of ^{12}C suppression- default is TANGO-Gradient (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gc2hsqc is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Using the multiplicity editing feature will result in a spectrum with the peaks from methylene-type carbons having the opposite phase to methyl and methine-type carbons. The experiment uses the CRISIS technique to minimize sensitivity loss due to unmatched coupling constants and echo time. CRISIS based multiplicity editing is on by default.

- The c2hsqc experiment has a sensitivity advantage (factor of ~1.4) over the gc2hsqc, but due to t1 noise from strong signals such as t-butyl, may produce potentially less clean spectra than its gradient counterpart.

- The gc2hsqc uses adiabatic or bip 180° carbon and proton pulses for much improved uniform inversion as well as for tolerance to RF inhomogeneity and/or pulse calibration imperfection.

- Datasets with moderate S/N are better processed with 2*ni linear prediction instead of the default (4*ni). One should remember to reset the window function along F1 axis if linear prediction parameters are changed.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

A. G. Palmer III, J. Cavanagh, P. E. Wright, M. Rance, J. Magn. Reson. 93 (1991) 151-170. (HSQC)

H. Hu, K. Krishnamurthy, Magn. Reson. Chem. 46 (2008) 683-689. (CRISIS2 with Multiplicity Editing)

Chapter 9
Hetero-TOCSY – (HC)HetToxys

Introduction

The (HC)HetToxys family of experiments can be used to resolve ambiguous assignments and/or overlap issues that are not resolved after the analysis of a more common set of 2D experiments such as a zTOCSY and a gHSQCAD. These experiments provide two pieces of information; first, typically a correlation is generated that is the same as in the gHSQCAD, e.g. "This proton is attached to that carbon". Second, a series of correlations are generated that are redundant in both F2, the proton dimension, and in F1, the carbon dimension. The information conveyed is "The proton at this chemical shift is coupled to a proton whose carbon is at that chemical shift".

The most sensitive experiment in the family is the HSQCADTOXY, which, along with the gHSQCADTOXY, has adiabatic 180° carbon pulses. The gradient enhanced version of the experiment does experience better artifact suppression, but at the expense of a loss of sensitivity (factor of ~1.4). The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version, with the HSQCADTOXY as the recommended choice. The non-adiabatic experiments in the HetToxy family, however, provide an option to invert or suppress only the responses directly attributed to the H-C pair (HSQC peaks), which can be advantageous for cases of severe overlap in the ^{1}H dimension.

The (HC)HetToxy family of experiments are very powerful for helping to solve structural problems with many classes of chemical substances wherever there is resonance overlap in the proton spectrum. In compounds such as peptides it is often useful to acquire HSQCADTOXY data for both H-C and H-N. Provided the ^{15}N chemical shifts of each amide are resolved, it is possible to effectively separate the complete ^{1}H NMR spectrum for each amino acid in the F1 (^{15}N)

dimension. Subsequent consultation of the H-C HSQCADTOXY spectrum can make it straightforward to verify all assignments for the carbons as well.
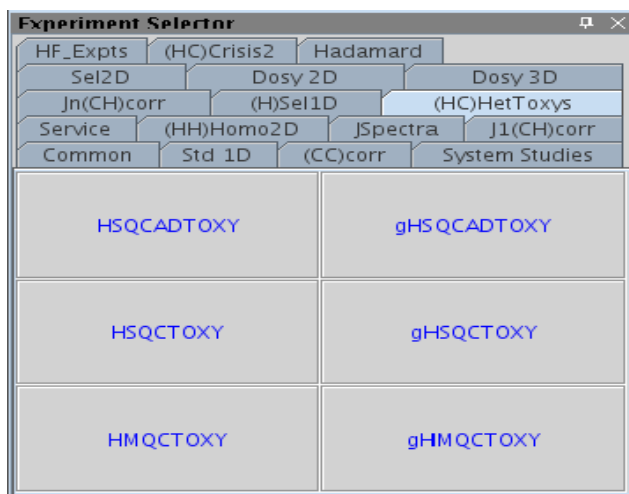
HSQCADTOXY
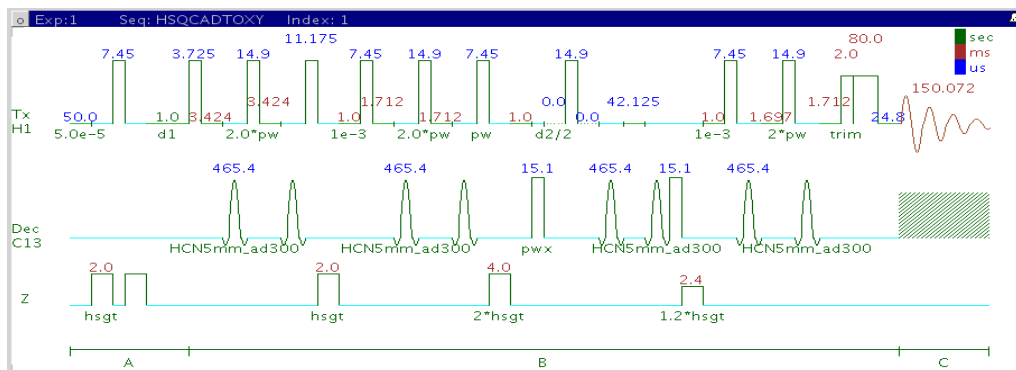
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and adiabatic 180º X-nuclei pulses.

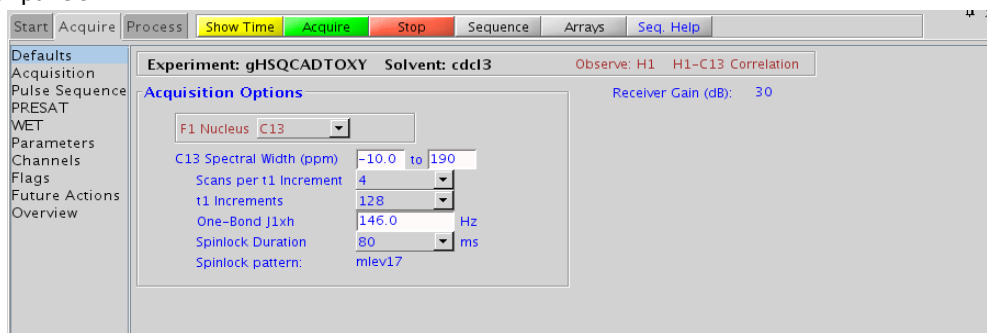**Usage:**

The HSQCADTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average ^{1}J$_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HSQCADTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment will transfer TOCSY-or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^1H-^{13}C (or ^1H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The HSQCADTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^1H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^1H-^1H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HSQCADTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCADTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

- The HSQCADTOXY uses adiabatic 180° carbon pulses and will often give better results than the HSQCTOXY, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

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gHSQCADTOXY

Description:

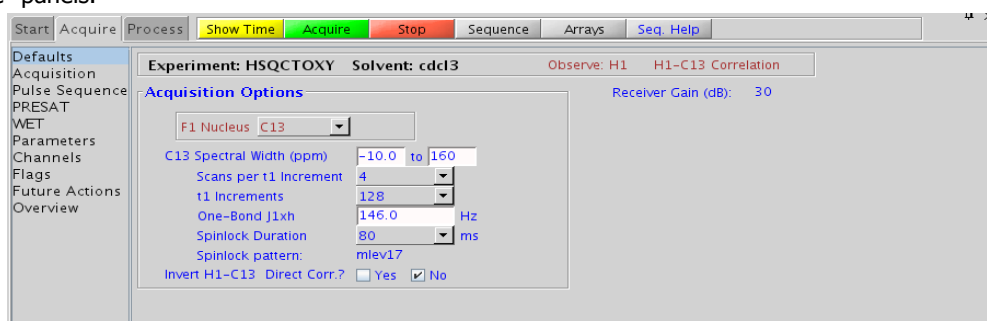Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock, adiabatic 180º X-nuclei pulses, and gradient coherence selection.

Usage:

The gHSQCADTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average ^{1}J$_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHSQCADTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment will transfer TOCSY-or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- The gHSQCADTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^{1}H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HSQCADTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCADTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

- The gHSQCADTOXY uses adiabatic 180° carbon pulses and will often give better results than the gHSQCTOXY, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

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HSQCTOXY
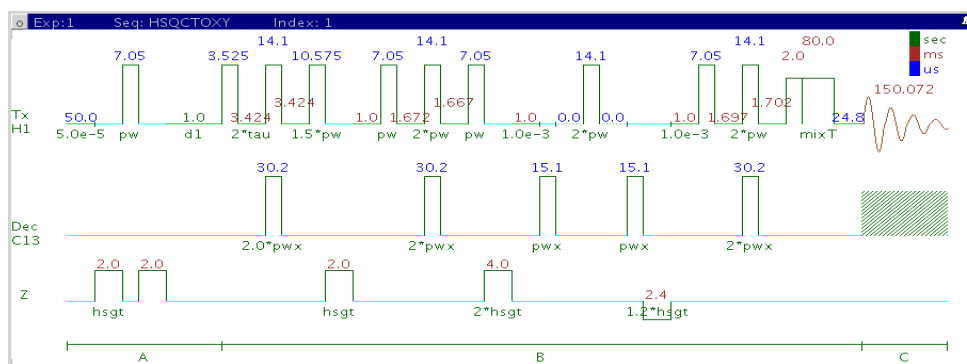
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and adiabatic 180° X-nuclei pulses.

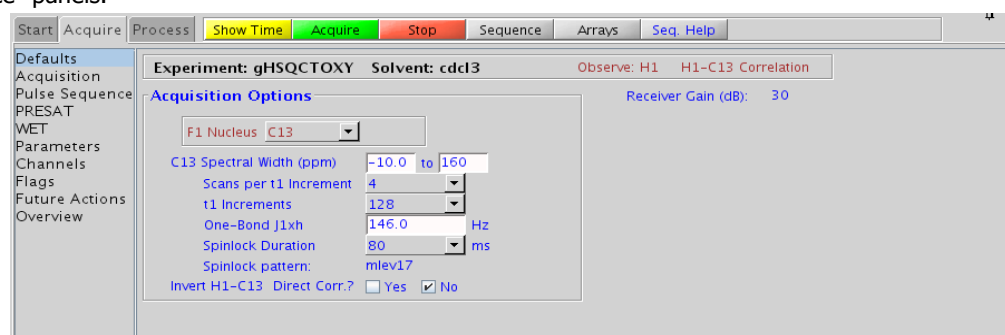**Usage:**

The HSQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average ^{1}J$_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Invert H1-C13 Direct Corr.?:	Check box to invert direct proton-carbon correlation
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HSQCTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment will transfer TOCSY-or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- On the "Pulse Sequence" tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^{1}H chemical shifts. This can allow the observation of ^{1}H-^{1}H J-connectivity between protons with very close overlap.

- The HSQCTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^{1}H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HSQCTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

- The HSQCADTOXY and gHSQCADTOXY use adiabatic 180° carbon pulses and will often give better results than the HSQC, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)
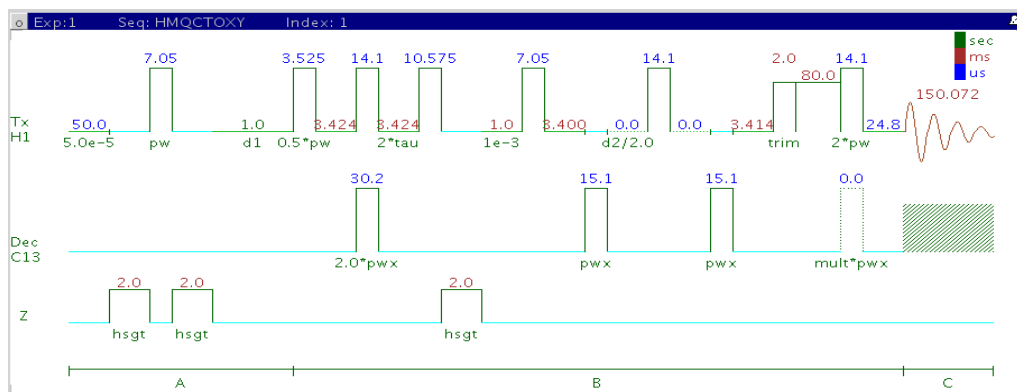
K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (Multiplicity Editing)

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gHSQCTOXY

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock, adiabatic 180º X-nuclei pulses, and gradient coherence selection.

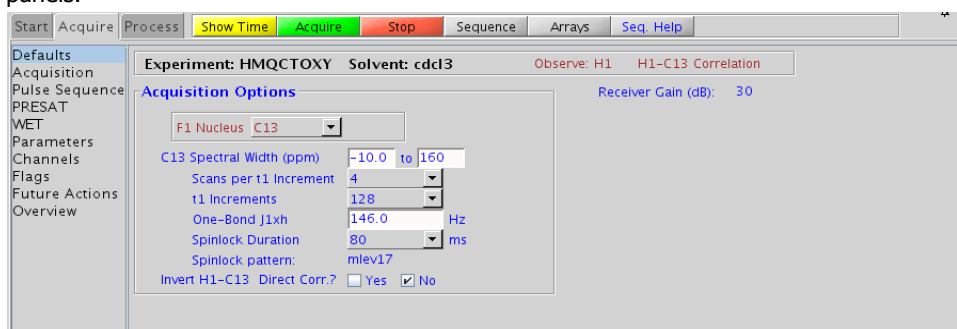**Usage:**

The gHSQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average ^{1}J$_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Invert H1-C13 Direct Corr.?:	Check box to invert direct proton-carbon correlation
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHSQCTOXY is set up from this experiment. Setup from a TOCSY-type or HSQC-type experiment will transfer TOCSY-or HSQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- On the "Pulse Sequence" tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^{1}H chemical shifts. This can allow the observation of ^{1}H-^{1}H J-connectivity between protons with very close overlap.

- The gHSQCTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^{1}H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HSQCTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHSQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

- The HSQCADTOXY and gHSQCADTOXY use adiabatic 180° carbon pulses and will often give better results than the HSQC, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

E. Kupce, R. Freeman, J. Magn. Reson. Ser. A 117 (1995) 246-256. (Adiabatic Pulses)

K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

R. D. Boyer, R. Johnson, K. Krishnamurthy, J. Magn. Reson. 165 (2003) 253-259 (Multiplicity Editing)

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HMQCTOXY

Description:

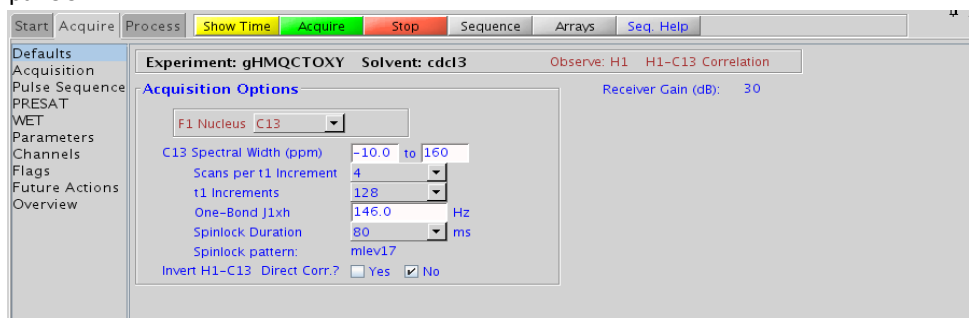Two Dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock.

Usage:

The HMQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average $^1J_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Invert H1-C13 Direct Corr.?:	Check box to invert direct proton-carbon correlation
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the HMQCTOXY is set up from this experiment. Setup from a TOCSY-type or HMQC-type experiment will transfer TOCSY-or HMQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value (≥30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- On the "Pulse Sequence" tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^{1}H chemical shifts. This can allow the observation of ^{1}H-^{1}H J-connectivity between protons with very close overlap.

- The HMQCTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HMQC stage is then distributed along the ^{1}H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HMQCTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHMQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

Reference(s):

L. Lerner, A. Bax, J. Magn. Reson. 69 (1986) 375-380. (HMQC-TOCSY)

R. C. Crouch, T. D. Spitzer, G. E. Martin, Magn. Reson. Chem. 30 (1992) S71-S73. (Multiplicity Edited HMQC-TOCSY)

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gHMQCTOXY
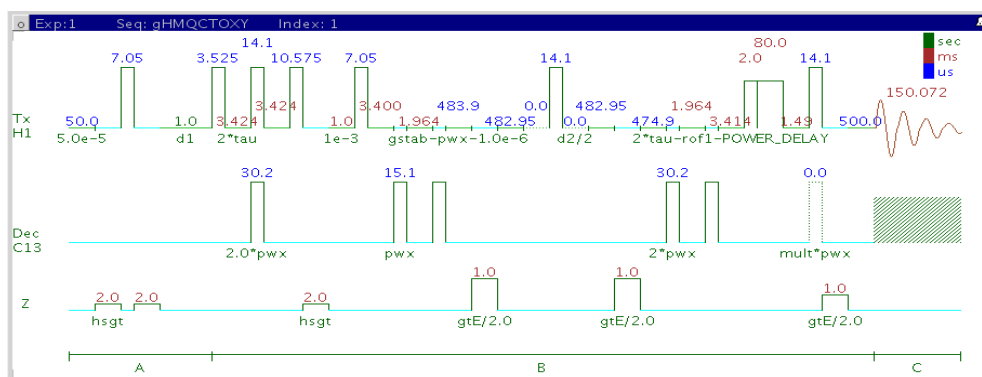
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear multiple-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and gradient coherence selection.

Usage:

The gHMQCTOXY experiment is used to acquire a 2-dimensional spectrum to collect information correlating ^{1}H-^{1}H TOCSY and ^{13}C chemical shift information, e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H-^{1}H TOCSY spectrum by spreading them out over the carbon (or other X-nuclei) chemical shift range. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull-down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Set value for the average ^{1}J$_{X,H}$- 146 Hz works well for many carbons
Spinlock Duration (mixT):	Select from pull down menu- 80ms will work best for large spin systems*
Invert H1-C13 Direct Corr.?:	Check box to invert direct proton-carbon correlation
C12-H1 suppression:	Check box to turn on/off on the "Pulse Sequence" panel- controls TANGO-Gradient ^{12}C suppression, default is on (highly recommended)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the gHMQCTOXY is set up from this experiment. Setup from a TOCSY-type or HMQC-type experiment will transfer TOCSY-or HMQC-related parameters, respectively. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

- The spinlock time (in addition to the magnitude of the coupling constants) ultimately controls how far along the spin system the correlations will extend. Spinlock durations exceeding 150ms should be used with caution as this could result in sample heating. The efficiency of the TOCSY transfer will be directly proportional to the size of the coupling constants between the pairs of protons. To show primarily vicinal and geminal couplings, select a shorter spinlock time, e.g. 30ms or less.

- The type of spinlock can be changed from mlev17 (for smaller molecules) to clean mlev17 (for larger molecules on the "Pulse Sequence" tab.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. However, higher power carbon decoupling may cause some perturbations that might necessitate more ss scans to reach equilibrium. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- On the "Pulse Sequence" tab there is a pull-down menu to select in-phase, inverted, or suppressed XH direct correlations. There is a minor sensitivity loss with this feature, however the extra information is obtained with no additional cost in experiment time. When direct correlations suppression is chosen the ^{13}C decoupling can be turned off to place any residual direct correlations away from the true ^{1}H chemical shifts. This can allow the observation of ^{1}H-^{1}H J-connectivity between protons with very close overlap.

- The gHMQCTOXY experiment, like all other heteronuclear TOCSY's, is an experiment with a lower sensitivity than the respective simple, single bond correlation experiment. This is because the magnetization generated during the initial HSQC stage is then distributed along the ^{1}H coupling network. Thus, a higher number of transients per increment are required unless the sample is very concentrated.

- In general, the HSQC-type experiments provide spectra with narrower peaks shapes (by suppressing ^{1}H-^{1}H homonuclear coupling modulation in F1) than the HMQC-types, typically leading to better S/N and resolution.

- The HMQCTOXY experiment has a sensitivity advantage (factor of ~1.4) over the gHMQCTOXY, but may produce potentially less clean spectra (due to t1 noise from strong signals such as t-butyl) than its gradient counterpart. The gradient enhanced version of these experiments can also suffer from additional sensitivity loss due to diffusion during the mixing time if the analyte is small and/or the solvent is non-viscous. Typically, one should consider using the non-gradient coherence selection version.

Reference(s):

L. Lerner, A. Bax, J. Magn. Reson. 69 (1986) 375-380. (HMQC-TOCSY)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

R. C. Crouch, T. D. Spitzer, G. E. Martin, Magn. Reson. Chem. 30 (1992) S71-S73. (Multiplicity Edited HMQC-TOCSY)

Chapter 10
Carbon-carbon correlation - (CC)corr

Introduction

This tab contains three experiments to map direct correlations from carbon to carbon. Two experiments are ^{13}C-detected (INADEQUATE and INADEQUATEAD) and the third is ^{1}H-detected (ADEQUATEAD). To unambiguously elucidate the structure of small-molecules, the INADEQUATE and INADEQUATE(AD) experiments have no peer except perhaps X-ray crystallography. They are, however, highly insensitive and thus infrequently used.   A good rule of thumb to determine if the sample is concentrated enough for this class of experiment to be successful within a reasonable time (overnight acquisition) is that the S/N for the CARBON spectrum should be ~25:1 after a single scan.   The proton-detected ADEQUATEAD experiment is more sensitive. However it has the requirement that a proton must be nearby for the carbon to be detected. The "AD" experiments include adiabatic carbon 180° pulses and are highly recommended, as they provide more uniform inversion profiles for a wide ^{13}C spectral width.
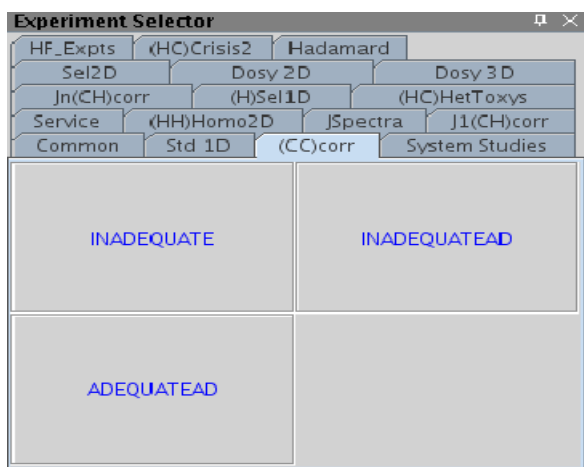
INADEQUATE
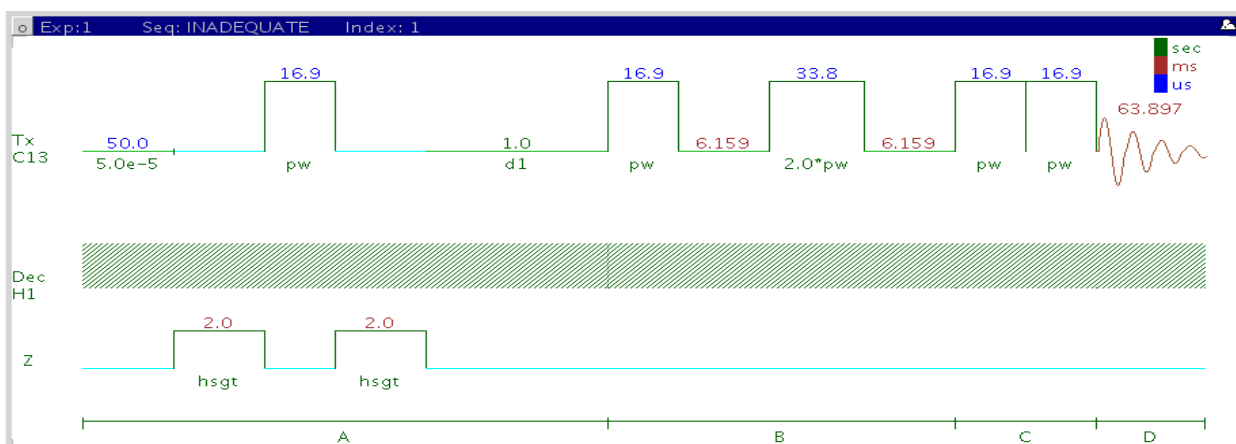
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional homonuclear multiple-quantum J-correlation spectroscopy.

Usage:

The INADEQUATE experiment shows correlations between carbons that are directly coupled to one another. The data have great utility for structure elucidation as the carbon connectivities are established directly from the correlations. These connectivities can then be used to map the molecular framework, similar to x-ray crystallography. The drawback to the experiment is that as the sensitivity is very low; it should only be attempted with samples of higher concentrations. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

C13 Spectral Width (sw):	Type in desired values in ppm
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond C-C coupling Jcc (jcc):	Type in value for the average J^1_{C-C}- 55 Hz works well for most molecules
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 2k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

- Spectral width (sw) setting will transfer from a CARBON if the INADEQUATE is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30).

- Spectral width in the F1 dimension (sw1) is set, by default, to sw*2.   However, a value of sw1=sw (while generating aliased peaks along F1) can be used with no ambiguities in connectivity interpretations.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Observing quaternary carbons is uniquely challenging. Use of some relaxation agent may be helpful while running the INADEQUATE experiment.

- A good rule of thumb to determine if the sample is concentrated enough for an INADEQUATE experiment is that the S/N for the CARBON spectrum should be ~25:1 after a single scan.

- The INADEQUATEAD uses adiabatic 180° carbon pulses and will often give better results than the INADEQUATE, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

A. Bax, R. Freeman, S. P. Kempsell, J. Am. Chem. Soc. 102 (1980) 4849-4851 (INADEQUATE)

J. Buddrus, J. Lambert, Magn. Reson. Chem. 40 (2002) 3-23. (INADEQUATE – Recent Developments)

A. D. Bain D. W. Hughes, C. K. Anand, Z. Nie, V. J. Robertson, Magn. Reson. Chem. 48 (2010) 630-641 (INADEQUATE – Practical Aspects)

INADEQUATEAD
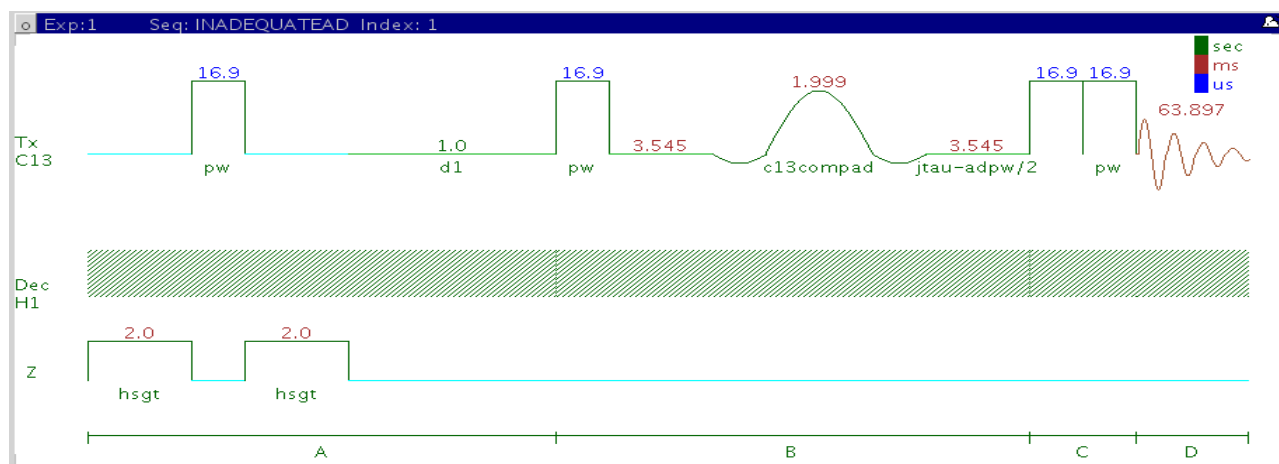
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional homonuclear multiple-quantum J-correlation spectroscopy with adiabatic 180º X-nuclei pulses.

Usage:

The INADEQUATE experiment shows correlations between carbons that are directly coupled to one another. The data have great utility for structure elucidation, as the carbon connectivities are established directly from the correlations. These connectivities can then be used to map the molecular framework, similar to x-ray crystallography. The drawback to the experiment is that as the sensitivity is very low; it should only be attempted with samples of higher concentrations. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

C13 Spectral Width (sw):	Type in desired values in ppm
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.064s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
One-Bond C-C coupling Jcc (jcc):	Type in value for the average J^1_{C-C}- 55 Hz works well for most molecules
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 2k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a CARBON if the INADEQUATEAD is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30).

- Spectral width in the F1 dimension (sw1) is set, by default, to sw*2.   However, a value of sw1=sw (while generating aliased peaks along F1) can be used with no ambiguities in connectivity interpretations.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- Observing quaternary carbons is uniquely challenging. Use of some relaxation agent may be helpful while running the INADEQUATEAD experiment.

- A good rule of thumb to determine if the sample is concentrated enough for an INADEQUATEAD experiment to be successful within a reasonable time (overnight acquisition) is that the S/N for the CARBON spectrum should be ~25:1 after a single scan.

- The INADEQUATEAD uses adiabatic 180° carbon pulses and will often give better results than the INADEQUATE, particularly when the carbon pulses are not perfectly calibrated for the given sample. In addition, the adiabatic 180° pulses provide a much more uniform inversion over a wide ^{13}C spectral width.

Reference(s):

A. Bax, R. Freeman, S. P. Kempsell, J. Am. Chem. Soc. 102 (1980) 4849-4851 (INADEQUATE)

L. Jin, D. Uhrin, Magn. Reson. Chem. 45 (2007) 628-633 (IPAP INADEQUATE with Adiabatic Pulses)

D. Uhrin, Ann. Rep. NMR. Spectrosc. 70 (2010) 1-34 (INADEQUATE – Review)

ADEQUATEAD
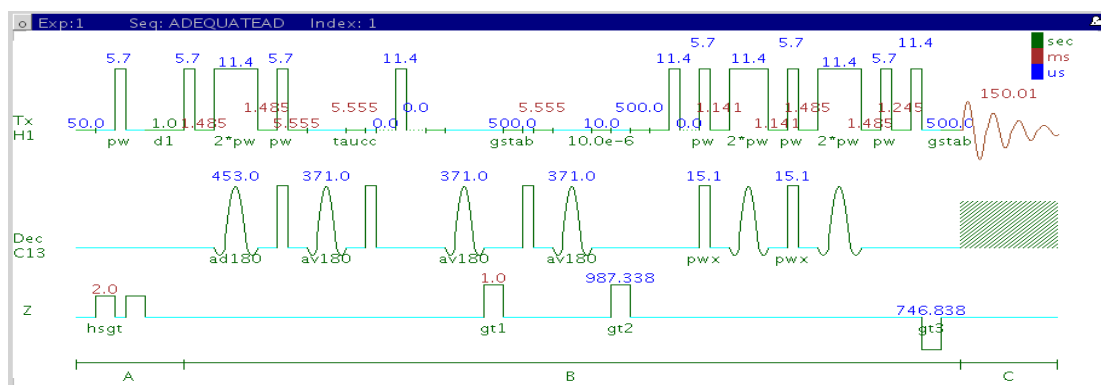
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single or double-quantum J-correlation spectroscopy with adiabatic 180º X-nuclei pulses.

Usage:

The ADEQUATEAD experiment can be acquired either as a single quantum experiment or a double quantum experiment. The single quantum experiment selectively transfers coherences from two adjacent carbons to protons directly attached to one of the carbons. The double quantum experiment can connect carbons that are separated by multiple bonds. The data have great utility for structure elucidation, as the carbon connectivities established indirectly from the correlations can then be used to map the molecular framework. The experiment is more sensitive (theoretically a factor of 32) than the INADEQUATEAD. However, the ADEQUATEAD experiment relies on at least one nearby attached proton, so correlations between quaternary carbons are not observed. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.15s
F1 Nucleus (dn):	Pull- down menu to set F1 nucleus to ^{13}C, ^{15}N, or other*
C13 Spectral Width (ppm):	Type in desired chemical shift range for F1 dimension
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
One-Bond J1xh (j1xh):	Type in value for the average $^{1}J_{X,H}$- 146 Hz works well for most molecules
C-C evolution:	Set to single quantum (SQ) or double quantum (DQ)
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (1k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a PROTON if the ADEQUATEAD is set up from this experiment. Receiver gain (gain) is usually set to a high value (≥30).

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

Reference(s):

B. Reif, M. Kock, R. Kerssebaum, H. Kang, W. Fenical, C. Griesinger, J. Magn. Reson. A118 (1996) 282-285 (ADEQUATE)

M. Kock, R. Kerssebaum, W. Bermel, Magn. Reson. Chem. 41 (2003) 65-69. (ADEQUATE with Adiabatic Pulses)

T. Parella, F. S. Ferrando, J. Magn. Reson, 166 (2004) 123-128. (ADEQUATE with Multiplicity Editing)

Chapter 11
Proton-fluorine -
HF_Expts

Introduction

This tab of the experiment selector includes both 1D and 2D experiments involving ^{1}H and^{19}F. All the experiments have been designed such that the data may be acquired without the need for a second high-band channel in the spectrometer. The 1D experiments are with fluorine observe, proton decouple (FObs_HDec), and *vice versa* (HObs_FDec). The 2D experiments available include both J-coupling (FH_gHETCOR) and through-space (FH_HOESY) correlation experiments.

These experiments can provide a unique handle for structure elucidation and peak assignment of fluorinated compounds. When acquiring impure samples of fluorinated compounds from a biological origin, these experiments can be used to see resonances from the molecule of interest, while excluding background signals. These experiments require a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file. There are unique calibration routines in the channel sharing mode. Please refer to the VnmrJ Installation and Administration manual, Chapter 9, for information on calibration.
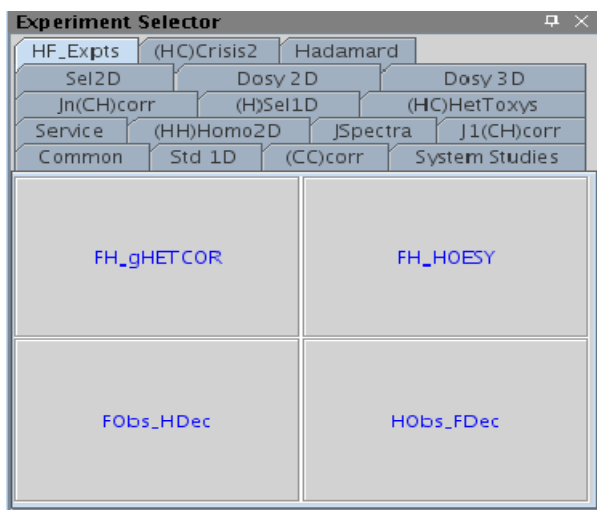
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FH_gHETCOR
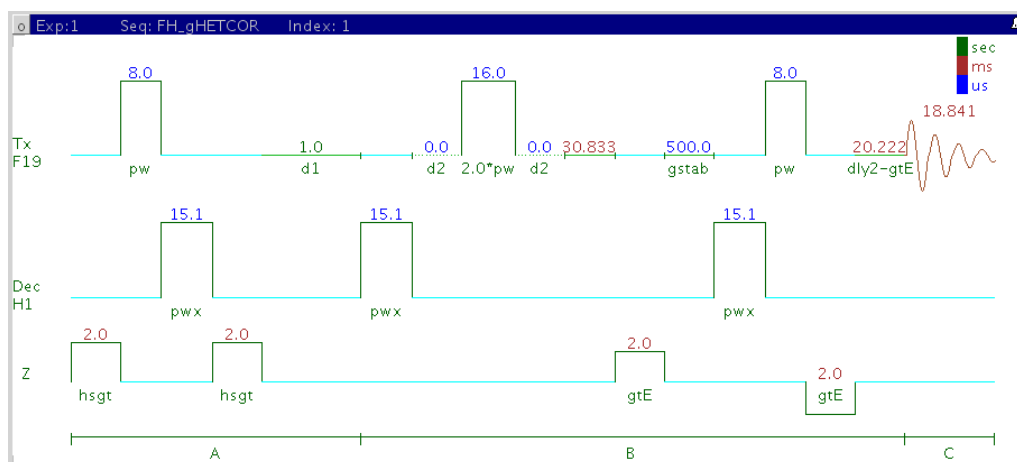
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear ^{1}H-^{19}F shift correlation with fluorine observe and gradient coherence selection.

Usage:

The FH_gHETCOR experiment is used to acquire a 2-dimensional spectrum to correlate protons coupled to fluorines with fluorine detection, meaning the F1 dimension is ^{1}H and the F2 dimension is ^{19}F. This scheme allows for higher resolution in the fluorine dimension as the chemical shift range is much larger. This experiment is useful to confirm the attachment location(s) for fluorine atom(s) in a molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired FLUORINE or Fobs_HDec*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.019 s
H1 Spectral Width [ppm] [F1]:	Select typical choices from pull down menu or type in values
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu* - controls the resolution in F1
F-H Coupling Constant (jFH):	Set value for the average ^{n}J$_{F,H}$
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

## PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 4*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.

- Spectral width (sw) and receiver gain (gain) settings will transfer from a FLUORINE or FObs_HDec data set if the FH_gHETCOR is set up from these experiments. As ^{19}F has a large chemical shift range, it is highly recommended that the sw is optimized prior to acquiring this experiment. Using the Fobs_HDec experiment for setup will preserve the decoupling parameters. However, decoupling may result in sensitivity losses and unintended signal cancellations. Decoupling can be turned off by setting the parameter dm to the value 'nnn'.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

Reference(s):

J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23. (Multidimensional ^{19}F NMR)

FH_HOESY
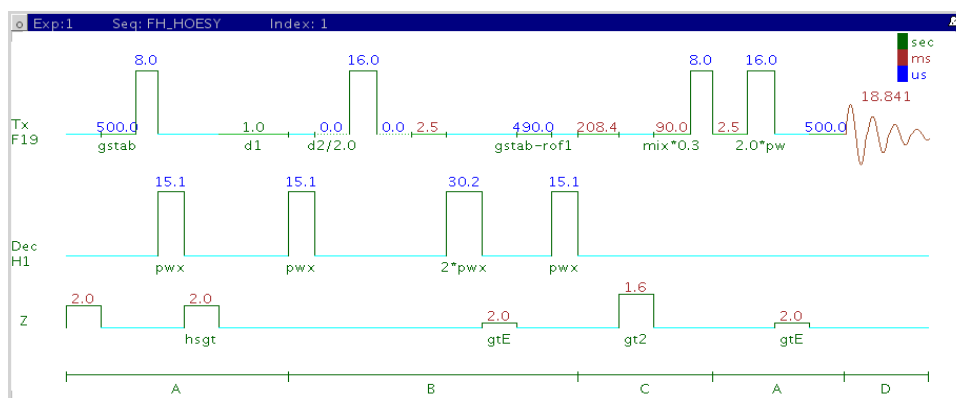
Description:

Two Dimensional heteronucler ^{1}H-^{19}F through-space correlation spectroscopy with fluorine observe.

Usage:

The FH_HOESY experiment will produce a 2-dimensional spectrum with crosspeaks between fluorines and protons that are close in space (typically <5 Å). The experiment is configured with fluorine detection, meaning the F1 dimension is ^{1}H and the F2 dimension is ^{19}F. This scheme allows for higher resolution in the fluorine dimension as the chemical shift range is much larger. The FH_HOESY experiment if often used to help determine stereochemistry for rigid ring systems or to confirm attachment locations for fluorines on unsaturated carbons (e.g. aromatic) of a molecule. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired FLUORINE or Fobs_HDec*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.019 s
Scans per increment (nt):	Select typical choices from pull down menu*
Number of increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1 (minimum ~64)
H1 Spectral Width [ppm] [F1]:	Select typical choices from pull down menu or type in values
HOESY mixing time (mix):	Select from pull down menu*
Make coherence selection with gradient:	Check box to turn gradient coherence selection on/off
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans
Relaxation time (d1):	Set from "Acquisition" panel controls the delay between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" or "Pulse Sequence" panels.

PROCESSING

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment, except for minor manual phasing changes that may be required as the NOE peaks will be negative for smaller molecules. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (2k x 1k is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is 2*ni). Menus to quickly change the weighting functions in F2/F1 are also present (defaults are gaussians); the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.

- Spectral width (sw) and receiver gain (gain) settings will transfer from a FLUORINE or FObs_HDec data set if the FH_gHETCOR is set up from these experiments. As ^{19}F has a large chemical shift range, it is highly recommended that the sw is optimized prior to acquiring this experiment. Using the Fobs_HDec experiment for setup will preserve the decoupling parameters. However, decoupling may result in sensitivity losses. Decoupling can be turned off by setting the parameter dm to the value 'nnn'.

- The default number of "steady state" or "dummy scans" that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times and d1 relaxation delays (defaults are 0.15s and 1s, respectively).

Reference(s):

J. Battiste, R. A. Newmark, Prog. NMR. Spectrosc. 48 (2006) 1-23. (Multidimensional ^{19}F NMR)

W. Bauer, Magn. Reson. Chem. 34 (1996) 532-537. (Gradient Inverse HOESY)

FObs_HDec
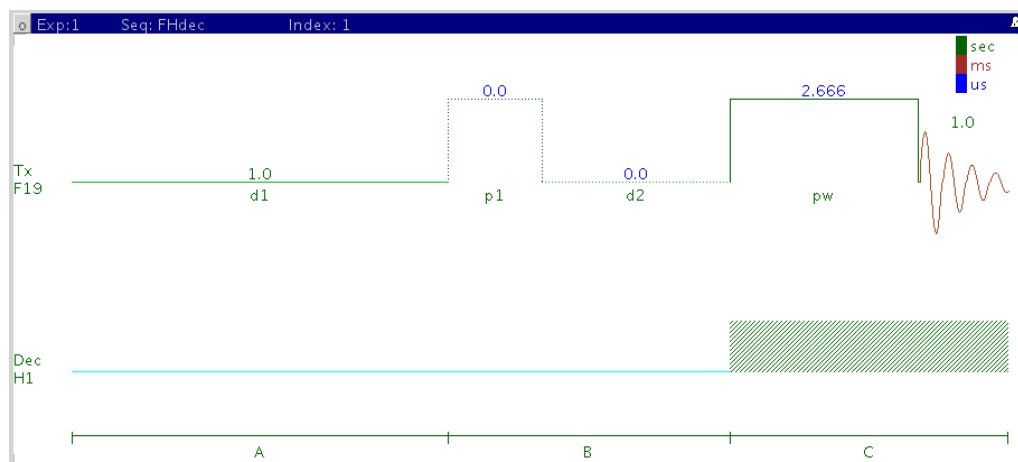
Description:

Uses the standard s2pul pulse sequence to acquire a 1D-^{19}F dataset with proton decoupling.

Usage:

The FObs_HDec experiment is typically used to confirm the presence of fluorine in a molecule. The large fluorine chemical shift range is highly sensitive to nearby functional groups. The experiment is nearly as sensitive as a 1D PROTON experiment, and the decoupling increases the sensitivity by collapsing the ^{19}F peaks into singlets. All parameters are set from the "Acquire" tab/"Default F19" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from the "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
H1 Decoupler offset (dof):	Type in value for the center of the proton chemical shift range to optimize decoupling
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel. Suggested choices for resolution enhancement (at the expense of S/N) are "pseudo echo" and "res-enhance." When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the "Interactive Weighting" button. CAUTION: the spectral integration will be affected with resolution enhancing functions- do not use these types of apodization for quantification.

Many NMR probes contain fluorinated materials, which cause broad signals that can distort the baseline (especially when lying next to or outside the spectral window). The default processing parameters are set to use linear prediction to back predict the first seven points of the spectrum in order to flatten the baseline. These settings may be modified under the "Process" tab on the "More 1D" panel.

TIPS, TRICKS AND NEXT STEPS

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.

- This fluorine experiment will produce a proton-decoupled ^{19}F spectrum. See the FLUORINE experiment (Chapter 2) for a proton-decoupled version of the experiment, or set the parameter dm to the value 'nnn'. Parameters to optimize the decoupling can be found on the "Pulse Sequence" tab. However, as the proton chemical shift range is quite small, the default parameters should usually work well (default shape covers an 8000 Hz range).

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the MoveSW button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is very large, once the fluorine signals are located, one should consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.

- The acquisition time, which controls the digital resolution (Hz/point, which is equal to 1/at), can be set on the "Acquisition" page of the "Acquire" tab. Because FLUORINE spectra are often acquired with large spectral widths, the default digital resolution (set from the chosen spectral width) may be insufficient to see small couplings; one should consider lengthening the acquisition time and/or narrowing the spectral width.

- It must be noted that the *total* interscan delay is the sum of the acquisition time (at) and the relaxation delay (d1). When acquiring a multi-scan FObs_HDec experiment for quantification, the interscan delay should be at least 5X the longest fluorine T1 value in the molecule.

- When acquiring impure samples of fluorinated compounds from a biological origin, one should consider using fluorine spectra in lieu of a more complicated proton spectrum as appropriate.

HObs_FDec
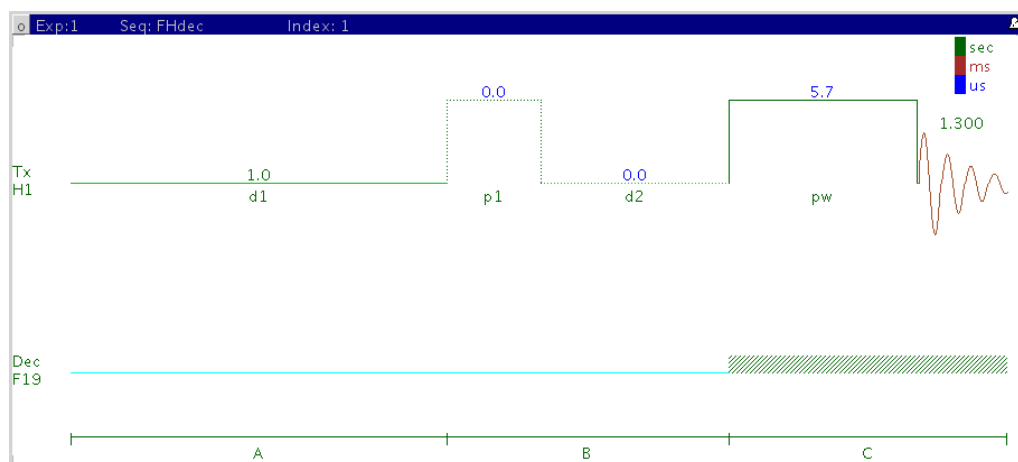
GENERAL DESCRIPTION AND USAGE

Description:

Uses the standard s2pul pulse sequence to acquire a 1D-^{1}H dataset with ^{19}F decoupling.

Usage:

The HObs_FDec experiment can be used to simplify proton spectra with complicated fluorine coupling and to establish which protons are coupled to fluorine. All parameters are set from the "Acquire" tab/"Default F19" panel unless otherwise specified.

KEY PARAMETERS

Solvent (solvent):	Set from the "Start" tab/"Standard" panel
Spectral Width (sw):	Type in specific ppm values
Number of scans (nt):	Select typical choices from pull down menu*
Relaxation Delay (d1):	Select typical choices from pull down menu*-controls the delay between scans
Pulse Angle:	Select typical choices from pull down menu*-controls the tip angle for the pulse
F19 Decoupler offset (dof):	Type in value for the center of the fluorine chemical shift range to optimize decoupling
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the digital resolution (Hz/point, 1/at)

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

A typical processing apodization choice which provides S/N enhancement (at the expense of resolution) for a 1D spectrum is line broadening, with an lb setting from ~0.5-1 Hz. A suggested optimal value is an lb equal to the peak linewidth at half-height. Other types of apodization are available under the "Process" tab on the "Weighting" panel. Suggested choices for resolution enhancement (at the expense of S/N) are "pseudo echo" and "res-enhance." When the weighting function is selected, values for these parameters are calculated based on the acquisition time. They may also be set manually with the "Interactive Weighting" button. CAUTION: the spectral integration will be affected with resolution enhancing functions- do not use these types of apodization for quantification.

TIPS, TRICKS AND NEXT STEPS

- Note: this experiment requires a probe that can be simultaneously tuned to H and F and has the appropriate H-F calibrations in the probe file.

- The HObs_FDec experiment will produce a fluorine-decoupled ^{1}H spectrum. See the PROTON experiment (Chapter 2) for a proton-decoupled version of the experiment, or set the parameter dm to the value 'nnn'. Parameters to optimize the decoupling can be found on the "Pulse Sequence" tab. As the fluorine chemical shift range is quite large, it is important to locate the chemical shift(s) of the fluorine(s) for decoupling and set the decoupler offset properly. If there are multiple fluorines in the molecule with large chemical shift differences, one should consider decoupling a single fluorine at a time. Alternatively, an adiabatic decoupling shape may be created. See the VnmrJ manual for instructions on using Pbox.

- To further optimize the spectral width (sw), place one cursor on each side of the desired region on the spectrum, click the MoveSW button located under the spectrum window and reacquire the data. The default range is set to cover the typical range of fluorine chemical shifts seen in organic molecules. As this range is very large, once the fluorine signals are located, one should consider narrowing the chemical shift range and reacquiring the spectrum for better resolution.

- ^{19}F shaped decoupling (due to large chemical shift range of ^{19}F) would typically necessitate higher powers to be used. However decoupling powers above 46 dB are not allowed in the shared mode (thus providing an instrument safety margin). Using the default parameters, the sensitivity of these decoupled experiments is ca. 60% that of the non-decoupled experiment. In case one wishes to change the decoupling bandwidths (for wider ^{19}F decoupling width) and/or duty cycle (to gain sensitivity and/or lower decoupling power) the decoupling shape needs to be recreated via the Pulse sequence panel.

Chapter 12
JSpectra

Introduction

This tab of the experiment selector includes two experiments that provide information regarding coupling constants (J's) in the F1 dimension. The HOMO2DJ experiment has proton chemical shifts in F2 and proton coupling constants in F1, while HETERO2DJ version has carbon chemical shifts in F2 and proton coupling constants in F1. As HETERO2DJ is carbon-detected, it is a very low sensitivity experiment. These experiments are usually used to resolve complex, overlapping multiplets. The HOMO2DJ is also used to help resolve complicated, multi-component samples, such as from a biological matrix. In addition, with appropriate post-acquisition processing, the F2 projection in HOMO2DJ can be interpreted as a broadband homonuclear decoupled ^{1}H spectrum.
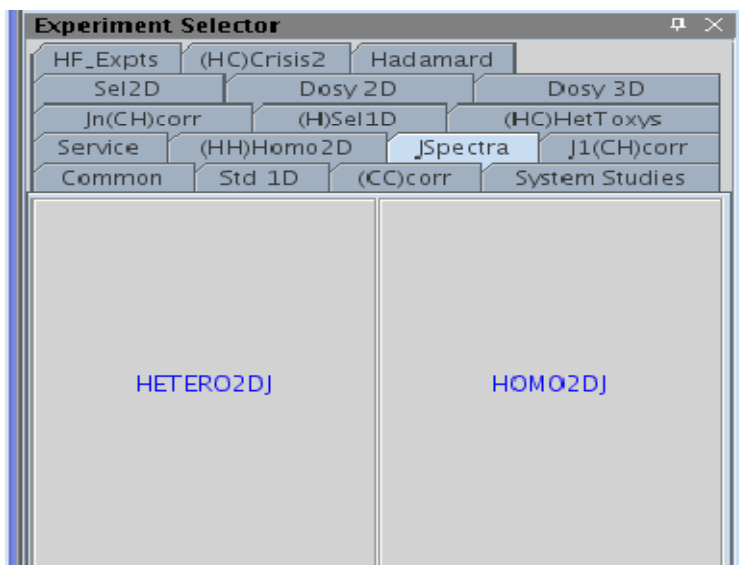
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HETERO2DJ
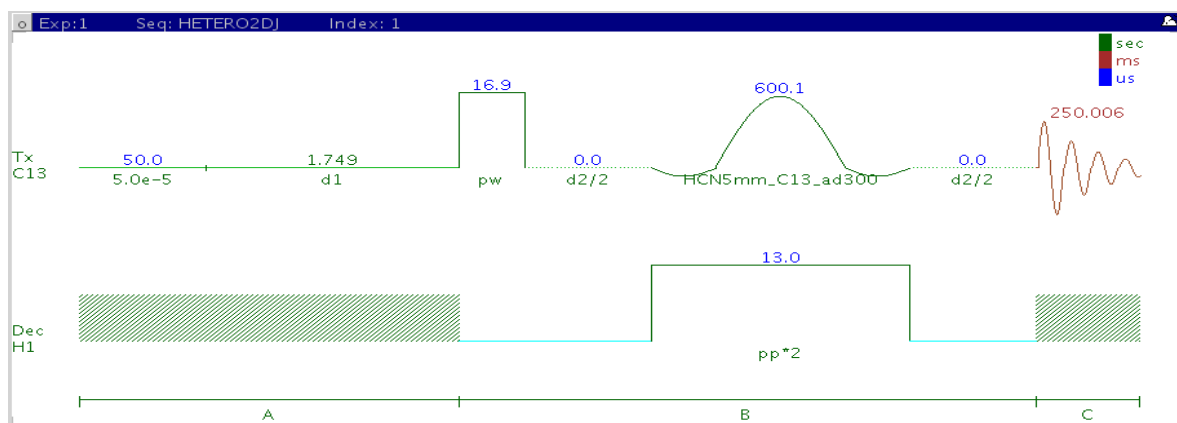
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear J-resolved spectroscopy.

Usage:

The HETERO2DJ experiment is used to acquire a 2-dimensional spectrum with the ^{13}C chemical shifts in the F2 dimension and the ^{1}H couplings in F1. The experiment is fairly insensitive as it is carbon-detected. Proton-carbon coupling constants are often used to help define *e/z* stereochemistry of double bonds or molecular conformations, e.g. the torsion angles of peptides. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired CARBON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.25s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
F1 spectral width:	Set value for the average range of $^nJ_{C,H}$
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

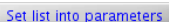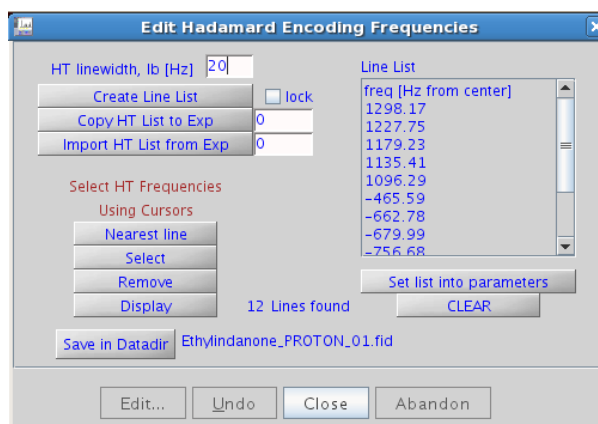Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (8k x 128 is the default), as well as a check box to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is off). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions are calculated automatically from the acquisition time and number of increments. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. This arrangement allows for the best resolution and sensitivity combination. As such, the optimal weighting functions will likely be the defaults of a squared shifted sinebell for F2 and gaussian for F1. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) setting will transfer from a CARBON if the HETERO2DJ is set up from this experiment. Receiver gain (gain) is usually set to a high value ($\geq$30), unless the sample is extremely concentrated or ^{13}C-labelled.

- A typical value for sw1 should be at least 3 times the largest expected one-bond coupling constant to avoid any potential folding of methyl quartets.

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. The default setting for this parameter is off.

- The (HC)EXSIDE experiment (see Chapter 7) can also be used to measure selective long-range H-C coupling constants and may provide better sensitivity, particularly when there are only one or two specific couplings of interest.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

L. Muller, A. Kumar, R. R. Ernst, J. Magn. Reson. 25 (1977) 383-390. (2DJ)

G. Bodenhausen, G. A. Morris, R. Freeman, D. L. Turner, J. Magn. Reson. 28 (1977) 17-28. (2DJ)

HOMO2DJ
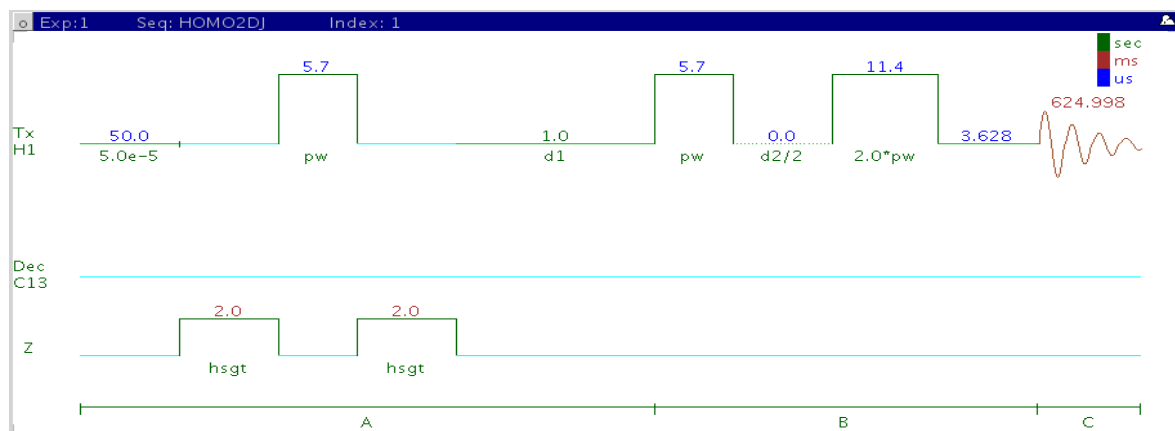
Description:
Two Dimensional homonuclear J-resolved spectroscopy.

Usage:
The HOMO2DJ experiment is used to acquire a 2-dimensional spectrum with the ^{1}H chemical shifts in the F2 dimension and the ^{1}H couplings in F1. This experiment can be used to obtain accurate measurements of couplings when there are complex multiplets and/or overlap in the 1D PROTON spectrum. This experiment can also be used to help resolve overlap in complex multi-component spectra, such as spectra of biofluids. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Spectral Width (sw):	Set from previously acquired PROTON*
Acquisition time (at):	Set from "Pulse Sequence" panel, controls the resolution in F2, default 0.625s
Scans per t1 increment (nt):	Select typical choices from pull down menu*
t1 Increments (ni):	Select typical choices from pull down menu*- controls the resolution in F1
F1 spectral width:	Set value for the average range of $^nJ_{H,H}$ – don't forget couplings constants are both positive and negative
Steady State (ss):	Set from "Pulse Sequence" panel, turn on/off gradient cleanup between scans

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. The "Process" tab/"Basic" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (8k x 128 is the default), as well as a check b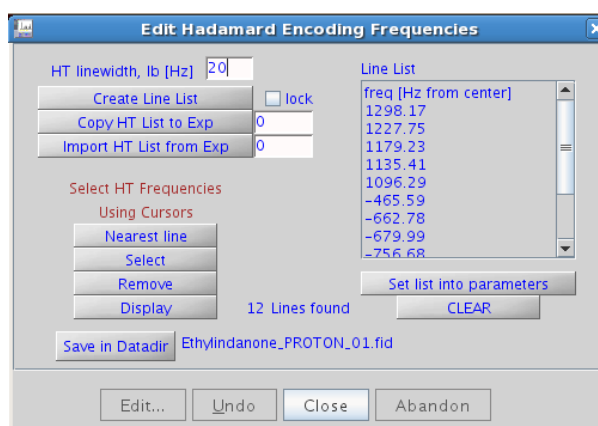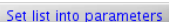ox to turn on/off linear prediction and a pull-down to control the extent of linear prediction (default is off). Menus to quickly change the weighting functions in F2/F1 are also present; the parameters for the weighting functions (defaults are squared sinebells) are calculated automatically from the acquisition time and number of increments. It is important to note that this experiment employs mix-mode processing, with the F2 dimension absolute value and F1 phase-sensitive. More processing details can be found under some of the other "Process" panels, such as the "Default", "Weighting", and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- Spectral width (sw) and receiver gain (gain) settings will transfer from a PROTON if the HOMO2DJ is set up from this experiment.

- It is highly recommended to adjust sw1 and ni such that ni/sw1 is equal to acquisition time (at) to accommodate the "45° rotate" that is typically applied during processing. Such equal resolution in both F1 and F2 dimension results in much better F2 projection (which mimics a proton decoupled proton spectrum).

- The default number of steady state scans (ss) that are acquired prior to data acquisition to establish sample equilibrium is 32, which should work for most samples. This value can be changed on the "Acquisition" panel of the "Acquire" tab if necessary.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.625s and 1s respectively).

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

L. Muller, A. Kumar, R. R. Ernst, J. Magn. Reson. 25 (1977) 383-390. (2DJ)

G. Bodenhausen, G. A. Morris, R. Freeman, D. L. Turner, J. Magn. Reson. 28 (1977) 17-28. (2DJ)

Chapter 13
Hadamard Encoded - Hadamard

Introduction

This tab of the Experiment Selector contains a suite of 2D experiments that employ Hadamard encoding. The concept behind Hadamard encoding is to excite the interesting chemical shifts with an array of frequencies. The Hadamard method of acquiring 2D spectra does offer a 2D spectrum recorded at a fraction of the time of the similar traditional 2D experiment. The sensitivity of a Hadamard experiment is comparable to that of a traditional experiment per unit of time. Consequently the advantage of the Hadamard experiment is realized only when there is enough sensitivity (i.e., sample concentration) to record a fast 2D spectrum.

The specific frequencies for the Hadamard matrix are obtained by selection from a PROTON or a CARBON spectrum as appropriate. The experiments include a full set of standard two-dimensional pulse sequences including homonuclear experiments (dqcosyHT, gcosyHT, tocsyHT, noesyHT, and roesyHT), heteronuclear experiments (hsqcHT, hmbcHT, and hetcorHT), as well as an hsqctocsyHT experiment. All the experiments are processed using the Hadamard transformation, which is set by default in the processing parameters.
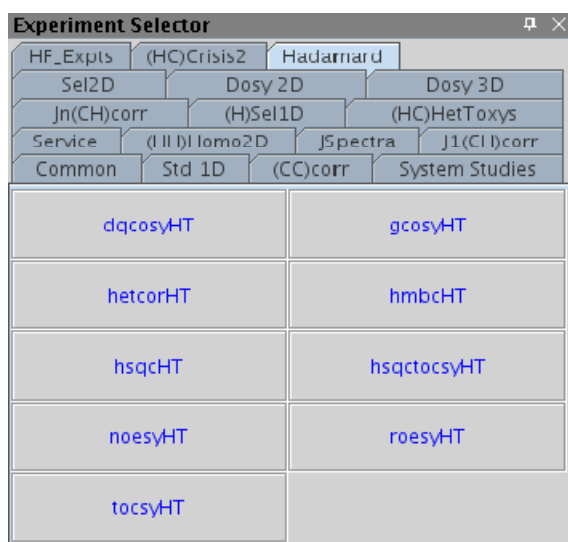
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dqcosyHT
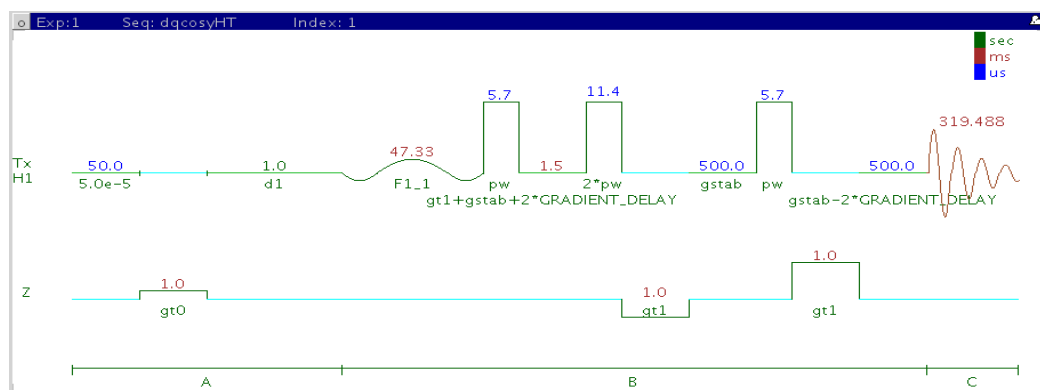
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional double-quantum filtered J-correlation spectroscopy with Hadamard encoding.

Usage:

The dqcosyHT will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between j-coupled protons. The Hadamard matrix is created from a previously acquired PROTON data set. The advantage of the dqcosyHT over the gcosyHT is that peaks with no double-quantum transitions (e.g. singlets) will be suppressed, producing cleaner spectra. The experiment is also phase sensitive, resulting in narrower peak shapes. The 90° pulse width should be calibrated accurately for this experiment. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1 pulse width (pw):	Type in calibrated 90° pulse value
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

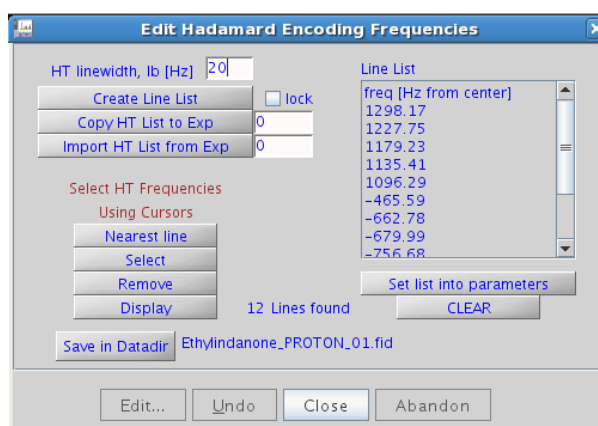*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard- transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click ▢ Create Line List to automatically create the frequency list:

- Uncheck ▢ lock to edit the line list. Place the cursor on the peak to be added or removed and click Select or Remove as appropriate. Click Set list into parameters, save the Hadamard frequency list in the Data directory using Save in Datadir, and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

- The main advantage of the dqcosyHT versus the gcosyHT is better resolution (phase sensitive versus absolute value experiment) and the absence of singlets that display no couplings. For instance, t-butyl signals, which can be a large source of t1 noise because of their intensity, are suppressed in a dqcosyHT.

- The 90° pulse width needs to be calibrated accurately for best results from the dqcosyHT experiment. See the PROTON experiment in Chapter 2 to calibrate the pulse.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

U. Piantini, O. W. Sorensen, R. R. Ernst, J. Am. Chem. Soc. 104 (1982) 6800-6801. (Double Quantum Filter)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)
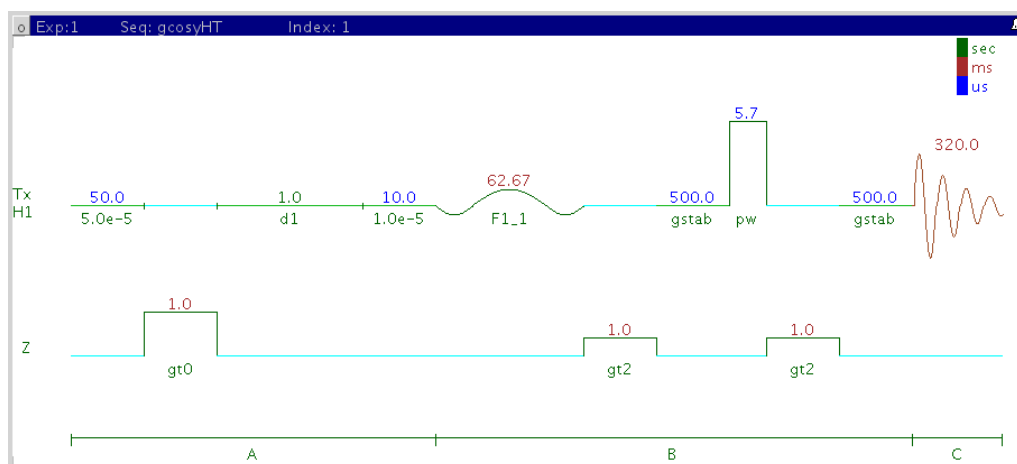
gcosyHT

Description:

Two Dimensional J-correlation spectroscopy with Hadamard encoding and gradient coherence selection.

Usage:

The gcosyHT experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON spectrum and crosspeaks between j-coupled protons The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1 pulse width (pw):	Type in calibrated 90° pulse value
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click [Create Line List] to automatically create the frequency list:

  Uncheck [☐ lock] to edit the line list. Place the cursor on the peak to be added or removed and click [Select] or [Remove] as appropriate. Click [Set list into parameters], save the Hadamard frequency list in the Data directory using [Save in Datadir], and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

Reference(s):

W. P. Aue, J. Karhan, R. R. Ernst, J. Chem. Phys. 64 (1976) 4226-4227. (COSY)

R. E. Hurd, J. Magn. Reson. 87 (1990) 422-428. (Gradient based Coherence Selection)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)
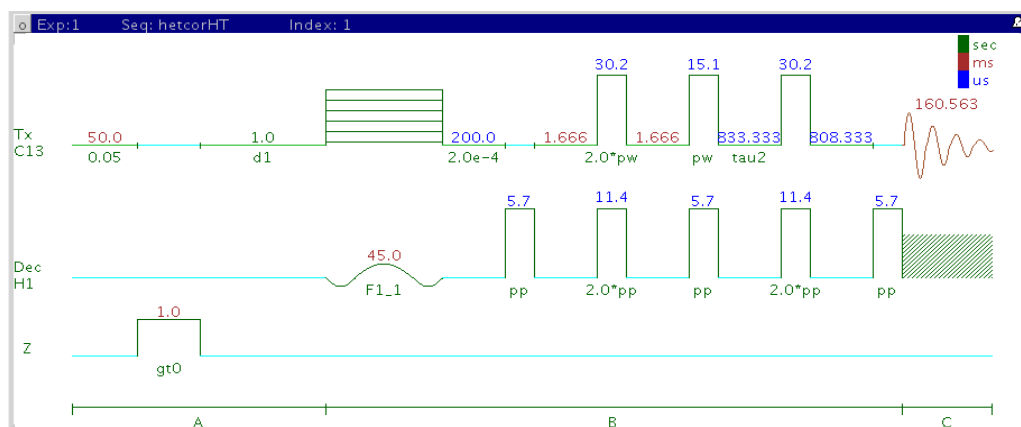
hetcorHT

GENERAL DESCRIPTION AND USAGE

Description:

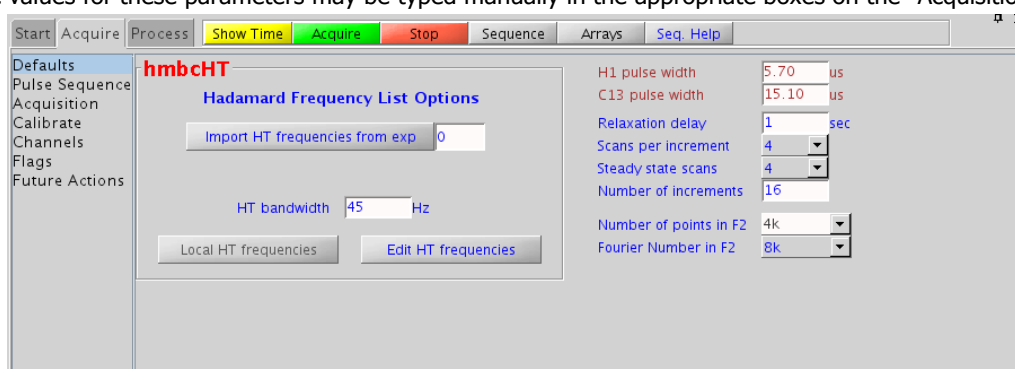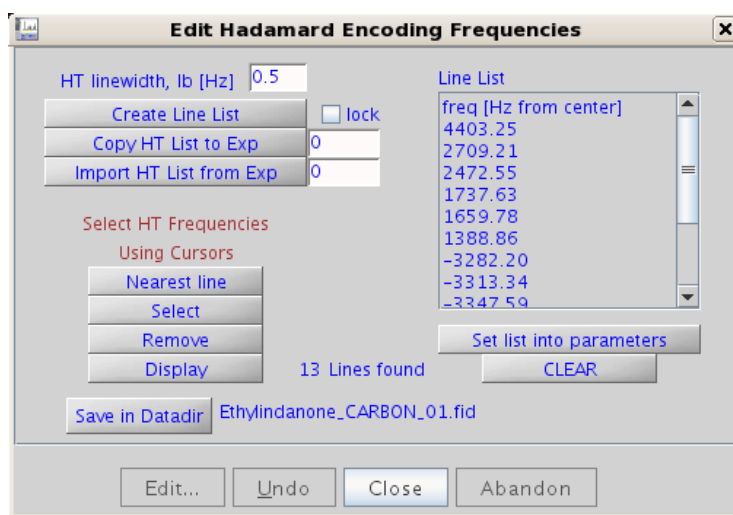Two Dimensional heteronuclear 1-bond correlation with Hadamard encoding (carbon observe).

Usage:

The hetcorHT experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons, however unlike the HSQC/HMQC experiments it is carbon-detected, meaning the F1 dimension is ^{1}H and the F2 dimension is ^{13}C. The Hadamard matrix is created from a previously acquired PROTON data set. The experiment is much less sensitive than the proton-detected hsqcHT, and should only be used under special circumstances where higher resolution is desired in the carbon dimension. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1/C13 pulse widths (pp/pw):	Type in calibrated 90º pulse values
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
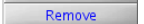
Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click Create Line List to automatically create the frequency list:

  Uncheck □ lock to edit the line list. Place the cursor on the peak to be added or removed and click Select or Remove as appropriate. Click Set list into parameters, save the Hadamard frequency list in the Data directory using Save in Datadir, and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

- The 90° pulse widths for ^{1}H and ^{13}C need to be calibrated accurately for best results from the hetcorHT experiment.

- In general, the hsqcHT experiment will be more sensitive than the hetcorHT. The advantage of the hetcorHT, though, is that a much higher resolution is achieved for the carbon, the F2, dimension. The resulting proton spectrum along the F1 dimensions is effectively a ^{1}H decoupled proton spectrum.

- Parameters to adjust the type and power for ^{13}C and/or ^{1}H decoupling can be found on the "Pulse Sequence" tab.

Reference(s):

A. Bax, G. A. Morris, J. Magn. Reson. 42 (1981) 501-505. (HETCOR)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)
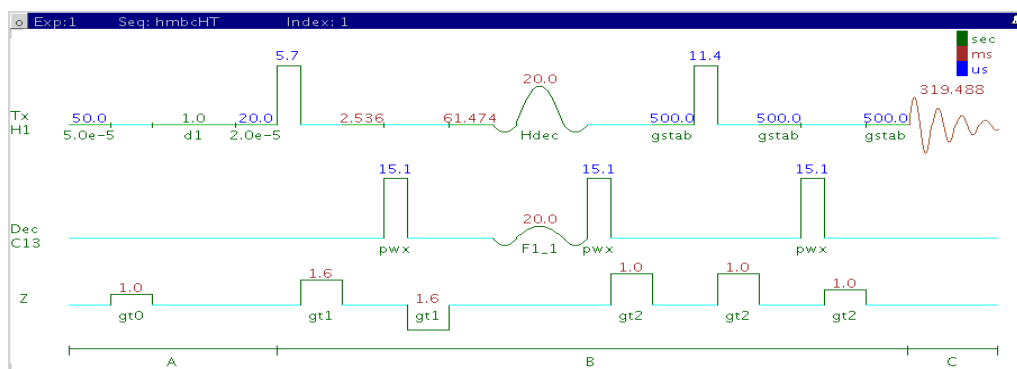
hmbcHT

GENERAL DESCRIPTION AND USAGE

Description:

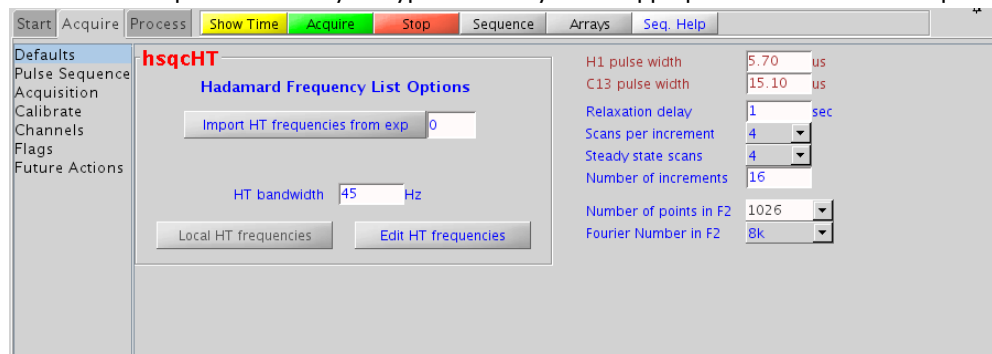Two Dimensional heteronuclear multiple-bond J-correlation spectroscopy with Hadamard encoding.

Usage:

The hmbcHT experiment is used to acquire a two-dimensional spectrum to correlate protons to carbons over long range couplings, usually over 2-3 bonds. The Hadamard matrix is created from a previously acquired CARBON data set prior to acquisition. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired CARBON- see Tips, Tricks and Next Steps for setup description
H1/C13 pulse widths (pw/pwx):	Type in calibrated 90° pulse values
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
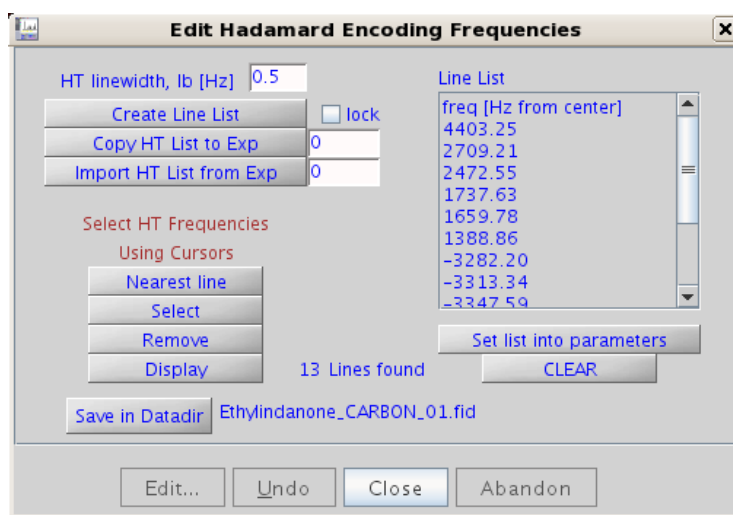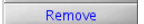
Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click Create Line List to automatically create the frequency list:

- Uncheck ☐ lock to edit the line list. Place the cursor on the peak to be added or removed and click Select or Remove as appropriate. Click Set list into parameters, save the Hadamard frequency list in the Data directory using Save in Datadir, and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with CARBON indirect dimension.

- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.

- Parameters to adjust the J-couplings $^{1}J_{C,H}$ and $^{n}J_{C,H}$ (defaults are 140 Hz and 8 Hz) can be found on the "Pulse Sequence" tab. The jnxh sets the optimal value of the proton-carbon coupling constant for detection. Using smaller values may increase the chances of seeing longer-range correlations, but at the expense of correlations with larger coupling constants, and, more importantly, at the expense of sensitivity.

Reference(s):

A. Bax, M. F. Summers, J. Am. Chem. Soc. 108 (1986) 2093-2094. (HMBC)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

hsqcHT
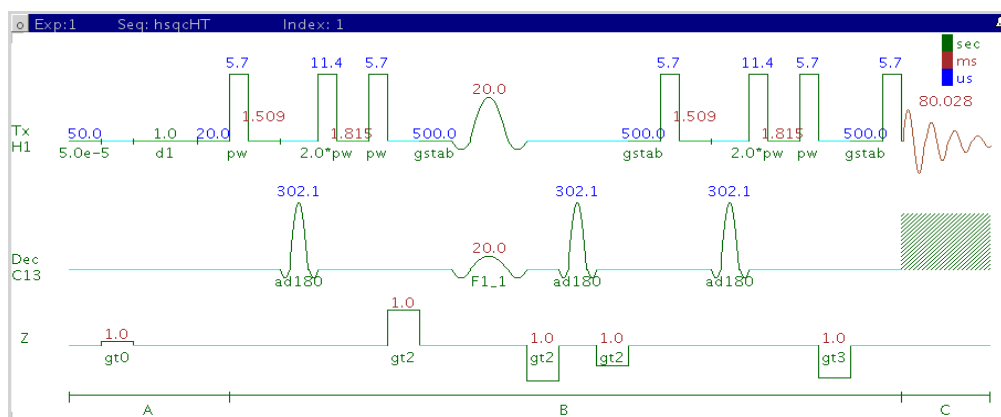
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with Hadamard encoding.

Usage:

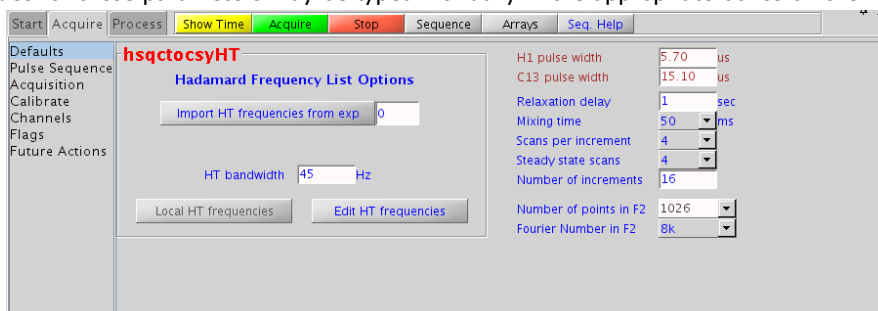The hsqcHT experiment is used to acquire a 2-dimensional spectrum to correlate protons to directly attached carbons.. The Hadamard matrix is created from a previously acquired CARBON data set prior to acquisition. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1/C13 pulse widths (pw/pwx):	Type in calibrated 90° pulse values
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

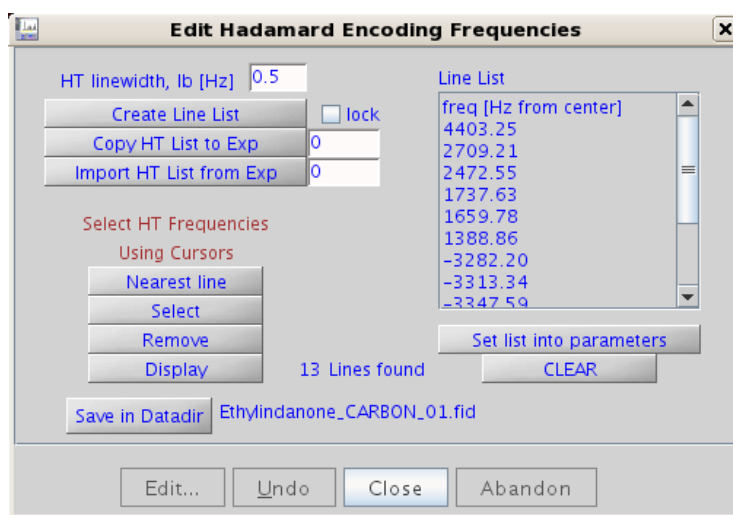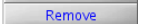Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click Create Line List to automatically create the frequency list:

- Uncheck □ lock to edit the line list. Place the cursor on the peak to be added or removed and click Select or Remove as appropriate. Click Set list into parameters, save the Hadamard frequency list in the Data directory using Save in Datadir, and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with CARBON indirect dimension.

- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.

- Parameters to adjust the type and power for ^{13}C decoupling can be found on the "Pulse Sequence" tab. To measure ^{1}H-^{13}C (or ^{1}H-X) coupling constants, the carbon decoupling can be turned off by entering "nnn" in the Channel 2 "Dec On/Off" entry on the "Channels" panel or alternatively by typing dm='nnn' on the command line.

Reference(s):

G. Bodenhausen, D. J. Ruben, Chem. Phys. Lett. 69 (1980) 185-189. (HSQC)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

hsqctocsyHT
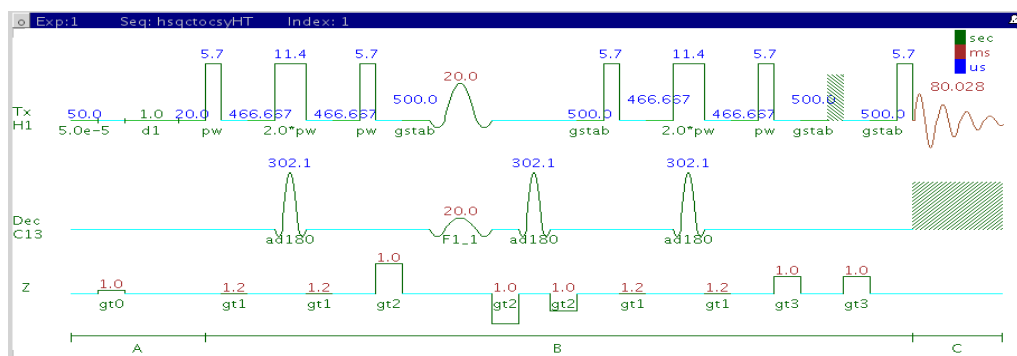
GENERAL DESCRIPTION AND USAGE

Description:

Two Dimensional heteronuclear single-quantum 1-bond J-correlation spectroscopy with a TOCSY spinlock and Hadamard encoding.

Usage:

The hsqctocsyHT experiment is used to acquire a 2-dimensional spectrum to connect ^{1}H TOCSY correlations with ^{13}C nuclei chemical shift information For e.g. Proton A is in the same spin system as proton B at carbon chemical shift X. The experiment is useful to resolve overlapping spin systems in the ^{1}H TOCSY by spreading them out over the carbon (or other X-nuclei) chemical shift range. The Hadamard matrix is created from a previously acquired CARBON data set . All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired CARBON- see Tips, Tricks and Next Steps for setup description
H1/C13 pulse widths (pw/pwx):	Type in calibrated 90º pulse values
Relaxation delay (d1):	Type in value- controls the delay between scans
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Mixing time (mix):	Select from pull down menu- 80ms will work best for large spin systems*
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np
Hadamard Frequency List:	Set from previously acquired CARBON- see Tips, Tricks and Next Steps for setup description
H1/C13 pulse widths (pw/pwx):	Type in calibrated 90º pulse values

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

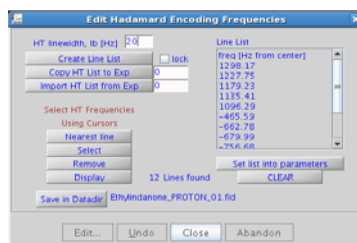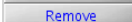Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a CARBON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click ⬚ Create Line List ⬚ to automatically create the frequency list:


  Uncheck ☐ lock to edit the line list. Place the cursor on the peak to be added or removed and click ⬚ Select ⬚ or ⬚ Remove ⬚ as appropriate. Click ⬚ Set list into parameters ⬚, save the Hadamard frequency list in the Data directory using ⬚ Save in Datadir ⬚, and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with CARBON indirect dimension.

- While editing the CARBON frequency list, it is important to remove solvent resonances from the list.

Reference(s):

K. E. Kover, O. Prakash, V, J. Hruby, J. Magn. Reson. A103 (1993) 92-96. (z-filtered HSQC-TOCSY)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

noesyHT
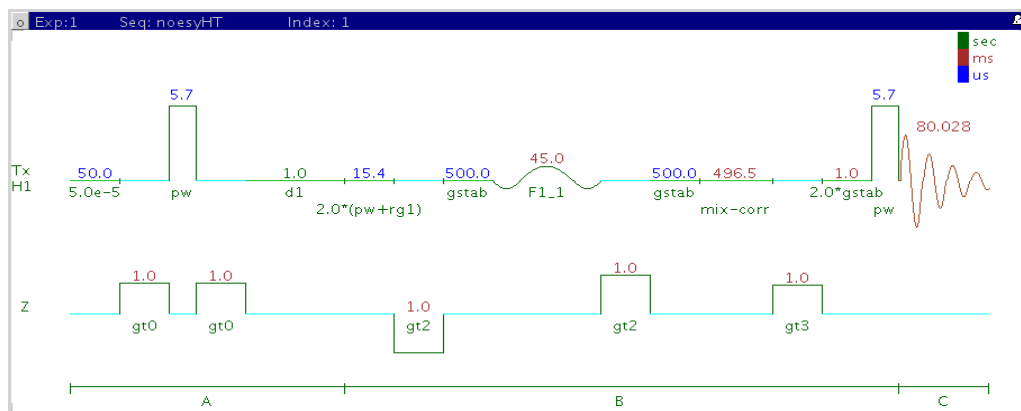
Description:
Two Dimensional through-space correlation spectroscopy with Hadamard encoding.

Usage:
The noesyHT experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <5 Å). The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1 pulse width (pw):	Type in calibrated 90° pulse value
Relaxation delay (d1):	Type in value- controls the delay between scans
Mixing time (mix):	Select from pull down menu- 500ms works well as a starting point*
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click ▭ Create Line List to automatically create the frequency list:

  Uncheck ▭ lock to edit the line list. Place the cursor on the peak to be added or removed and click ▭ Select or ▭ Remove as appropriate. Click ▭ Set list into parameters , save the Hadamard frequency list in the Data directory using ▭ Save in Datadir , and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

- The default parameters use a Grad-90-Grad steady state between scans, which helps cleanup un-relaxed magnetization. This can result in much cleaner spectra, especially when using shorter acquisition times (at) and relaxation delays (d1) (defaults are 0.15s and 1s, respectively).

- A mixing time of 500ms is a good starting place for most small molecules. Very small molecules and/or weak NOE interactions may require longer mixing times (up to ~1s). Longer mixing times, however, increase the possibility of spin diffusion for larger molecules, a situation where the magnetization is transferred through space, and then through coupling, resulting in spurious correlations between protons. The NOE correlation can be confirmed by acquiring a build-up curve, whereby the mixing time is slowly increased in 50-100ms increments until the NOE intensity ceases to increase and levels off. This is the optimal mixing time.

- Medium-sized molecules (MW ~500 depending on the molecule and spectrometer frequency) can exhibit zero or very small NOE's. One should consider acquiring a roesyHT for these molecules. Alternatively one may consider, (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Small molecules may have crosspeaks arising from chemical exchange during the NOE mixing time and will have the same phase as the diagonal. The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

Reference(s):

S. Macura, Y. Huang, D. Suter, R. R. Ernst, J. Magn. Reson. 43 (1981) 259-281. (NOESY)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)
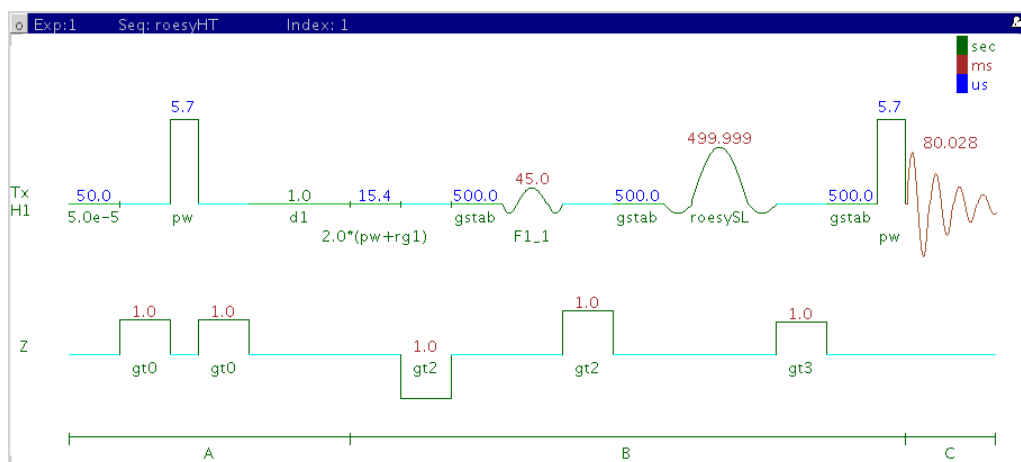
roesyHT

Description:
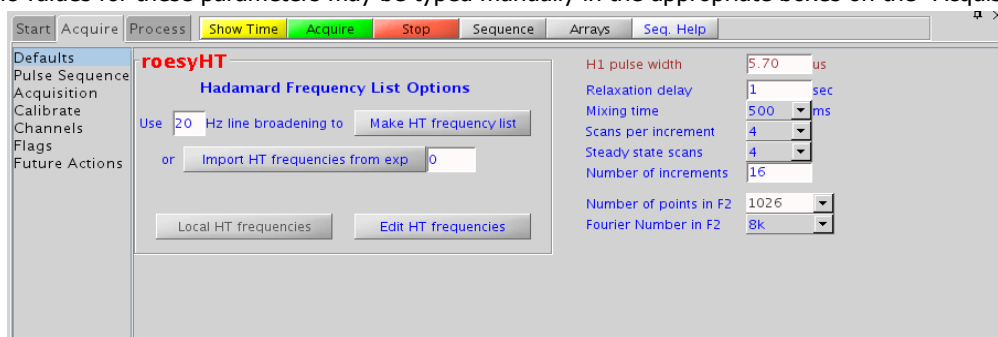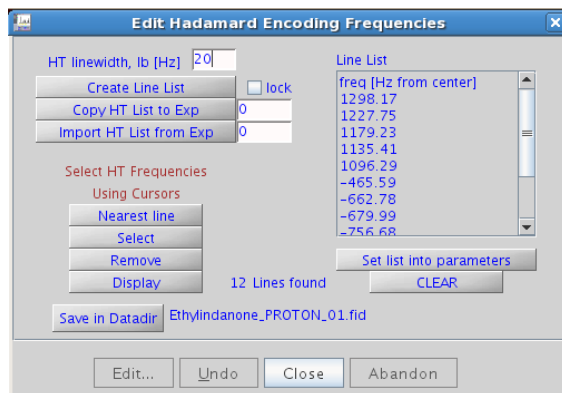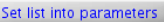Two Dimensional through-space correlation spectroscopy in the rotating frame with Hadamard encoding.

Usage:
The roesyHT experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between protons that are close in space (typically <4 Å). The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1 pulse width (pw):	Type in calibrated 90° pulse value
Relaxation delay (d1):	Type in value- controls the delay between scans
Mixing time (mix):	Select from pull down menu- 200ms works well for most small molecules* DO NOT EXCEED 500ms*
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.

Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click [Create Line List] to automatically create the frequency list:

  Uncheck [☐ lock] to edit the line list. Place the cursor on the peak to be added or removed and click [Select] or [Remove] as appropriate. Click [Set list into parameters], save the Hadamard frequency list in the Data directory using [Save in Datadir], and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

- A mixing time of 200ms is a good starting place for most small molecules. Very weak ROE interactions may require longer mixing times (up to ~500ms), but keep the mixing time shorter as longer spinlocks may result in sample heating. It is a good idea not to exceed a 500ms spinlock.

- Because the mixing time for the roesyHT is limited, weaker correlations may not be observable. roesyHT is the experiment of choice over noesyHT for medium-sized molecules (MW ~800-1000 depending on the molecule and spectrometer frequency), as they can exhibit zero or very small NOE's. One may consider running noesyHT experiments (which can be run with longer mixing times) by (i) choosing a more viscous solvent such as d_6-DMSO or d_6-DMSO with ~10-20% D_2O (which results in slower molecular tumbling and hence generates a positive NOE enhancement like larger molecules), (ii) selecting a lower temperature (if the solvent allows it), or (iii) running the experiment on a spectrometer with higher field strength (if available).

- Crosspeaks in the roesyHT spectrum that have the same phase as the diagonal may arise from chemical exchange during the ROE mixing time, or due to TOCSY-type correlations (couplings). The exchange crosspeaks provide information about inter-converting molecular conformations and could be used to recognize such molecular dynamic processes.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 207-213. (ROESY)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

tocsyHT

Description:

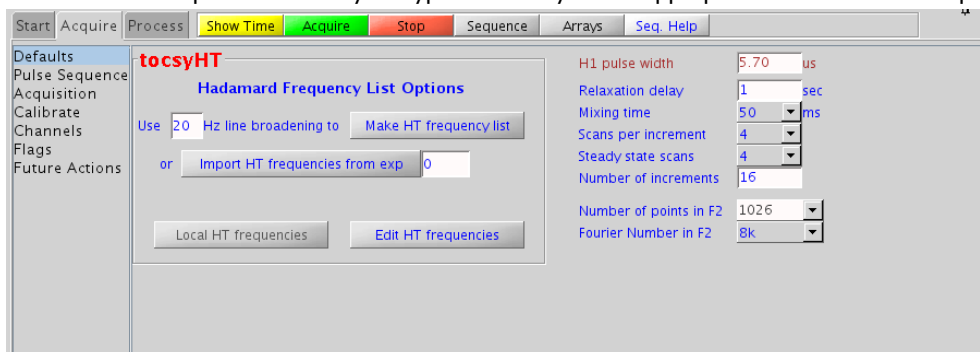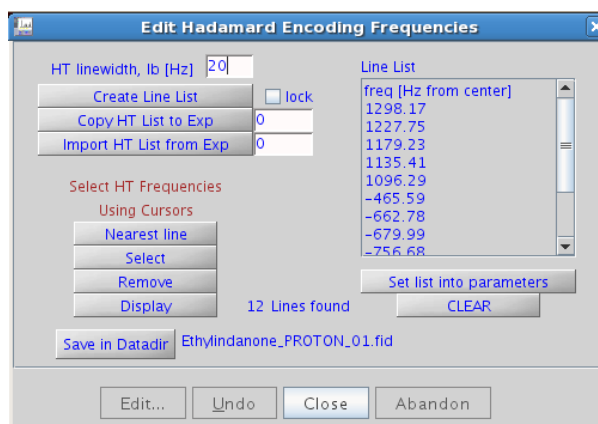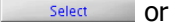Two Dimensional total J-correlation spectroscopy for scalar coupled spin systems with Hadamard encoding.

Usage:

The tocsyHT experiment will produce a 2-dimensional spectrum with peaks along the diagonal corresponding to the peaks in a 1D PROTON and crosspeaks between all protons in a scalar coupled spin system. The Hadamard matrix is created from a previously acquired PROTON data set. All parameters are set from the "Acquire" tab/"Defaults" panel unless otherwise specified.

KEY PARAMETERS

Hadamard Frequency List:	Set from previously acquired PROTON- see Tips, Tricks and Next Steps for setup description
H1 pulse width (pw):	Type in calibrated 90° pulse value
Relaxation delay (d1):	Type in value- controls the delay between scans
Mixing time (mix):	Select from pull down menu- 80ms will work best for large spin systems*
Scans per increment (nt):	Select typical choices from pull down menu*
Steady state scans (ss):	Select typical choices from pull down menu*
Number of increments (ni):	Unlike ordinary 2D experiments the parameter ni defines the Hadamard matrix size (dictated by the number of regions that will be excited)
Number of points in F2 (np):	Select typical choices from pull down menu- controls the resolution in F2
Fourier Number in F2:	Select typical choices from pull down menu- usually 2*np

*Note: Specific values for these parameters may be typed manually in the appropriate boxes on the "Acquisition" panel.
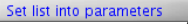
Automated processing is turned on by default, and the parameters are set to reasonable values, such that the resulting data after the acquisition will usually require no further adjustment. Note that a Hadamard transform is used, such that the parameter "proc1" should be set to "ht", which is the default. The "Process" tab/"Default" panel contains many of the commonly modified processing parameters, such as a pull-down menu to change the FT data size (4k x 256 is the default). Avoid arbitrarily setting the fn1 parameter, because its value is based upon the actual separation and bandwidths of frequencies in the Hadamard dimension, and is set automatically. More processing details can be found under some of the other "Process" panels, such as the "Weighting" and "More 2D" panels.

TIPS, TRICKS AND NEXT STEPS

- To setup the Hadamard encoding frequencies, record, process, and autosave a PROTON spectrum. With the PROTON spectrum in current workspace select Experiments menu → Hadamard Experiments → Set Hadamard Encoding from the menu to bring up the dialog box below. Click [Create Line List] to automatically create the frequency list:

  Uncheck [☐ lock] to edit the line list. Place the cursor on the peak to be added or removed and click [Select] or [Remove] as appropriate. Click [Set list into parameters], save the Hadamard frequency list in the Data directory using [Save in Datadir], and close the dialog box. This frequency list will be used for all subsequent Hadamard experiments with PROTON indirect dimension.

- The spinlock time ultimately controls how far along the spin system the correlations will extend. A value of 50 or 80ms is typically a good choice.

- A pull-down menu to change the waveform for the tocsyHT spinlock can be found on the "Pulse Sequence". The DIPSI2 spinlock pattern is a good choice for small molecules and FLOPSY8 is typically used for larger molecules.

Reference(s):

A. Bax, D. G. Davis, J. Magn. Reson. 63 (1985) 355-360. (TOCSY with MLEV17)

E. Kupce, R. Freeman, J. Magn. Reson. A105 (1993) 310-315. (Hadamard)

E. Kupce, T. Nishida, R. Freeman, Prog. NMR. Spectrosc. 42 (2003) 95-122. (Hadamard – Review)

Alphabetical Index of Experiments

Alphabetical Index of Experiments (cont.)